Cryptococcal Meningitis in The Tropics: Defining the Problem and Refining the Management

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1. Acknowledgements

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<td>ABPA</td>
<td>Allergic bronchopulmonary aspergillosis</td>
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<td>AE</td>
<td>Adverse event</td>
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<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
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<tr>
<td>AML</td>
<td>Acute myeloid leukaemia</td>
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<tr>
<td>ART</td>
<td>Anti-retroviral therapy</td>
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<tr>
<td>ARV</td>
<td>Anti-retroviral</td>
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<td>CFU</td>
<td>Colony forming units</td>
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<td>CGB</td>
<td>Canavanine-Glycine-Bromothymal</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CM</td>
<td>Cryptococcal meningitis</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<tr>
<td>CPA</td>
<td>Chronic pulmonary aspergillosis</td>
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<tr>
<td>CrAg</td>
<td>Cryptococcal antigen</td>
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<td>CRF</td>
<td>Case report form</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DALY</td>
<td>Disability adjusted life years</td>
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<td>DMEC</td>
<td>Data monitoring and ethics committee</td>
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<td>DSMB</td>
<td>Data safety and monitoring board</td>
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<td>EFA</td>
<td>Early fungicidal activity</td>
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<tr>
<td>GAFFI</td>
<td>Global Action Fund for Fungal Infections</td>
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<td>GalXM</td>
<td>Galactoxylomannan</td>
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<td>GCS</td>
<td>Glasgow coma score</td>
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<td>GDP</td>
<td>Gross domestic product</td>
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<td>GXM</td>
<td>Glucuronoxylomannan</td>
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<tr>
<td>HCMC</td>
<td>Ho Chi Minh City, Vietnam</td>
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<tr>
<td>HIV</td>
<td>Human immune-deficiency virus</td>
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<td>HR</td>
<td>Hazard ratio</td>
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<tr>
<td>HTD</td>
<td>Hospital for Tropical Diseases (HCMC, Vietnam)</td>
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<tr>
<td>IA</td>
<td>Invasive aspergillosis</td>
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<tr>
<td>ICP</td>
<td>Intracranial pressure</td>
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<tr>
<td>ICTRIP</td>
<td>International clinical trials registry platform</td>
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<tr>
<td>ICU</td>
<td>Intensive care unit</td>
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<td>IDSA</td>
<td>Infectious Disease Society of America</td>
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<td>IFN</td>
<td>Interferon</td>
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<td>IL</td>
<td>Interleukin</td>
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<td>IQR</td>
<td>Interquartile range</td>
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<tr>
<td>IRIS</td>
<td>Immune reconstitution inflammatory syndrome</td>
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<tr>
<td>ITT</td>
<td>Intention to treat</td>
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<td>LFA</td>
<td>Lateral flow antigen</td>
</tr>
<tr>
<td>LLN</td>
<td>Lower limit of normal</td>
</tr>
<tr>
<td>LTA4H</td>
<td>Leukotriene-A4 Hydrolase</td>
</tr>
<tr>
<td>MedRA</td>
<td>Medical dictionary for regulatory activities</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>MLST</td>
<td>Multi-locus sequence typing</td>
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<tr>
<td>MP</td>
<td>Mannoproteins</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PCP</td>
<td>Pneumocystis pneumonia</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PTB</td>
<td>Pulmonary tuberculosis</td>
</tr>
<tr>
<td>QALY</td>
<td>Quality adjusted life years</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real time polymerase chain reaction</td>
</tr>
<tr>
<td>SAE</td>
<td>Severe adverse event</td>
</tr>
<tr>
<td>SAFS</td>
<td>Severe asthma with fungal sensitization</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Th1/2/17</td>
<td>T-helper cell type 1/2/17</td>
</tr>
<tr>
<td>TMB</td>
<td>Tuberculous meningitis</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper limit of normal</td>
</tr>
<tr>
<td>USAE</td>
<td>Unexpected severe adverse event</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VVC</td>
<td>Vulvo-vaginal candidiasis</td>
</tr>
<tr>
<td>WCC</td>
<td>White cell count</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
</tbody>
</table>
4. Introduction

4.1 Executive Summary of Thesis

The basis of my thesis is the CryptoDex trial, a randomized controlled trial of adjunctive dexamethasone in HIV-associated cryptococcal meningitis, which I implemented and ran in 13 sites in 6 countries in Southeast Asia and Africa. This trial is presented in detail in Chapter 1. In order to contextualize the trial, and to get a better sense of the burden of cryptococcal meningitis in Vietnam, I estimated the burden of all the major invasive fungal infections in Vietnam. My approach to making these estimates, and my findings, are presented in Chapter 1. The final two data chapters arose from the CryptoDex trial. The CryptoDex trial was stopped early on the basis of an excess of adverse events in the intervention arm. Therefore, in chapter 7 I investigate the ethical, statistical, and logistical issues around stopping clinical trials early, and provide a detailed case study of our experience with early termination. In an approach to better understand the effects of dexamethasone, and how it may have lead to worse outcomes, I then characterized markers of immune response in the cerebrospinal fluid of the study patients and describe their relationship with clinical and microbiological outcomes. These findings are presented in chapter 8. Each chapter contains its own introduction and methods sections; the remainder of this introduction places each chapter in context.
4.2 Global Burden of Fungal Infections

Fungal infections can be broadly categorized into five main types, with some overlap between them. Infections may be primarily mucosal, cutaneous, allergic, deep-tissue, or invasive. Throughout this thesis, I will refer to invasive, deep-tissue and allergic fungal infections as ‘serious.’ Deep-tissue and invasive mycoses are particularly serious because they are often associated with high mortality, and are frequently difficult to treat. Even on treatment, invasive mycoses have case fatality ratios up to 70% (5). Estimates suggest that over 90% of fatal fungal infections are caused by four genera: *Candida*, *Cryptococcus*, *Aspergillus*, and *Pneumocystis* - together they cause over two million life-threatening infections globally, each year (6). The incidence of oesophageal candidiasis, cryptococcal meningitis (CM), and *Pneumocystis jiroveci* pneumonia (PCP) are closely associated with HIV seroprevalence (7), and much of the burden of *Aspergillus* is linked to pulmonary tuberculosis (TB) (8,9), meaning that most cases of serious mycoses are likely to occur in lower-income areas where HIV and TB are prevalent (10).

Despite the amount of illness and death associated with fungal infections, they have a low profile and even well-resourced healthcare settings frequently neglect systematic surveillance (6). Fungal infections attract relatively little funding, with one estimate suggesting that fungal infections are allocated just 1.4-2.5% of the ‘immunology and infection’ research resources of major funders (6), even though they cause a comparable number of deaths to either TB or malaria (6,10). Given that fungal infections are likely to disproportionately affect countries with limited resources, where TB and HIV are prevalent (10), the lack of research and maldistribution of available treatments (11) raises issues of research equity (12).

4.3 Burden of Fungal Infections in Vietnam

There is no surveillance programme for fungal infections in Vietnam, and the epidemiology of fungal infections is largely unknown. There is an increasing volume of data
related to penicilliosis (4,6) and cryptococcosis (2,3,13) but accurate national estimates of incidence are missing, as are local epidemiological data on Candida, Aspergillus, and Pneumocystis. In a rapidly developing country such as Vietnam (14), the incidence of serious fungal infections is likely to increase further in tandem with rising access to complex medical interventions like prolonged intensive care and immunosuppressive therapies (15). An assessment of the baseline incidence of serious fungal infections is vital to facilitate the work of health care planners and public health professionals.

National population-based active surveillance programmes are the gold-standard for estimating disease burden, but they are extremely expensive and difficult to implement. Methods using sentinel surveillance have been described to provide data at a lower cost (16–19). Using this approach, one or more sites considered to be representative for a specific area report the number of cases observed of some condition of interest. The number observed at the sentinel sites is then multiplied to account for the cases that would not have been identified at the site, to arrive at an estimate for the whole area. A detailed study of catchment areas, patient flows, and diagnostic accuracy is required to make such estimates valid, which is challenging even in a simple healthcare setting. A sentinel surveillance approach is especially complicated to implement in the urbanized, densely populated communities found in much of Asia, where overlapping healthcare providers are the norm (20,21).

The Global Action Fund for Fungal Infections (GAFFI, www.gaffi.org) is co-ordinating a global drive to describe the burden of serious fungal infections. A growing number of researchers have applied actuarial methods for estimating the burden of fungal infections at the national level (22–39). These methods combine publicly available population and risk factor data to arrive at an actuarial estimate for the burden of that condition, and are fully
described in section 5.3 of chapter 1 where I estimate the prevalence and incidence of serious mycoses in Vietnam.

In many ways Vietnam is an ideal research setting for infectious diseases. With regards to estimating the burden of fungal diseases, the tropical climate, dense population, centralized health care system, and established research infrastructure mean that even rare but serious diseases, including most serious fungal infections, can be studied.

4.4 Cryptococcus spp. as a pathogen

There are over 70 species in the genus of Cryptococcus, but the vast majority are unable to cause disease in humans, just existing as environmental saprophytes (40). When human disease occurs, cryptococcal meningitis is the most severe manifestation. In this section I will give an overview of its history, microbiology, epidemiology, human immune responses, and treatment.

4.4.1 History

Cryptococcus neoformans was first described in 1894 following isolation of the yeast from peach juice (41,42), and almost contemporaneously identified as a pathogen in a patient with osteomyelitis (43). It was initially classified as a Saccharomyces spp, but it was a poor fit for the genus in that it did not cause fermentation and formed basidiospores rather than ascospores. For these reasons, in 1901 it was transferred to the genus Cryptococcus (40). The organism continued to be referred to by a number of different names in the decades following its discovery. The most common of these was Torula histolytica (which referred to a mistaken understanding that the organism lysed surrounding tissue) and this name persisted until the 1950s when consensus was finally achieved in the name Cryptococcus neoformans (44).

Although ~70 cryptococcal species have now been identified, just two, Cryptococcus neoformans and Cryptococcus gattii, account for almost all cases of human disease. They are
amongst the most significant pathogenic yeasts in man, responsible for a large burden of mortality and morbidity (45–47). These two species are widely dispersed throughout the environment; *C. neoformans* is associated with avian guano (44), while *C. gattii’s* first identified environmental niche was the Australian Red River gum tree (48,49). However, both species have subsequently been isolated from a wide variety of trees (50–52). The vast majority of human cryptococcal disease occurs in immunocompromised hosts, although disease in the immunocompetent is recognized with increasing frequency (2,40,44,53). In general *Cryptococcus* spp. are not considered to be primary human pathogens; rather the ability to cause disease is thought to be an accidental result of some adaptation to their usual saprophytic niche.

### 4.4.2 Light microscopy

Cryptococci are readily identified (and distinguished from other medically important yeasts) by their polysaccharide capsule (44). The capsule is easily seen following India Ink staining of clinical specimens. The yeast cells are round to oval, usually of a few microns in diameter, and characteristic budding is often seen. The capsule is a key determinant of virulence, and it is postulated that it enables survival of the yeast within free-living amoebae or other simple organisms that it encounters in its usual environmental niche. The capsular size varies depending on the yeast’s growth environment.

### 4.4.3 Other laboratory features

Biochemical profiling can be used to distinguish *Cryptococcus* species, with the most obvious example being the use of Canavanine-Glycine-Bromothymol Blue (CGB) Agar to distinguish *C. neoformans* from *C. gattii*. Because *C. gattii* is resistant to canavanine and can utilize glycine, its successful growth effects a change in pH which turns the medium blue, a pattern not seen with *C. neoformans* (54). Commercial identification systems, such as API 20C AUX™ (bioMérieux SA, Marcy-l’Etoile, France) and Vitek™ (bioMérieux Inc., Hazelwood, USA)
Pathogenic *Cryptococci* can also be distinguished serologically. In 1935 Benham described serotypes A, B and C, using sera from inoculated rabbits (56). The resolution of this method was improved by Evans in the 1950s (57,58), enabling the identification of serotype D in the late 60s, and a hybrid form (AD) in the 1980s (59,60). *Cryptococcus neoformans* var. *grubii* is generally of serotype A, *Cryptococcus neoformans* var. *neoformans* generally D, and *Cryptococcus gattii* B or C. However, exceptions do occur, which makes serological typing alone inadequate for accurate speciation (61).

Molecular typing techniques, notably restriction fragment length polymorphism analysis (RFLP) (62), amplified fragment length polymorphism analysis (AFLP) (63) and multi-locus sequence typing (MLST) (64) have generally supported speciation established by classical microbiology and serology. However, they have also been instrumental in identifying new species, revising incorrect inter-species distinctions, and informing the decision to raise *Cryptococcus neoformans* var. *gattii* from a varietal form to a species in its own right (40,65,66). Earlier observations that *C. neoformans* var. *gattii* isolates were antigenically dissimilar, had different ecological niches, and caused a different spectrum of disease have been validated by sequence-based technologies demonstrating that the two forms are evolutionarily distinct (67,68), having diverged 40-80 million years ago (69,70).

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis of the URA5 gene has been particularly useful (62). This simple tool divides *C. neoformans* into five genotypes (VNI-VNIV and VNB) and *C. gattii* into four (VGI-VGIV). The genotypes provide a basis from which to understand phenotypes, correlating with features such as outbreak epidemiology (40), antifungal susceptibility and virulence (71,72). However, it has recently been noted that these genotypes have variable degrees of genetic heterogeneity, and some
may benefit from subdivision (e.g. VGII being divided into a, b and c in the Vancouver Island outbreak – see below). Some genotypes may be sufficiently distinct to be classified as varieties, or even ‘cryptic species’ - VG genotypes demonstrate distinctiveness at least equivalent to that seen between \textit{C. neoformans} vars. \textit{grubii} and \textit{neoformans} (66,73,74). Taxonomical debates continue as classification systems keep pace with developing technology (65,75).

4.4.4 Determinants of pathogenicity in humans

Since \textit{Cryptococcus} spp. are primarily environmental saprophytes and transmission between humans does not occur, the adaptive pressures conferring pathogenicity must come from the non-human environment: so-called ‘bystander pathogenicity’ (76,77). The virulence factors that allow \textit{C. neoformans} to evade the human immune response include its external polysaccharide capsule, its ability to grow at mammalian body temperature, its production of melanin, and a number of secreted factors (including phospholipase B and urease) (40,78). However, none of these are likely to have developed in response to selective pressure from mammalian immune systems. \textit{Cryptococcus} spp. can infect hosts ranging from amoebae to humans, but mammalian hosts are not required at any stage of the life cycle. Even pigeons, considered to have an important role in the global dispersal of \textit{C. neoformans}, are not susceptible to cryptococcosis so are unlikely to exert evolutionary pressure (44,79). It is more likely that interactions between \textit{Cryptococci} and environmental organisms have selected for attributes that are coincidentally useful in mammalian infection. Some of these attributes are described below.

4.4.4.1 Uptake and subsequent intracellular proliferation

In 1982, Ruiz et al. demonstrated that several organisms commonly found in pigeon guano were fungivorous for \textit{Cryptococcus neoformans} (80), which is of interest because these organisms would be expected to exert selective pressure on \textit{Cryptococcus neoformans}
populations. Amongst these fungivorous organisms was the amoeba *Acanthamoeba castellani*, which ingests *C. neoformans*, after which intracellular proliferation and the accumulation of cytosolic vesicles containing shed capsular polysaccharide are observed (81). However, intracellular proliferation is reduced in isolates deficient in capsules or phospholipase, and acapsular isolates are easily destroyed (82,83). Melanin production also protects *C. neoformans* from predatory amoebae with melanized isolates are more likely to survive ingestion (82,83). The human pathogenicity factors described here most likely evolved under selective pressures imposed by amoebae.

There are similarities in the way *C. neoformans* interacts with amoebae and mammalian host macrophages. *C. neoformans* is a facultative intracellular pathogen in mammals, and is able to survive phagocytosis by mammalian immune cells and proliferate intracellularly (40). This transferable intracellular survival skill may help to explain the broad host range of *C. neoformans*, since most mammalian immune responses rely on phagocytic cells. Once ingested by phagocytes, Cryptococci can disseminate by non-lytic extrusion of yeast cells into the extracellular space and adjacent host cells (‘vomocytosis’) (82,84–89). This ‘Trojan horse’ mechanism is thought to be key in establishing central nervous system infections (90–92).

### 4.4.4.2 Capsule

*C. neoformans*’ unique capsule is comprised of polysaccharides (glucuronoxylomannan (GXM) and galactoxylomannan (GalXM)) and mannoproteins (MP) (93). Beyond its role in amoebic and host cell phagocytosis, the capsule also prevents dehydration and desiccation in dry conditions (94). The capsule has a well-established role in mammalian pathogenesis (93,95,96). Encapsulated *C. neoformans* strains are more pathogenic in mice. The capsule promotes yeast survival in their lungs, increases dissemination, and subdues both cellular and complement-based immune responses (97–99). Capsular polysaccharide components directly stimulate naive T-helper cells to differentiate into the Th2 phenotype, which favours
intracellular parasitism and dissemination (100). The fact that acapsular C. neoformans strains are only capable of causing disease in mice with an absent or deficient thymus further demonstrates the pathogenic role of the capsule (44). Despite this evidence from animal models, links between capsule size and human virulence were not demonstrated until recently. Robertson et al. showed that a larger ex vivo capsule size was associated with higher opening pressure of CSF and a deficient CSF inflammatory response (96). In their studies, the larger capsule was associated with increased shedding of capsular antigens and greater CSF viscosity, potentially explaining the elevated opening pressure of CSF (96).

4.4.4.3 Growth at 37°C

The ability to grow at 37°C or higher is essential for any human pathogen (101–104). Why Cryptococcus spp. developed tolerance to such high temperatures is unknown, though several theories exist. Many fungal saprophytes are thought to have adapted to infect endothermic hosts (101). However, such an adaptation would only be vital for organisms that require mammalian hosts within their replicative cycle, so for Cryptococcus specifically, adaptations to warmer environments may offer a likelier explanation.

Strains of C. neoformans can grow in temperature ranges from 30°C to 40°C (104). Thermotolerance varies between different C. neoformans strains, and this may be partly determined by geography. For instance, C. neoformans var. neoformans strains are generally less thermotolerant than C. neoformans var. grubii, and are more prevalent in temperate regions (105). Given the evidence that Cryptococcus spp. originated in sub-Saharan Africa, these organisms would have likely experienced selection pressure for thermotolerance as a result of high ambient temperatures (50,106).

4.4.4.4 Melanin production

C. neoformans synthesizes melanin with the enzyme laccase (107–109). Melanization likely developed to protect cells from UV radiation and supports growth at higher
temperatures, but also enhances pathogenicity (110–112). Defects in melanin production lead to improved host survival in mouse models (109). Melanin has been shown to protect *Cryptococcus* spp. against enzymatic degradation, antimicrobial peptides, oxidative stress, and heavy metal toxicity (113–116). Of note, it also decreases the efficacy of amphotericin B in *vitro* (115). Finally, higher laccase activity in human clinical isolates is associated with higher *ex vivo* CSF survival and anti-fungal resistance (117).

### 4.4.4.5 Morphology switching

Although the majority of cryptococcal pathogenic adaptations seem to occur independent of mammalian hosts, morphological transformations do occur during mammalian infection. One important transformation is the up-regulation of capsule synthesis, producing giant cells (also known as titan cells, see Figure 4-1), the diameter of which are 40-50 μm on average (83,93,118,119), but can reach up to 100 μm (120).

In mice, giant cells can constitute 10-80% of the total pulmonary fungal population, depending on the length of infection, inflammatory response, and fungal burden (83). Lower fungal burdens and reduced inflammation are associated with a higher proportion of giant cells (83). Giant cells often have a single polyploid nucleus, indicating DNA replication occurs without subsequent mitosis and/or cytokinesis. Variable ploidy in the cryptococcal giant cell subpopulation shows both flexibility and stability of the genome (78,83,120).
The formation of giant cells may facilitate evasion of host immune defenses. Giant cells are frequently found in extracellular spaces and are more resistant to phagocytosis than standard yeast cells. It is hypothesized that this extracellular subpopulation co-operates with the remaining population of normal cryptococci following their dissemination via phagocytosis. Morphological heterogeneity presents an obstacle for the immune response and thereby increases pathogenicity and persistence of disease (116).

4.4.4.6 Genome “flexible stability”

Both the size and number of chromosomes are highly variable in clinical and environmental cryptococcal isolates, suggesting considerable genomic flexibility (121,122). The karyotype may also change within a single infection, as observed in multiple human cases and mouse infection models (123). Major chromosomal rearrangements have been observed in closely related isolates from the same patient, separated by just 77 days. Together, these findings suggest that host-derived selective pressures may be among the drivers of genome flexibility in mammalian infections (124–126).
Genomic flexibility may act as a virulence factor in cryptococcal infections, and may have important clinical repercussions. For example, aneuploidy may be a factor in developing resistance to fluconazole (127–129). This phenomenon is seen in *Candida albicans*, where chromosomal rearrangement and duplication allows an infecting populations to respond to selective pressures (130). In this process, termed heteroresistance, a subpopulation of resistant organisms exists amongst a larger population of susceptible siblings as a result of genomic plasticity. Studies on azole heteroresistance in *C. neoformans* have demonstrated disomy in chromosomes 1 and 4 of resistant subpopulations, whilst the most resistant clones had disomies in chromosomes 1, 4, 10, and 14 (127,131). Thus far two mechanisms to explain the link between these genome changes and azole resistance have been discovered. Amplification of efflux pump genes on chromosome 1 are associated with azole resistance (127), while disomy of chromosome 4 upregulates genes which maintain endoplasmic reticulum integrity under azole stress (131).

4.4.5 Emergence of Cryptococcus spp. as a public health challenge

Since its discovery, *Cryptococcus* has transformed from a rare pathogen to a major public health challenge. In their 2017 paper, Rajasingham *et al* estimated that cryptococcal meningitis causes 15% of AIDS related deaths (45). Their estimates indicated that in 2014 alone, over 220,000 people were affected by the disease, and over 180,000 of them died. Their findings showed the largest number of deaths occurred in sub-Saharan Africa, followed by the Asia-Pacific region. The global HIV pandemic is the most dramatic example of *Cryptococcus* exploiting a new environmental niche, and AIDS continues to be the commonest risk factor globally. However, in rich countries ongoing emergence is increasingly driven by non-communicable causes of immune-deficiency (46,132–136), including iatrogenic immunosuppression due to solid organ transplants or biological immunomodulatory therapies (134,137,138). There are no accurate estimates for the global incidence of non-HIV associated cryptococciosis: sporadic cases in apparently immune-competent hosts persist.
(46,133,135,136,139,140), but large outbreaks in immune-competent populations have also been described and are discussed in section 4.4.5.2.

4.4.5.1 Cryptococcus as a globally endemic pathogen

The global distribution of Cryptococcus spp. may have been facilitated by a combination of the great human migrations, expansion of domesticated pigeon populations and the huge expansion of international trade in Africa driven by European imperialism (141–146). This theory has been further supported by research in Thailand, where a genetic bottleneck, suggested to result from the founder-effect, was identified in \textit{C. neoformans} var. \textit{grubii} isolates (142). Strong signatures of clonality were detected in stark contrast to the genetic heterogeneity seen in African isolates (142). The mean time to the most recent common ancestor was estimated to be approximately 7000 years ago, well after the currently estimated dates for the human out-of-Africa migration (143,147,148), suggesting Cryptococcus left Africa with man, pigeons and perhaps other vectors to ultimately expand clonally in new geographic locations. This ex-African clonality has been described in other geographic locations, and is believed to demonstrate an epidemic population structure for the organism (53,64,149,150)

4.4.5.2 Cryptococcus as an outbreak pathogen

The story of \textit{Cryptococcus gattii} provides another example of the cryptococcal capacity to exploit new niches. \textit{C. gattii} is estimated to have diverged from \textit{C. neoformans} approximately 40 to 80 million years ago (69,70). It was first described in 1970 in a Congolese patient with cryptococcosis; the infecting isolate was found to have a different morphology to \textit{C. neoformans} (151,152). Instead of the usual uniform round or oval forms seen with \textit{C. neoformans}, this new isolate produced frequent elongated or bacilliform morphotypes. The pathogen subsequently reached prominence in Australia, and pioneering work was undertaken to identify its environmental niche in 1990 (48,49). Following extensive sampling
of plants, their debris, soil and air, the major niche was identified as the Red River Gum tree, *Eucalyptus camaldulensis*. Since then, numerous other tree species have been identified as habitats; the particular niche of the organism appears to be rotting bark and wood, as well as soil beneath the canopy (51,52,74,153–157). Historically, the incidence rates of *C. gattii* cryptococcosis have been highest in Papua New Guinea (42.8/million/year) and Australia’s Northern Territory (8.5/million population/year) (158). Unlike disease due to *C. neoformans*, patients diagnosed with *C. gattii* usually have no identified immune-deficiency, and pulmonary involvement is more common (159,160). Host factors may have an impact on the risk of disease - in Australia the incidence rate in the aboriginal population is 10.4/million/year compared to the non-indigenous population rate of 0.7/million/year, and the difference is not thought to be wholly explained by differences in geography (158).

It was previously believed that *C. gattii* was limited to the tropics and subtropics, but it is increasingly being recognized in temperate regions. The ongoing outbreak on Canada’s Vancouver Island and in the Pacific Northwest of the USA, described in more detail below, provides an excellent illustration of a pathogen exploiting a new environmental niche; incidence there is now 25.1/million/year (161).

The various *C. gattii* genotypes differ in their global distributions, reproductive behaviour and pathogenicity. The VGII genotype appears to be the oldest, estimated to have diverged from a common ancestor 12.5 million years ago; VGIV diverged 11.7 million years ago; and VGI and VGIII diverged from each other 8.5 million years ago (69). VGI is the genotype most prevalent in Australasia and Europe, VGII is most prevalent in South and North America (including the current Pacific Northwest outbreak), VGIII is more common in North and South America than other regions (but not predominant), and VGIV is the most frequently described in Africa (66,162). The geographic spread of *C. gattii* and *C. neoformans* is depicted Figure 4-2. The data (review by Cogliati, 2013) contains a combination of clinical, veterinary and
environmental samples from 2012 and earlier, which were not necessarily collected under formal surveillance nor randomised sampling programmes. Despite these limitations, it is interesting to note the regional variations, especially in *C. gattii* (163).

Since 1999 there has been an outbreak of *C. gattii* disease centred around Vancouver Island, Canada, and by 2009 it had spread to northwestern USA (164). This outbreak has largely resulted from clonal expansions of three subtypes of VGII (VGIIa, VGIIb and VGIIc). VGIIa dominates, and has been termed the “major” strain. Laboratory models suggest it has enhanced virulence - it has a high intracellular proliferation rate (IPR) (meaning it replicates rapidly within macrophages), which is associated with shorter survival times in the mouse infection model. Interestingly, this increased virulence may in part be explained by mutations in its mitochondrial genome (72,165). Voelz et al. recently clarified the previously described link between VGIIa’s increased IPR and its capacity to transform its mitochondrial morphotype to tubular (from globular) (165). It had already been noted that a tubular mitochondrial
morphotype was more common in the pathogenic outbreak strain (72). Using time-lapse images, it was noted that VGIIa cells can rapidly tubularize their mitochondria in response to oxidative stress, becoming significantly less likely to be killed by the macrophage and yet slower to replicate than those with globular mitochondria. The finding of reduced fecundity in a strain with a higher IPR was explained by the observation that the remaining yeast cells (with globular mitochondria) replicated very rapidly. In the presence of the resistant but non-replicative VGIIa tubular mitochondrial morphotype, even non-outbreak strains are stimulated to increased IPRs, suggesting a signaling pathway whereby yeast cells establish a ‘division of labour’ (165). This may have implications for other infections, and especially co-infections occurring in the presence of C. gattii (165).

VGIIb, which is termed the “minor” strain in this outbreak, demonstrates lower virulence than VGIIa both in vitro and in vivo (72,166), although a difference in human outcomes has been more difficult to demonstrate (161). VGIIb is responsible for less than 10% of cases in this outbreak, and because many of those affected are in older age groups it is difficult to compare clinical outcomes (161). VGIIc is genotypically and phenotypically similar to VGIIa, but is unique to the United States. It was first isolated in Oregon and appears to be the result of a recombination event, either locally or prior to its spread into this region (72). Currently in Oregon, VGIIc causes 27% of infections and VGIIa approximately 63% (167), compared to 0% and 86.3%, respectively, in British Columbia (161).

The origin of the outbreak strains has been the source of debate. Until recently, evidence was balanced between the likeliest candidates: South America, Africa and Australia (which all showed evidence of recombination and high genetic diversity). However, the case for South America has recently been strengthened (69) by the discovery of a strain characterising a basal genetic lineage in virgin Amazonian rainforest, where contamination from imported wood is thought to be extremely unlikely (168). Ultimately it is hoped that better understanding of the
relationships between subtypes and their environmental niches will enable the public health community to predict the likely range of current outbreaks, as well as regions that may be at risk of outbreaks in the future (169).

4.5 HIV Associated Cryptococcal Meningitis

The remainder of this introduction will focus specifically on HIV-associated cryptococcal meningitis, which is overwhelmingly caused by Cryptococcus neoformans var. grubii (40).

4.5.1 Clinical features and diagnosis

The clinical features of HIV associated cryptococcal disease, gathered from multiple sources, were summarized in the 2011 textbook “Cryptococcus – from Human Pathogen to Model Yeast”. The authors note that there is involvement of the CNS in 65-84% of cases at presentation, but lung involvement is only seen in 4-18% of cases. The commonest symptom is headache, usually sub-acute, and this is observed in 67-100% of cases. Fever is seen in 56-95% of cases, an altered level of consciousness in 10-23%, and seizures in 4-9%. Disease is difficult to distinguish clinically from TB meningitis, which would be the primary differential diagnosis for HIV positive patients in tropical settings such as Vietnam (170,171).

Luckily, cryptococcal meningitis is relatively easy to confirm on examination of the CSF. The CSF opening pressure is typically raised to over 25cm/CSF (60% of patients) (40). Typical CSF lab features include a mildly elevated white cell count (4-11 cells/mm³) and normal or elevated protein (40). The diagnosis can be confirmed by a positive India Ink stain for cryptococci (positive in 66-88% of cases) or a positive lateral flow cryptococcal antigen (CrAg) test (positive in 91-100% of cases). Conveniently, the CrAg remains sensitive across all serotypes, including C. gattii (40).
4.5.2 Immune responses

The clinical features of cryptococcal meningitis, like other infectious diseases, arise from a combination of both host and pathogen factors. One of the most important host determinants is the immune response. In Chapter 8 of this thesis, I investigate the immune responses in cryptococcal meningitis by measuring the cytokine concentrations in the CSF over time.

4.5.2.1 Cytokines and the immune response

Cytokines are intra-cellular messenger proteins that influence target cells to proliferate, differentiate, relocate, or alter their gene expression. This is depicted by the schematic in Figure 4-3.

Figure 4-3 Schematic of cytokine communication between source and target cells. Stimuli, including cytokines, activate receptors (yellow) on source cell membranes that alter the expression of cytokine genes. Corresponding changes to the cytokine output (small blue arrows) of the source cell interact with receptors on target cell membranes, causing a change in cell behavior with or without a change in cytokine output, which may in turn influence the source cell (dotted line).

Cytokines can act via autocrine, paracrine, or endocrine routes, but because cytokines act via alterations of gene expression, their effects are not immediate (172). Cytokines are primarily involved in communication between immune cells, although any cell can produce, or
be affected by, cytokines. The nomenclature surrounding cytokines is varied, and they have historically been categorized according to source, function and structure (173). A recent system for categorizing them (174) suggests they be divided into:

- **haematopoietic growth factors** which stimulate the proliferation and differentiation of blood cells

- **interferons** which were originally identified because of their anti-viral activity, but have been found to be involved in host defence against other infections

- **lymphokines** which are involved primarily in communication between lymphocytes, and are responsible for inducing differentiation of lymphocytes

- **monokines** which are predominantly produced by mononuclear phagocytes, but have varied functions

- **chemokines** whose major role is attracting leukocytes, but are also involved in leukocyte differentiation

- and **other cytokines** that don’t fall into any of the above categories.

Cytokines were initially recognized as ‘soluble factors’ in pus, although their role was initially unclear. Since the first soluble factor (IL-2) was characterized in the 1970s (173), there has been extensive research on individual cytokines, both in terms of their role in pathophysiology and their therapeutic potential. However, on a systems level, the interactions between cytokines are complicated (172,174) and have been difficult to explain in many infectious diseases.

Cytokines act via their cellular targets by promoting the differentiation and proliferation of cell types with particular behaviours. Amongst the cell types most important to the cytokine
mediated immune response are CD4+ T-lymphocytes and macrophages. CD4+ T-lymphocytes differentiate into pro-inflammatory T-helper type 1 (Th1) and Th17 cells, anti-inflammatory Th2 cells, and immunoregulatory T-regulatory (TReg) cells. Each of the Th1, Th2, and Th17 cell types release a milieu of cytokines, often forming positive feedback loops that polarize the immune response. The TReg cell has a vital role in terminating these positive feedback loops and regulating the immune response (175). Macrophages are also affected by the cytokine profile, differentiating into proinflammatory M1 macrophages, or anti-inflammatory M2 macrophages. M1 macrophages in particular also exhibit positive feedback loops, attracting circulating macrophages and promoting their differentiation to the M1 phenotype (176). These interactions are illustrated in outline in Figure 4-4.
Figure 4-4 Illustration of the cytokine receptor (in yellow) dependent differentiation of naïve CD4+ T-lymphocytes into T-helper lymphocytes type 1 (Th1), Th2, and Th17, influenced by and adding to the cytokine milieu. Pro-inflammatory cell types are shown in red and anti-inflammatory cell types in orange. TReg cells restore balance in the immune response by terminating positive feedback cytokine loops. Also showing cytokine receptor dependent recruitment and differentiation of circulating macrophages into M1 (pro-inflammatory, red) or M2 (anti-inflammatory, orange) cell types.

Measuring cytokine concentrations in plasma or at the site of infection is a common approach for inferring the nature of the underlying cellular immune responses.

4.5.2.2 Immune response in HIV-associated cryptococcal meningitis

The nature of the immune response may be an important prognostic indicator in CM. In general, a Th1 response is thought to be protective, while a Th2 response is not. In the mouse model, Th1 associated pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), and IL-12 are associated with improved outcomes and yeast clearance (44,177,178), whereas Th2 associated cytokines IL-4 and IL-10 are associated with higher fungal burdens (179). Corticosteroids such as dexamethasone skew the immune
response from Th1 to Th2 in healthy human adults (180). However, despite our understanding that Th1 responses are helpful and that dexamethasone attenuates this response, mice with cryptococcal disease treated with dexamethasone in the absence of antifungals have improved survival (181).

In humans, the role of the Th1/Th2 paradigm is less clear. It has previously been shown that higher endogenous IFN-γ concentrations improve early fungicidal activity (a microbiological surrogate marker of treatment success) (182). Furthermore, administration of recombinant IFN-γ has a beneficial effect on early fungicidal activity (183). Jarvis et al recently characterized the baseline immune profiles in the CSF of adults with HIV–associated cryptococcal meningitis. The authors did principal component analyses (rather than simply using individual cytokine concentrations) and were able to identify a pro-inflammatory cluster associated with reduced two week mortality, and improved rates of fungal clearance. The pro-inflammatory principal component was driven by IL-6, IFN-γ, IL-8, IL-10, RANTES, and (to a lesser degree) TNF-α. Jarvis et al found no corollary association between Th2 type responses and poor outcomes (184).

Weisner et al reported that a specific genotype of C. neoformans var. grubii was associated with increased IL-4 and IL-10 expression on in vitro stimulation of whole blood from healthy volunteers, and that this genotype was also associated with worse clinical outcomes (185). In their experiments, IFN-γ levels were also higher in infections due to this genotype, although the difference failed to reach statistical significance, possibly due to the small study size. The currently available mixed results allow only a partial understanding of the immune response in patients with cryptococcal meningitis.

4.5.3 Management

Medical research into the appropriate management of cryptococcal disease has increased rapidly since the onset of the HIV epidemic (5). Unlike many CNS infections, diagnosis of
cryptococcal meningitis is generally straightforward, with high burdens of an easily identified pathogen. Infection can be shown using a number of staining and culture methods, or with highly sensitive antigen detection kits (44). However, clinical outcomes remain unacceptably poor, with a 90-day case fatality rate of 30-70% (3,5,47,161). Even when the best available treatments are provided in well-resourced settings, mortality remains at 10-15% (47,161). Achieving good outcomes in the management of HIV-associated cryptococcal meningitis relies on effective anti-fungal therapy, judiciously timed anti-retroviral therapy (ART), and careful management of complications including raised intracranial pressure, immune reconstitution inflammatory syndrome, and cryptococcomas (186,187).

4.5.3.1 Antifungal therapy

Optimal antifungal therapy is delivered in three phases: induction, consolidation, and maintenance. The best drugs, combinations, and durations have received significant research attention since amphotericin B became available in the late 1950s, as the first treatment available for cryptococcosis (44). The arrival of azoles in the 1980s, with their better oral bioavailability and tolerability, revolutionized cryptococcal treatment. They enabled shorter durations of amphotericin treatment and the long-term suppressive therapy required for immune-suppressed patients. By 2015 there had been 22 randomised controlled trials in HIV-associated cryptococcal disease, although in general these had been small (the median number of patients randomized is 87), and were mostly not powered to specifically investigate survival (see Table 4-1). Rather, trials tended to use composite endpoints consisting of survival, clinical improvement, and rate of sterilization of cerebrospinal fluid. One such surrogate marker is Early Fungicidal Activity (EFA), which was developed as an early indicator of treatment efficacy (188). Following its conception in Thailand and its subsequent use in numerous intervention studies, many hope that it will eventually serve as a robust surrogate marker of the efficacy of antifungal treatments (188–193).
<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated number of cases of CM per year in HIV patients</td>
<td>223,100</td>
</tr>
<tr>
<td>Estimated number of CM deaths per year</td>
<td>180,000</td>
</tr>
<tr>
<td>Number of patients with CM randomized in treatment trials</td>
<td>5,328</td>
</tr>
<tr>
<td>Number of RCTs in HIV-associated CM</td>
<td>22</td>
</tr>
<tr>
<td>Median number of patients per RCT</td>
<td>87</td>
</tr>
<tr>
<td>Number of RCTs powered to mortality (all post 2009)</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4-1. HIV-associated cryptococcal meningitis (CM) RCT statistics 1979 – 2015, extracted from International Clinical Trial Registration Platform (ICTRP) on 1st February 2015.

A major focus for the early research in HIV-associated cryptococcal meningitis was testing increased doses of amphotericin, shortening treatment regimens, and combination treatment with flucytosine (195–197). The majority of this early research was case-studies or treatment cohorts. However, from the late 80s increasing numbers of RCTs were performed, and these are summarized in . The landmark paper was the 1997 AIDS Clinical Trials Group and Mycoses Study Group study of 379 participants, which compared 2 weeks 0.7mg/kg amphotericin combined with either flucytosine 100mg/kg or placebo (197). The current Infectious Diseases Society of American (IDSA) treatment guidelines for CM state that this trial “defined [the] preferred regimen of Amphotericin B and flucytosine”. However, the trial was not powered to demonstrate a survival effect, and was not conducted in a high-burden setting. Furthermore, the primary end-point of CSF sterilization by 2 weeks was not statistically significant in the primary analysis (60% in combination therapy; 51% in amphotericin mono-therapy, p=0.06). A secondary multiple regression analysis showed that combination therapy was predictive of sterilization at 2 weeks, but not of clinical improvement. The overall death rate in this study was too low in the second phase, at 9%, to allow comparison of survival outcomes. Despite these drawbacks, the conclusions of the investigators were later confirmed by other trials.
showing that combination therapy was associated with significantly faster rates of fungal clearance (191).
<table>
<thead>
<tr>
<th>Trial ID number</th>
<th>Title of trial</th>
<th>Start date</th>
<th>End date</th>
<th>Number enrolled</th>
<th>Comparison</th>
<th>Primary End Point</th>
<th>Result of intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT 00000708</td>
<td>Multi-center Comparison of Fluconazole (UK-49858) and Amphotericin B as Treatment for Acute Cryptococcal Meningitis</td>
<td>01-04-88</td>
<td>31-11-89</td>
<td>194</td>
<td>Arm 1 oral fluconazole 200mg Arm 2 0.4mg/kg AmB</td>
<td>CSF clearance at 2 weeks</td>
<td>Fluconazole not superior (though time to clearance was longer in fluconazole group)</td>
</tr>
</tbody>
</table>
| NCT 00000639    | A Randomized Double Blind Protocol Comparing Amphotericin B With Flucytosine to Amphotericin B Alone Followed by a Comparison of Fluconazole and Itraconazole in the Treatment of Acute Cryptococcal Meningitis | 01-10-91   | 31-08-94 | 381             | **Stage 1 induction**  
Arm 1 0.7mg/kg AmB + flucytosine  
Arm 2 AmB  
**Stage 2 consolidation**  
Arm 1 fluconazole; Arm 2 itraconazole | **Stage 1** CSF clearance at 2 weeks and symptom resolution.  
**Stage 2** CSF clearance at 10 weeks and symptom resolution | Combination not superior in Stage 1; fluconazole superior in stage 2 (secondary analysis, flucytosine was associated with faster fungal clearance in stage 1) |
| NCT 00000776    | Dexamethasone in Cryptococcal Meningitis                                                                 | 30-09-96   | not stated | 36              | Arm 1 dexamethasone Arm 2 placebo                                           | Rate of reduction of intracranial pressure     | Results not available                              |
| NCT 0012467     | Safety and Antifungal Activity of Recombinant Interferon-Gamma 1b (rIFN-Gamma 1b) Given With Standard Therapy in Patients With Cryptococcal Meningitis | 01-01-00   | 31-07-01 | 79              | Arm 1 high dose Interferon 1b  
Arm 2 low dose interferon 1b  
Arm 3 placebo       | CSF clearance at 2 weeks, and safety                                                                  | Non-significant trend towards increased rate of clearance, no significant safety concerns |
| ISRCTN 95123928 | Combination anti-fungal therapy in cryptococcal meningitis                                               | 22-04-04   | 01-12-09 | 299             | Arm 1 AmB 4weeks  
Arm 2 AmB + fluconazole 2 weeks  
Arm 3 AmB + flucytosine 2 weeks | Mortality at 10 weeks                                                                               | AmB + flucytosine superior to AmB monotherapy    |
<p>| NCT 00145249    | Amphotericin Alone or in Combination With Fluconazole for AIDS-Associated Meningitis                     | 01-05-05   | 30-04-08 | 143             | Arm 1 AmB Arm 2 AmB + 800mg fluconazole Arm 3 AmB + 400mg fluconazole      | CSF clearance at 2 weeks, symptom resolution, and safety | Non-significant trend towards superior fungal clearance in high dose fluconazole arm. No safety concerns |</p>
<table>
<thead>
<tr>
<th>Study ID</th>
<th>Description</th>
<th>Intervention</th>
<th>Study Duration</th>
<th>Outcome Measures</th>
</tr>
</thead>
</table>
| ISRCTN 68133435 | High dose amphotericin B with flucytosine and amphotericin B plus high dose fluconazole for treatment of cryptococcal meningitis in human immunodeficiency (HIV)-infected patients | Arm 1: 0.7mg/kg AmB + standard oral therapy  
Arm 2: 1mg/kg AmB + standard oral therapy | 01-06-05 to 01-06-06 | Rate of CSF clearance until 2 weeks  
1mg/kg AmB superior to 0.7mg/kg |
| ISRCTN 52812742 | A multicentric open comparative randomized study to optimize dose duration safety efficacy and cost of two doses of liposomal amphotericin in the treatment of systemic infection in India | Arm 1: 1mg/kg liposomal AmB  
Arm 2: 3mg/kg liposomal AmB | 07-03-06 to 06-03-07 | CSF clearance at 2 weeks  
No significant difference |
| NCT 00847678 | Efficacy and Safety of Mycograb as Adjunctive Therapy for Cryptococcal Meningitis in Patients With AIDS | Arm 1: Mycograb + standard anti-fungal  
Arm 2: placebo + standard anti-fungal | 01-08-06 to 31-10-09 | Rate of CSF clearance until 2 weeks, safety  
Terminated for undisclosed reasons |
| NCT 00830856 | Early Versus Delayed Antiretroviral Therapy (ART) in the Treatment of Cryptococcal Meningitis in Africa | Arm 1: early (within 72hrs) ART  
Arm 2: delayed (>10 weeks) ART | 01-10-06 to 30-09-07 | Mortality up to 3 years  
Delayed ART superior to early ART |
| NCT 00324025 | Efficacy and Safety of Mycograb as Adjunctive Therapy for Cryptococcal Meningitis in Patients With AIDS | Arm 1: Mycograb + standard anti-fungal  
Arm 2: placebo + standard anti-fungal | 01-03-07 to 30-09-07 | Rate of CSF clearance until 2 weeks, safety  
Terminated for undisclosed reasons |
| ISRCTN 72024361 | Short course interferon-gamma for human immunodeficiency virus (HIV)-associated cryptococcal meningitis | Arm 1: 1-3 dose IFN-g + standard therapy  
Arm 2: 1-6 dose IFN-g + standard therapy  
Arm 3: standard therapy | 10-07-07 to 31-05-10 | Rate of CSF clearance until 2 weeks, safety  
Interferon arms were superior to standard therapy, but not dose-related. No safety concerns |
| NCT 01075152 | Cryptococcal Optimal ART Timing Trial | Arm 1: early (1-2 weeks) ART  
Arm 2: delayed (5-6 weeks) ART | 01-11-07 to 27-04-12 | Mortality at 26 weeks  
Delayed ART superior to early ART |
| ISRCTN 02725351 | High dose fluconazole with or without flucytosine in the treatment of human immunodeficiency virus (HIV)-associated cryptococcal meningitis | 18-02-08 | 02-12-08 | 41 | Arm 1 1200mg fluconazole  
Arm 2 1200mg fluconazole + flucytosine 200mg/kg | Rate of CSF clearance until 2 weeks | Combination superior to monotherapy |
|------------------|-------------------------------------------------------------------------------------------------|---------|---------|-----|-------------------------------------------------|-----------------------------------|-----------------------------|
| NCT 00976040     | Optimal Time to Start Antiretroviral Therapy in HIV-infected Adults With Cryptococcal Meningitis | 01-09-09 | 26-04-11 | 28 | Arm 1 early (within 7 days) ART  
Arm 2 delayed (>28 days) ART | Rate of CSF clearance until 4 weeks | No difference in rate of clearance |
| NCT 00885703     | High-Dose Fluconazole for the Treatment of Cryptococcal Meningitis in HIV-Infected Individuals | 01-02-10 | not stated | 168 | Arm 1 Fluconazole 1200mg or 1600mg or 2000mg  
Arm 2 Fluconazole 1200mg or 1600mg or 2000mg + AmB | Rate of CSF clearance until 2 weeks, survival | Results not available |
| NCT 02136030     | Liposomal Amphotericin B for the Treatment of Cryptococcal Meningitis | 01-02-11 | not stated | 84 | Arm 1 liposomal AmB 4mg/kg  
Arm 2 standard AmB | Rate of CSF clearance until 2 weeks, clinical response | Results not available |
| ISRCTN 20410413  | Home care and routine: cryptococcal meningitis screening among patients starting antiretroviral therapy with advanced disease | 01-02-12 | 30-09-13 | *1999 | Arm 1 CrAg screening and pre-emptive treatment + community support + standard care  
Arm 2 standard care | Mortality at 12 months | Screening and community support superior to standard care alone |
| ISRCTN 45035509  | A phase III randomised controlled trial for the treatment of HIV-associated cryptococcal meningitis: oral fluconazole plus flucytosine or one week amphotericin B-based therapy vs two weeks amphotericin B-based therapy | 28-01-13 | 31-12-16 | 721 | Arm 1 (Oral) fluconazole (1200mg) + flucytosine for 2 weeks  
Arm 2 (1-week) AmB (1mg/kg) + fluconazole (1200mg) or flucytosine, til day 7; then fluconazole (1200mg) til day 14; Arm 3 (2-weeks) AmB (1mg/kg) + fluconazole (1200mg) or flucytosine til day 14 | Mortality at 10 weeks | Oral and one-week were non-inferior to 2 weeks (the current standard); flucytosine was superior to fluconazole; lowest mortality was in 1 week AmB and flucytosine. |
<table>
<thead>
<tr>
<th>ISRCTN</th>
<th>Study Title</th>
<th>Start Date</th>
<th>End Date</th>
<th>N</th>
<th>Arm 1</th>
<th>Arm 2</th>
<th>Arm 3</th>
<th>Arm 4</th>
<th>Outcome Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>59144167</td>
<td>Adjunctive dexamethasone in HIV-infected adults with cryptococcal meningitis</td>
<td>19-02-13</td>
<td>29-08-14</td>
<td>451</td>
<td>Arm 1 standard therapy + placebo</td>
<td>Arm 2 standard therapy + dexamethasone for 6 weeks</td>
<td>Mortality at 10 weeks</td>
<td>Dexamethasone did not reduce mortality; secondary analyses showed increased disability and adverse events with dexamethasone.</td>
<td></td>
</tr>
<tr>
<td>01802385</td>
<td>Adjunctive Sertraline for the Treatment of HIV-Associated Cryptococcal Meningitis</td>
<td>14-08-13</td>
<td>30-08-14</td>
<td>172</td>
<td>Arm 1 100mg serotonin + standard therapy</td>
<td>Arm 2 200mg serotonin + standard therapy</td>
<td>Arm 3 300mg serotonin + standard therapy</td>
<td>Arm 4 400mg serotonin + standard therapy</td>
<td>Rate of CSF clearance until 2 weeks</td>
</tr>
<tr>
<td>10248064</td>
<td>AMBITION-cm: AMBIsome Therapy Induction OptimizatioN - Intermittent high dose AmBisome on a high dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa</td>
<td>01-06-14</td>
<td>ongoing</td>
<td>80</td>
<td>Arm 1 Liposomal-AmB 10 mg/kg single dose</td>
<td>Arm 2 L-AmB 10 mg/kg two doses</td>
<td>Arm 3 L-AmB 10 mg/kg three doses</td>
<td>Arm 4 (control) L-AmB 3mg/kg for 14 days</td>
<td>Rate of CSF clearance until 2 weeks</td>
</tr>
</tbody>
</table>

Table 4-2 Summary of randomised controlled trials in HIV associated cryptococcal meningitis, 1979 to 2015. Extracted from International Clinical Trial Registration Platform (ICTRP) on 1st February 2015.
In 2013 researchers from Vietnam finally showed that combination anti-fungal therapy improved survival. In this trial of 299 patients the most effective treatment option for induction therapy was two weeks of amphotericin B (1mg/kg/day) in combination with flucytosine (100mg/kg/day). When compared to four weeks of amphotericin B monotherapy, this combination increased the probability of survival to 10 weeks from 0.56 to 0.69 (a hazard ratio for mortality of 0.61, p=0.04) (3). In this same trial, two weeks of fluconazole combined with amphotericin B was not superior to four weeks of amphotericin B monotherapy – the 10 week probability of survival increased from 0.56 to 0.67 (hazard ratio for mortality 0.71, p=0.13). However, amphotericin and fluconazole for two weeks was at least as effective as four weeks of amphotericin B monotherapy, meaning the duration of IV therapy can be halved. For pragmatic reasons, in developing settings where flucytosine is generally unavailable (198,199) the WHO and the IDSA recommend two weeks of induction therapy with amphotericin B with fluconazole. An effective induction therapy not including amphotericin B could help to reduce drug toxicities, costs, and length of hospital stay. However, no such therapy has yet been proven, although results of an ongoing study are eagerly anticipated (200).

Following induction therapy, guidelines recommend consolidation therapy with fluconazole 800mg/day for a further 8 weeks (186). Itraconazole penetrates the CSF poorly, and van der Horst et al’s trial, described above (197), gave some indication that fluconazole was superior in consolidation therapy. Although not statistically significant, the proportion of patients with negative CSF by 10 weeks was higher in patients given fluconazole than in those given itraconazole (72% vs 60%). Fluconazole was predictive of negative CSF at 10 weeks in the trial’s multiple logistic regression model (odds ratio 1.78, p=0.02).

Because there is a high risk of relapse after completion of induction and consolidation therapy (201), patients are finally switched to maintenance (or secondary prophylaxis) (186).
This continues from week 10, until such a time as treatment of the underlying HIV has allowed immune function to be regained. Current guidelines recommend treatment with 200mg/day of fluconazole (186,187), which is superior to the alternatives of daily itraconazole (202) or weekly amphotericin B (203), for at least 12 months, with the HIV viral load suppressed for 3 months and CD4 count over 100cells/µm³.

4.5.3.2 Adjuvant therapies

Even with this evidence-based anti-fungal therapy, outcomes remain poor. Given the lack of development of new anti-fungal agents to treat cryptococcal disease, several groups have undertaken research into adjuvant therapies, including corticosteroids, IFN-γ (183,204), and acetazolamide (205). The rationale for and outcomes of the randomized controlled trial of corticosteroids are fully described in Chapter 1. IFN-γ was shown to have a statistically significant impact on EFA, but not on mortality (183,204). The acetazolamide trial was discontinued early due to an excess of adverse advents, likely due to additive toxicity with amphotericin (205). Although the data from this trial were analyzed before the pre-planned interim analysis, there was clear evidence that acetazolamide was causing harm and no evidence that it was reducing intracranial pressure. Currently none of these adjuvants can be recommended in routine clinical care.

The timing of initiation of anti-retrovirals (ARVs) in HIV co-infection has been a key area of interest over recent years, with a need to balance the benefits of ARVs (especially with regards to opportunistic infections) with the possible harmful consequences of immune reconstitution syndromes. Early initiation appears to be beneficial in many opportunistic infections, including pulmonary tuberculosis (206–208), but not TB meningitis (209). In cryptococcal meningitis, the COAT trial (2014) compared early initiation of ARVs to deferring treatment for 5-6 weeks, and found that early initiation was associated with an increased risk of death, and appeared to carry no benefit in terms of reduced incidence of opportunistic
infections (210). This trial was stopped early because of harm in the early ARV group, and is discussed more fully in chapter 7. Two previous smaller studies also suggested probable increased risks of harm with early initiation of ARVs (211,212). Both studies struggled with slow recruitment. Makadzange et al’s study of 54 participants was stopped after the second independent interim analysis as the group receiving early ARVs had a hazard ratio for mortality of 2.85 (95% confidence interval 1.1–7.23) (211). Bisson et al’s study was also stopped early, having observed significantly more instances of IRIS in the early ARV group, and no benefit in terms of fungal clearance (212). It is likely that the increased mortality associated with early ARV therapy is related to IRIS. In keeping with this theory, Boulware et al. noted that the increased mortality was especially pronounced in patients with a baseline CSF white cell count <5 cells/mm³ (210), an established risk factor for IRIS (213,214).

Perhaps the most supported adjuvant in the treatment of cryptococcal meningitis is the careful management of raised intracranial pressure. International guidelines recommend that pressure should be maintained below 25cm of CSF, with daily lumbar punctures if necessary (186). Pressure is elevated at presentation in over half of patients (3,210,215), and failure to comply with management guidelines has been associated with adverse outcomes (216). Persistent anxiety amongst clinicians and patients that frequent lumbar punctures lead to harm appear to be unfounded (217,218). However, there have not been any prospective trials comparing different pressure management methods.

4.5.3.3 Early detection and treatment

Current antifungal and adjuvant options have yet to achieve the large reductions in mortality required in cryptococcal meningitis, but perhaps an upstream approach could improve upon this. Early diagnosis is invariably beneficial in the management of infectious diseases. There is significant interest in the use of lateral flow antigen (LFA) detection tests for cryptococcal antigen (CrAg) to diagnose a pre-symptomatic stage of cryptococcal meningitis in
which treatment efficacy may be improved. Undoubtedly, the LFA test offers an opportunity to improve standard diagnostics in resource limited settings (219), as it is cheap, requires little equipment, no electrical power, and minimal training. CrAg positivity has been shown to predate symptoms of cryptococcal meningitis by several weeks (220) and is associated with increased mortality (221,222). Modelling suggests that systematic screening and treatment could be a cost-effective intervention in selected patient populations (13,223,224), and screening of asymptomatic patients has been introduced in South Africa on a country-wide scale (detailed in the National Strategic Plan 2012-2016, published by South African National AIDS Council and accessible at [http://sanac.org.za/wp-content/uploads/2015/11/National-Strategic-Plan-on-HIV-STIs-and-TB.pdf](http://sanac.org.za/wp-content/uploads/2015/11/National-Strategic-Plan-on-HIV-STIs-and-TB.pdf)). However, the best treatment for asymptomatic antigenaemia is unknown. A prospective observational cohort suggested a benefit of pre-emptive administration of fluconazole in such patients, but dosing schedules were uncontrolled and varied considerably, preventing the formulation of a treatment recommendation (223). Another area of concern was illustrated in studies from Cambodia and South Africa showing that many outpatients with antigenaemia but without headache have evidence of CNS disease on CSF examination (225,226). An acceptable CrAg screening strategy would need to ensure patients with CM continued to receive evidence-based therapy.

A common format for a screen-and-treat approach is depicted in Figure 4-5 (227). Importantly, it acknowledges that optimal treatment for asymptomatic antigenaemia has not been established and that lumbar puncture should be performed whenever feasible to exclude CM (228). Such a programme was predicted to be cost-effective in a Ugandan study (223), and two studies in Southeast Asia suggested implementation would cost less than 300 USD per life year gained (depending on the actual prevalence of CrAg positivity and fluconazole drug costs). Even at the top end of the estimated range, the intervention would be classified as ‘very cost-effective’ under WHO guidelines (13,224).
However, implementation of the screen-and-treat approach is not universally supported by research evidence. In Kenya, 782 ambulatory patients were tested between 2009-2010, and were offered high-dose fluconazole followed by a maintenance dose if they tested positive (229). The study failed to demonstrate any overall mortality benefit to the intervention and was compromised by poor uptake of the intervention and the fact that historical controls were used. Still, in this ‘real world’ setting, there was little evidence to suggest any benefit. A different approach in Tanzania, where universal screening of hospitalized HIV patients was undertaken between 2009-2010, failed to improve diagnostic accuracy beyond the standard of care, and did not demonstrate a mortality benefit (230). In both studies, all patients received fluconazole monotherapy even if meningitis was diagnosed. Mortality was high in CM patients, and inadequate drug therapy may have masked any mortality gains from early diagnosis, although this does not explain why the proposed benefit from preventing cases of CM was not realised.
Data emerging from screen-and-treat programmes in Tanzania/Zambia (231), Uganda (232), and South Africa (225) are confirming that cryptococcal antigenaemia at baseline is associated with increased mortality. In these studies, the proportion of antigen positive participants who had died by 12 months ranged from 25-32% compared to 7-12% for antigen negative participants. However, the increased mortality wasn’t entirely mitigated by pre-emptive fluconazole therapy. This may suggest that antigenaemia is just an independent marker of disease severity and that fluconazole therapy is inadequate to reduce mortality. Furthermore, these three studies have confirmed the high prevalence of infection of the central nervous system in asymptomatic antigenaemic patients. More work around the appropriate investigation and management of patients with positive cryptococcal antigen screening tests is urgently required.

The experience of clinical research in cryptococcal meningitis can help to guide the scientific response to future emerging infections. Firstly, the diagnostic tests and treatments must be available where the burden of disease is highest, and ideally research should be undertaken in these settings (12). Secondly, trials should be powered for relevant clinical end-points until surrogate end-points have been conclusively validated. Finally, it shows the importance of having proposed preventative medicine interventions, such as early initiation of ARVs and pre-symptomatic screening, trialed in a robust, real-world setting before they are rolled out, as results can be unexpected.

4.6 Thesis Aims

Invasive fungal infections are a neglected area of clinical research, especially in tropical settings such as Vietnam. Cryptococcosis in particular remains a challenging human health issue that is unlikely to spontaneously resolve whilst populations of iatrogenically immunosuppressed hosts increase and the pathogen itself continues to adapt to new environments and hosts. These unmet needs were the motivation for my thesis.
With this thesis, I estimate the burden of fungal infections in Vietnam, assess the role of dexamethasone in the management of cryptococcal meningitis, investigate the process of terminating clinical trials early, and consider how host responses interact with cryptococcal meningitis. The primary aims for each part of my thesis are listed below.

4.6.1 

**Estimating the burden of fungal disease in Vietnam**

Describe the incidence and prevalence, in Vietnam, of the most serious invasive fungal infections.

4.6.2 

**Adjunctive corticosteroids in HIV-associated cryptococcal meningitis: a randomized controlled trial in African and Southeast Asian countries**

Determine the effect of dexamethasone adjunctive therapy on survival until 10 weeks in adult patients with HIV-associated cryptococcal meningitis.

4.6.3 

**Stopping trials early**

Describe the relationships between early termination of trials and publication of results, and perform a case study on the early termination of the CryptoDex trial.

4.6.4 

**Immune responses in cryptococcal meningitis**

Assess the impact of dexamethasone on the immune response in CSF, and determine whether dynamic immune responses are associated with patient outcomes.
5. Estimating the Burden of Fungal Disease in Vietnam
5.1 Summary

Introduction Data regarding the prevalence of fungal infections in Vietnam are limited yet such infections are likely to occur more frequently as increasingly complex healthcare creates more iatrogenic risk factors. We sought to estimate baseline prevalence and incidence of selected serious fungal infections for the year 2012.

Materials and Methods We made estimates with a previously described actuarial method, using reports on the prevalence and incidence of various established risk factors for fungal infections from Vietnam, or similar environments, supplemented by personal communications. Global data were used if local data were unavailable.

Results We estimated that 2,352,748 episodes of serious fungal infection occurred in Vietnam in 2012. Frequent conditions included recurrent vaginal candidiasis (3,893 / 100,000 women annually), tinea capitis (457 / 100,000 annually), and chronic pulmonary aspergillosis (61 / 100,000 / five year period). We estimated a total 140 cases of cryptococcal meningitis, 206 of penicilliosis and 608 of \textit{Pneumocystis jiroveci} pneumonia occurred in 2012 in Vietnam.

Conclusions We present here the first summary of fungal infections in Vietnam. The majority of severe disease is due to \textit{Aspergillus} species, which may be driven by the high prevalence of pulmonary tuberculosis. The AIDS epidemic promotes opportunistic infections, such as penicilliosis and cryptococcosis, which may complicate immunosuppressive treatments. Our estimates provide a useful indication of disease prevalence to inform future research and resource allocation but should be validated by further epidemiological approaches.
5.2 Background

As highlighted in section 4.2, the global prevalence of fungal infections is high and invasive mycoses are associated with severe mortality and morbidity. It is likely that the burden of serious fungal infections fall heavily on resource-limited tropical settings, where HIV and tuberculosis are also prevalent. Furthermore, in rapidly developing countries such as Vietnam (14), the incidence of serious fungal infections is likely to increase further in tandem with rising access to complex medical interventions like prolonged intensive care and a range of iatrogenic immunosuppression (15). An assessment of the baseline incidence of serious fungal infections is vital to facilitate the work of health care planners and public health professionals.

There is no formal surveillance for these serious conditions in Vietnam, and there have been no estimates of their incidence and prevalence from any of Vietnam’s South East Asian neighbours. As part of a global drive to describe the burden of serious fungal infections, a growing number of researchers have been applying an actuarial method for estimating the burden of fungal infections at the national level (22–39). Here the term actuarial means population demographic and disease risk factor data are used to arrive at a mathematical estimate for the burden of that condition (analogous to the work of an actuary in insurance, who predicts how many and what type of liabilities the company is likely to face, based on what is known about the insured population). Given the importance of standardized data collection and data sharing, as highlighted in reviews from major medical journals in recent years (233,234), I used similar methods to those of researchers from Europe, the Middle East, South America and the Caribbean (22–39). Since the goal of this global effort is to generate comparable and consistent international data, the model focuses only the prevalence and incidence of disease. More detailed analyses, including mortality and morbidity outcome data, would be required to fully understand ‘burden’, but such analyses are beyond the scope of the current work.
The conditions of interest for this study are broadly grouped as AIDS defining mycoses, infections related to Aspergillus, infections related to Candida, and other mycoses. The AIDS defining mycoses I included are PCP, cryptococcal meningitis, and penicilliosis. I estimate the burden of disease related to Aspergillus in four categories: invasive aspergillosis (IA); allergic bronchopulmonary aspergillosis (ABPA); severe asthma with fungal sensitization (SAFS); and chronic pulmonary aspergillosis (CPA). I categorize candidal infections as candidaemia, Candida peritonitis, oesophageal candidiasis, oral candidiasis, and recurrent vulvovaginal candidiasis (VVC). I also estimate the burden of fungal keratitis and tinea capitis.

In this chapter I describe how the actuarial methods used to estimate fungal disease burden elsewhere were adapted for Vietnam, and how appropriate sources of data were identified. I present the resulting estimates of the burden of selected serious mycoses in Vietnam; the first attempt of its kind in South East Asia. Vietnam is classified as a lower-middle income country by the World Bank and is undergoing pivotal changes in terms of economic development (14). Without an estimate of baseline burden of fungal disease it will be difficult to make robust plans for their management within Vietnam’s developing healthcare system. The actuarial estimates I present here are a first step towards informing healthcare policy with respect to fungal infections. I also present a proposal for the ‘next step’ as a study concept for a formal sentinel surveillance programme. Implementing a sentinel surveillance programme was beyond the scope of this body of work, but such disease surveillance results could generate the missing morbidity and mortality outcome data, and help to validate the accuracy of the prevalence and incidence estimates I made using this low-cost and convenient approach.
5.3 Methods

5.3.1 Actuarial method

The actuarial method is illustrated in Figure 5-1 using the process for estimating the burden of chronic pulmonary aspergillosis (CPA). This method was originally described by Denning et al in 2011 (9) and has been adapted for Vietnam (39). The model first calculates the annual incidence of pulmonary tuberculosis (PTB) associated CPA by multiplying the incidence of PTB with and without cavities by the incidence of CPA post-TB in those with and without cavities. The incidence of non-PTB associated CPA is then derived from this number, based on the expected proportion of patients with an established diagnosis of CPA whose disease is related to TB. These two numbers are added together for an annual incidence, which is converted to a five year period prevalence to allow comparability with other published data.

In the CPA worked example, I split the annual cases of PTB survivors into two groups: cavitatory and non-cavitatory. Vietnamese data show that 40% (235) of PTB patients have

![Figure 5-1 actuarial approach for estimating period prevalence of chronic pulmonary aspergillosis (CPA) using pulmonary tuberculosis (PTB) data (9)](image-url)
cavitatory disease, so the number of PTB survivors is multiplied by 0.4 and 0.6 to arrive at a number of survivors in the cavitary and non-cavitatory groups, respectively. Data indicate that the incidence of CPA in PTB survivors with cavities is 22%, and the incidence of CPA in PTB survivors without cavities is 2% (9), so I multiply the numbers in the cavitary group by 0.22 and the non-cavitary group by 0.02. The annual incidence of non-PTB associated CPA is estimated from the proportion of patients diagnosed with CPA thought to be associated with PTB. In Asia, approximately 75% (236–238) of CPA cases follow PTB – therefore multiplying the incidence PTB associated CPA by 0.33 gives the incidence of non-PTB associated CPA. The incidences of PTB and non-PTB associated CPA are then combined, and converted to a period prevalence. For this calculation, I assumed that the combined CPA incidence was stable over the five year period and that annual mortality for CPA patients was 15% (9). I took the combined annual incidence of CPA and subtracted 15% before rolling it over into the next year and adding it to the new cases. I repeated this process five times to arrive at a 5-year period prevalence, resulting in a multiplier of 3.152 to convert annual incidence to five year period prevalence. The detailed methods for every other condition are described under individual subject headings below.

5.3.2 Population structure

I used the 2012 WHO population estimates for Vietnam (239), and described the population structure using the 2009 Vietnam census data (240). The 2009 census was a 15% sample survey of the population (240), and is the third census since reunification to use internationally recognized methodologies to produce a robust description of the population (240). The year 2012 was selected because it was the most recent year for which the majority of the required data were available.
5.3.3 Search strategy for local fungal disease epidemiology and risk factor data

An ad hoc review of the epidemiology of fungal infections in Vietnam showed that the available data were so few that a formal systematic review was not justified, so my review of the literature was purposive. I searched for Vietnam-specific data on the prevalence of fungal disease risk factors such as HIV/AIDS, chronic lung disease, haematological disease, organ transplantation, and intensive care. If published data were unavailable, I contacted local colleagues who had previously published in relevant fields for supplementary information. Wherever Vietnam-specific reports were not available, I searched the literature for relevant data from South East, East and South Asian countries. If no ‘local’ data source could be identified, I used the international data sources that were used to develop the original Global Action Fund for Fungal Infections (GAFFI) model, as the best available surrogate (Figure 5-2). The GAFFI model and its assumptions were first presented at the 12th European Congress on Clinical Microbiology and Infectious Diseases 2013, Berlin, where estimates of disease burden were made for Austria, Brazil, China, India, Ireland, Israel, Kenya, Netherlands, Nigeria, Senegal, Singapore, Spain, and Uganda. Where there were multiple data sources for one tier of the decision making tree, I took a subjective decision on which was the most robust and applicable to Vietnam. No attempt was made to combine newly identified data.
5.3.4 **Method for estimating the burden of AIDS defining mycoses**

I searched PubMed for data on the epidemiology of HIV/AIDS using the key words ("HIV" or “AIDS”) and ("epidemiology" or “incidence” or “prevalence”) and (“Vietnam” or “Asia”) with no predefined time-period. I also searched for data on the websites of the General Statistics Office of Vietnam (240), and the WHO (239). I included cryptococcal meningitis, PCP, and penicilliosis as AIDS-defining fungal infections. For each of these, the model estimates the number of cases by dividing the total number of AIDS cases in Vietnam by the expected percentage of the condition among new AIDS cases. Results are presented as annual number of cases and incidence per 100,000 population in 2012.

5.3.5 **Method for estimating burden of candidal infections**

I searched PubMed for data on the epidemiology of candidal infections using the key words ("Candida" or “Candidaemia”) and ("epidemiology" or “incidence”) and (“Vietnam” or “Asia”) with no predefined time-period. I searched the website of the General Statistics Office of Vietnam (240) for information on the number of ICU beds in Vietnam. I included candidaemia, *Candida* peritonitis, oesophageal candidiasis, oral candidiasis, and recurrent VVC.
as fungal infections related to *Candida*. I established the population incidence of candidaemia from the literature. I then calculated the number of cases in Vietnam and divided them into ICU and non-ICU associated cases based on proportions reported in the literature. I predicted the annual number of cases of *Candida* peritonitis based on the incidence of peritonitis in ICU patients with candidaemia. I estimated the annual number of patients with oesophageal candidiasis and oral candidiasis by applying established incidence rates for antiretroviral therapy (ART)-experienced and ART-naïve people living with HIV to those respective populations in Vietnam. I estimated the annual number of cases of recurrent VVC by establishing the number of women older than 16 years in Vietnam and multiplying this by an incidence rate for VVC in women older than 16 year from the literature. All results, except those for recurrent VVC, are presented as number of cases and incidence per 100,000 population in 2012. Recurrent VVC results are presented as number of cases and prevalence per 100,000 population in 2012.

### 5.3.6 Method for estimating burden of Aspergillus infections

I searched PubMed for data on the epidemiology of infections related to *Aspergillus* using the key words (“Aspergillus” or “invasive aspergillosis” or “allergic bronchopulmonary aspergillosis” or “severe asthma with fungal sensitization” or “chronic pulmonary aspergillosis”) and (“epidemiology” or “incidence” or “prevalence”) and (“Vietnam” or “Asia”) with no predefined time-period. In terms of risk factors for *Aspergillus* infections, I searched the literature for the incidence of haematological malignancies and the number of solid organ transplants occurring each year in Vietnam. Furthermore, I checked for any national or regional transplant registries that could help to ascertain the number of transplants occurring each year. I searched for data on the prevalence of chronic obstructive pulmonary disease (COPD) and asthma in Vietnam. I searched the website of the WHO (239) for data on the incidence of pulmonary TB in those with and without HIV.
The model for IA calculates the number of cases likely to result from respiratory disease and immunocompromise. To estimate the number of patients developing IA each year as a result of respiratory risk factors I established the annual number of COPD admissions then multiplied it by a value for prevalence of IA in COPD admissions from the literature. To this I added the number of patients expected to suffer from IA as a result of immunosuppression from haematological disease and organ transplantation. I established the number of patients with AML, HSCT, and renal, heart, lung, and liver transplants each year in Vietnam and multiplied this by an incidence of IA from the literature for each condition. Results are presented as annual number of cases and incidence per 100,000 population in 2012.

The models for ABPA and SAFS estimate the number of patients with these conditions from the local prevalence of adult asthma. I multiplied the estimated number of adults with asthma by the prevalence rate for each condition, as established from the literature. Results are presented as annual number of cases and point prevalence per 100,000 population in 2012.

The model for CPA was described fully in the introduction. Results are presented as annual number of cases and 5-year period prevalence per 100,000 population based on 2012.

5.3.7 Method for estimating burden of other mycoses

Other mycoses included fungal keratitis and tinea capitis. I searched PubMed for data on the epidemiology of fungal keratitis using the key words (“Fungal keratitis”) and (“epidemiology” or “incidence”) and (“Vietnam” or “Asia”). The search terms for tinea capitis were (“Tinea capitis”) and (“epidemiology” or “incidence” or “prevalence”) and (“Vietnam” or “Asia”) with no predefined time-period.

I estimated the annual number of cases of fungal keratitis by obtaining a population estimate of incidence from the literature and multiplying it by the population. I estimated the
number of individuals experiencing tinea capitis on an annual basis by multiplying the number of children in Vietnam by a prevalence estimate from the literature. Fungal keratitis results are presented as annual number of cases and incidence per 100,000 population in 2012. Results from the tinea capitis estimates are presented as total number of cases and point prevalence per 100,000 population in 2012.

5.3.8 Statistical and sensitivity analyses methods

Sensitivity analyses are often performed by disease burden researchers to determine which components of their models have the greatest impact on the overall estimate (241). The two main purposes of sensitivity analyses are to allow the quality of data on key assumptions to be improved for future models, and to give a sense of the uncertainty around an estimate. Narrowing the range for a component with a disproportionately large impact on the result of a model leads to more accurate estimates. There are several popular methods for performing sensitivity analyses in infectious disease models, of varying complexity (241). For this thesis, I took a three stage approach: first I looked at one-way sensitivity to individual variables across their ranges. Next I constructed a multivariable linear regression model in order to assess impact of individual variables, taking into account their interaction. Finally I made estimates for best and worst case scenarios, as a means of describing the accuracy of the model.

The ranges I used for the sensitivity analyses varied by component: where confidence intervals were available, or could be calculated, I used them; otherwise I used the extremes found in the literature. Both of these methods provided high and low estimates. Using Microsoft Excel (Microsoft Corp, Seattle, WA, 2007), I ran the models in triplicate using ‘low,’ ‘selected,’ and ‘high’ scenario values, and compared the overall estimates. The impacts on the overall estimates of using the highest and lowest values for each component, compared to the values actually selected, are presented in tornado charts.
After completing the one-way sensitivity analyses I went on to ascertain the ‘relative
importance’ of each component in a multivariable model. I used the ‘RelImpo’ (242,243)
package in R version 3.2.1 (244). The package performs variable decomposition on linear
regression models, taking into account interactions between variables, to produce a
percentage value for the relative importance of individual model components to the outcome.
I constructed linear regression models in R using the ‘lm’ function to approximate as closely as
possible the complex interactions in these models, and allow calculation of relative
importance (RI): I entered model components as independent variables, and the outcome as
the dependent variable. Because the linear regression model here is inherently a perfect fit,
and is only being used to explain the relative importance of various components of the model,
I did not formally assess the adequacy of the model.

The figures produced do not immediately provide insight into the accuracy of the
estimate. To make an estimate of 95% confidence intervals, I planned a Monte Carlo
simulation based on the range and distribution for each variable, as described by Mogasale et
al in their paper estimating the burden of typhoid fever (245).

5.4 Results

5.4.1 Model assumptions for population and country profile

The population of Vietnam in 2012 was 90,796,000. Assuming the population structure has
changed little since the 2009 census, there would be 20,791,284 (23%) persons below 15
years of age and 6,080,479 (17%) women over 50 years of age (240). Figure 5-3 shows the
population structure of Vietnam.
5.4.2 Model assumptions for AIDS defining mycoses

I collected data on the epidemiology of HIV, and the coverage of anti-retroviral therapy (ART), from Vietnamese Ministry of Health (246), UNAIDS (247), WHO (239), and Global Burden of Disease (10) reports. In 2012 there were an estimated 256,845 HIV-infected individuals and 4,677 new cases of AIDS in Vietnam (246). Of 256,845 HIV infected persons in Vietnam, 114,900 (45%) were receiving ART (246,247).

The number of new AIDS diagnoses is reported each year in Vietnam, but the type of AIDS defining illness is not. I found one report describing the prevalence of different AIDS defining illnesses in Vietnam (248). This paper by Klotz et al in 2007 presented findings from an outpatient setting with ambulatory patients so results are unlikely to be generalisable to all patients presenting with AIDS defining illnesses, and thus were not used. I identified a Vietnam-specific report on the epidemiology of Penicilliosis by Thuy et al from 2011 which used sentinel surveillance to estimate incidence (4). A report by Park et al from 2009 brought
together multiple data sources to make an estimate of the global burden of cryptococcal meningitis (5). These incidence estimates are broken down into regions, including South East Asia. I identified two other reports from Asia on the incidence of AIDS defining illnesses. The first report, by Kaplan et al in 1996 (249), described the incidence of AIDS defining illnesses in a cohort of 460 AIDS patients from Thailand and northern India. The other report, by Kumarasamy et al in 2003 (250), presented surveillance results for the incidence of AIDS defining illnesses in a cohort of 594 AIDS patients in southern India. The findings are summarized in Table 5-1.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcal meningitis</td>
<td>0%</td>
<td>-</td>
<td>3%</td>
<td>23%</td>
<td>5%</td>
</tr>
<tr>
<td>PCP</td>
<td>3%</td>
<td>-</td>
<td>-</td>
<td>13%</td>
<td>6%</td>
</tr>
<tr>
<td>Penicilliosis</td>
<td>0%</td>
<td>4%</td>
<td>4%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 5-1 Data sources with estimates for prevalence of cryptococcal meningitis, PCP and penicilliosis in AIDS patients, arranged from left to right according position in the decision making tree (Figure 5-2)

For each condition I selected the data source highest up the decision making tree (Figure 5-2). Therefore, excluding the estimates from Klotz, I assumed that of new AIDS diagnoses in 2012, 3% would be cryptococcal meningitis, 13% PCP, and 4% penicilliosis.

### 5.4.3 Model assumptions for candidal infection

I found no local data on the epidemiology of candidal infections so the original GAFFI data sources were utilized. Based on international data from 2010, I assumed an incidence rate of candidaemia of 5 per 100,000 population per year with 1.5 occurring in intensive care unit (ICU) patients and 3.5 in others (251). Data from a prospective cohort of 271 patients in France, suggested half of candidaemia cases in ICU patients resulted from candidal peritonitis (252). I obtained data about the number of ICU beds in 2012 from the General Statistics Office.
of Vietnam (240). Again based on international reports, I estimated that oral candidiasis would occur in 90% of HIV positive patients not yet receiving ART (253). The incidence rates for oesophageal candidiasis were estimated to be 20% for ART naive HIV patients and in 5% of those already on treatment (253,254). The prevalence of recurrent vulvovaginal candidiasis was estimated as 6% of women over 50 years based on a large internet survey from Western countries (255).

5.4.4 Model assumptions for Aspergillus infection

Although I did not identify any studies directly describing the epidemiology of Aspergillus infections in Vietnam or Asia, I did identify several local sources of data relating to risk factors. In terms of immunocompromise risk factors for IA, I found that Vietnam does not contribute systematically to any cancer or transplant registries. However, the incidence of acute myeloid leukaemia (AML) was estimated as 5 per 100,000 population and there were approximately 20-25 stem cell transplantations in 2012 [Personal Communication Dr. Huynh Van Man, Transplantation dept, Blood Transfusion and Hematology Hospital in HCMC]. In 2012, 130 kidney, 3 heart and 4 liver transplants were reported in an official press release (256) but with no national registry these figures are approximate. No lung transplant procedures have yet been reported. I applied the following multipliers, derived from data from the French Mycosis Study Group (257), to the above local disease statistics to reach an estimate of IA incidence: 10% of patients with acute myeloid leukaemia (AML) and 10% of patients with non-AML haematological malignancies (258,259), 0.5% of renal transplant patients (256), 4% of lung transplant patients (256), 6% of heart transplant patients(256) and 4% of liver transplant patients (256).

Tan et al estimated the prevalence of COPD in those over 30 years old in the Asia-Pacific region in 2003, giving Vietnam an estimate of 6.7% (260). A health economics study of the costs to Vietnam of smoking-related disease estimated the annual number of COPD
admissions as 348,992 (261). A paper by Xu et al in 2012 described the incidence of IA amongst 992 patients admitted with acute exacerbations of COPD as being 3.9% (262). I therefore estimated that IA would occur in 3.9% of all COPD admissions in Vietnam in 2012.

The models for ABPA and SAFS utilize data on asthma prevalence. The local prevalence of asthma was estimated as 1.04% based on data from a global WHO survey of 178,215 individuals (263). I found no local data about the epidemiology of ABPA or SAFS, and so used the original GAFFI data sources to estimate ABPA would occur in 2.5% of adult asthmatics and SAFS in 33% of the most severe 10% of adult asthmatics (8).

The model for CPA, as described in the introduction and elsewhere (8,9), requires local TB data. Vietnam’s annual TB incidence is 218/100,000 (239). The original GAFFI CPA model estimated that 12% of patients with TB would have cavitatory disease – however, amongst Vietnamese patients with pulmonary TB 40.8% have cavitatory disease (235,264) so the model was modified accordingly. In brief, I established the annual incidence of TB from 2012 WHO estimates (239), then estimated that 22% of those with cavities and 2% of those without would develop CPA (9). It is estimated that 15% of patients with CPA will die each year (9). The CPA model uses this as an attrition rate, and calculates five year period prevalence. Based on Asian data, a conservative estimate that 75% of CPA cases result from TB was made to facilitate estimation of non-TB cases (236,237) (Figure 5-1).

5.4.5 Model assumptions for other mycoses

An epidemiology paper from Northern Vietnam estimated the incidence of fungal keratitis as 7 per 100,000 population (265). The prevalence of tinea capitis has not been described in South East Asia – most international reports of school-based surveillance give estimates varying between 0.1% to 9-11% (266–268). Given the lack of local data, I used the original GAFFI model estimate of 2% of school-aged children, based on the mean incidence from several surveys in London (269).
### 5.4.6 Results of actuarial estimates

<table>
<thead>
<tr>
<th>Infection</th>
<th>Key assumptions for model</th>
<th>Total cases (range)</th>
<th>Incidence/Prevalence b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcal meningitis</td>
<td>3% of new AIDS diagnoses</td>
<td>140 (23-1319)</td>
<td>0.15</td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>13% of new AIDS diagnoses</td>
<td>608 (281-2748)</td>
<td>0.67</td>
</tr>
<tr>
<td>Penicilliosis</td>
<td>4% of new AIDS diagnoses</td>
<td>206 (159-594)</td>
<td>0.23</td>
</tr>
<tr>
<td>Candidaemia</td>
<td>5/100,000 general population: 3.5 in ICU patients, 1.5 in non-ICU patients</td>
<td>4,540 (1,735-10,150)</td>
<td>5</td>
</tr>
<tr>
<td>Oesophageal candidiasis</td>
<td>20% of HIV patients not on ARVs; 5% of those on ARVs</td>
<td>33,107 (9,524-61,173)</td>
<td>36</td>
</tr>
<tr>
<td>Oral candidiasis</td>
<td>90% of HIV positive not on ARVs</td>
<td>121,590 (7,454-260,028)</td>
<td></td>
</tr>
<tr>
<td>Recurrent vaginal candidiasis &gt;4/times/year</td>
<td>6% of adult woman</td>
<td>1,767,581 (1,194,070-3,229,512)</td>
<td>3,893 c</td>
</tr>
<tr>
<td>Invasive aspergillosis</td>
<td>3.9% severe COPD; 10% AML; 10% non-AML haematological malignancy; 0.5% renal transplants; 6% heart transplants</td>
<td>14,523 (3,745-18,556)</td>
<td>15</td>
</tr>
<tr>
<td>Allergic bronchopulmonary aspergillosis</td>
<td>2.5% of adult asthmatics; 15% of adults with cystic fibrosis</td>
<td>23,607 (4,981-66,208)</td>
<td>26 c</td>
</tr>
<tr>
<td>Severe asthma with fungal sensitisation</td>
<td>33% of the most severe 10% of adult asthmatics</td>
<td>31,161 (8,538-181,599)</td>
<td>34 c</td>
</tr>
<tr>
<td>Chronic pulmonary aspergillosis</td>
<td>22% of cases of cavitatory pulmonary TB; 2% of non-cavitatory cases</td>
<td>55,509 (9,162-127,519)</td>
<td>61 d</td>
</tr>
<tr>
<td>Fungal keratitis</td>
<td>7 cases per 100,000 population</td>
<td>6,356 (4859-7751)</td>
<td>7</td>
</tr>
<tr>
<td>Tinea capitis</td>
<td>2% children &lt;14 yrs old</td>
<td>415,301 (42,960-8,668,458)</td>
<td>457 c</td>
</tr>
</tbody>
</table>

**Estimated total cases**: 2,474,338

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*a Ranges derived from highest and lowest scenarios in sensitivity analyses
*b Unmarked figures are annual incidence rates per 100,000 population/year
*c Point prevalence per 100,000 population in 2012
*d 5 year period prevalence per 100,000 population in 2012

Table 5-2 Results from actuarial estimates of the burden of selected fungal infections in Vietnam, 2012.
I estimated that 2,474,338 episodes of serious fungal infection occurred in Vietnam in 2012. Full details of results, including the ranges for each condition, are found in Table 5-2. The more common conditions were those associated with lower case fatality ratios, but considerable morbidity, such as recurrent vaginal candidiasis (prevalence of 3,893 per 100,000 women) and tinea capitis (prevalence of 457 per 100,000 population). 6,356 cases of fungal keratitis were estimated.

Chronic pulmonary aspergillosis had a 5 year period prevalence of 61 per 100,000. I estimated that there were 4,540 cases of candidaemia, 608 of PCP, 206 of penicilliosis and 140 of cryptococcal meningitis in 2012.

5.4.7 Sensitivity Analyses

Figures 5.4 to 5.18 are tornado charts showing results of one-way sensitivity analyses on individual components for each condition. The invasive aspergillosis charts show the model as a whole (Figure 5.10), and once split into two smaller components. Figure 5.12 shows the cases estimated to result from transplants, and Figure 5-11 shows the remaining estimated cases. Because the majority of model components lacked data on distribution, I was unable to perform Monte Carlo estimates of confidence intervals around burden estimates.

5.4.8 General notes on the following tornado charts

The figure titles contain an estimated maximum range for the annual number of patients experiencing each condition. I determined the maximum range by running high and low scenarios for each model. The x-axes use a logarithmic scale, and show the impact on total numbers of every covariate at the limit of its range (the full range for each covariate is presented in square brackets at the left hand side of the chart). For ease of presentation, I have shown the percentage values for relative importance (RI) on the right hand side of the charts, although these results were obtained by variable decomposition of a multiple linear regression model.
Figure 5-4 Tornado chart: one-way sensitivity analysis for number of cases of cryptococcal meningitis, Vietnam, 2012 (range 23-1319)

- [0.5-12] Incidence of CM (%): 0.17 -> 4.00 RI 86%
- [4,677-10,990] Number of AIDS cases: 1 -> 2.35 RI 14%

Figure 5-5 Tornado chart: one-way sensitivity analysis for number of cases of PCP, Vietnam, 2012 (range 281-2748)

- [4,677-10,990] Number of AIDS cases: 1 -> 2.35 RI 17%
- [6-25] Incidence of PCP (%): 0.46 -> 1.92 RI 83%

Figure 5-6 Tornado chart of one-way sensitivity analysis for number of cases of penicilliousis, Vietnam, 2012 (range 159-594)

- [4,677-10,990] Number of AIDS cases: 0.68 -> 1.60 RI 81%
- [3.4-5.4] Incidence of PM (%): 0.77 -> 1.23 RI 19%
Figure 5-7 Tornado chart: one-way sensitivity analysis for number of cases of candidaemia, Vietnam, 2012 (range 1,735-10,150)

[2-11] Incidence of candidaemia /100k

0.1 1 10

0.40 2.20 RI 99.8%

[86.8m-92.3m] Population

0.96 1.02 RI 0.2%

Figure 5-8 Tornado chart: one-way sensitivity analysis for number of cases of oesophageal candidiasis, Vietnam, 2012 (range 9,524-61,173)

[19-95] % diagnosed cases on ARVs

0.45 1.33 RI 77.8%

[207,056-297,243] Current number HIV/AIDS

0.81 1.16 RI 11.6%

[16-24] Incidence in ARV naïve (%)

0.84 1.16 RI 10.1%

[4-6] Incidence in ARV treated (%)

0.96 1.04 RI 0.5%
Figure 5-9 Tornado chart: one-way sensitivity analysis for number of cases of oral candidiasis, Vietnam, 2012 (range 7,454-260,028)

- **[19-95] % diagnosed cases on ARVs**: 0.10 to 1.54, RI 91%
- **[207,056-297,243] Current number HIV/AIDS**: 0.81 to 1.16, RI 5%
- **[72-100] Incidence in ARV naïve (%)**: 0.80 to 1.11, RI 4%
Figure 5-10 Tornado chart: one-way sensitivity analysis for number of cases of invasive aspergillosis, Vietnam, 2012 (range 3,745-18,556)

- [1.3-3.9] Incidence of IA in COPD admissions (%): 0.38 (0.74), 1.00 (1.00)
- [279k-419k] COPD admissions to hospital per year: 0.81 (0.74), 1.19 (1.00)
- [5-24] Incidence of IA in AML (%): 0.97 (0.74), 1.09 (1.00)
- [1.3-5] AML population frequency /100k: 0.98 (0.74), 1.03 (1.00)
- [86.8m-92.3m] Population: 1.00 (0.74), 1.00 (1.00)
- [2.7-23] Incidence IA in Allogeneic HSCT: 1.00 (0.74), 1.00 (1.00)
- [0.2-1] Incidence of IA in renal Tx: 1.00 (0.74), 1.00 (1.00)
- [0.7-10] Incidence of IA in liver Tx: 1.00 (0.74), 1.00 (1.00)
- [0.4-15] Incidence of IA in heart Tx: 1.00 (0.74), 1.00 (1.00)
- [20-25] Allogeneic HSCT per year: 1.00 (0.74), 1.00 (1.00)
- [104-156] Renal Tx per year: 1.00 (0.74), 1.00 (1.00)
- [2.4-3.6] Heart Tx per year: 1.00 (0.74), 1.00 (1.00)
- [3.2-4.8] Liver Tx per year: 1.00 (0.74), 1.00 (1.00)
Figure 5-11 Tornado chart: one-way sensitivity analysis for number of cases of invasive aspergillosis from COPD and AML, Vietnam, 2012 (range 3,745-18,547)

- [1.3-3.9] Incidence of IA in COPD admissions (%): 0.38 (RI 81.2%)
- [279k-419k] COPD admissions to hospital per year: 0.81 (RI 15.8%)
- [5-24] Incidence of IA in AML (%): 0.97 (RI 2.6%)
- [1.3-5] AML population frequency /100k: 0.98 (RI 0.3%)
- [86.8m-92.3m] Population: 1.00 (RI <0.1%)
Figure 5-12 Tornado chart: one-way sensitivity analysis for number of cases of invasive aspergillosis from transplant, Vietnam, 2012 (range 0.8-8)

- [2.7-23] Incidence IA in Allogeneic HSCT (%): 0.49 → 2.40, RI 92.5%
- [0.2-1] Incidence of IA in renal Tx (%): 0.97 → 1.38, RI 5.4%
- [0.7-10] Incidence of IA in liver Tx (%): 1.00 → 1.15, RI 0.7%
- [0.4-15] Incidence of IA in heart Tx (%): 0.94 → 1.13, RI 0.7%
- [20-25] Allogeneic HSCT per year: 0.92 → 1.08, RI 0.6%
- [104-156] Renal Tx per year: 0.97 → 1.03, RI <0.1%
- [2.4-3.6] Heart Tx per year: 0.99 → 1.01, RI <0.1%
- [3.2-4.8] Liver Tx per year: 1.00 → 1.00, RI <0.1%

Figure 5-13 Tornado chart: one-way sensitivity analysis for number of cases of allergic bronchopulmonary aspergillosis, Vietnam, 2012 (range 4,981-66,208)

- [0.7-3.5] Prevalence of ABPA in asthmatics (%): 0.28 → 1.40, RI 57.7%
- [0.82-2.05] Asthma Prevalence (%): 0.79 → 1.97, RI 42%
- [86.8m-92.3m] Population: 0.96 → 1.02, RI 0.3%
Figure 5-14 Tornado chart: one-way sensitivity analysis for number of cases of severe asthma with fungal sensitization, Vietnam, 2012 (range 8,538-181,599)

- [0.82-2.05] Asthma Prevalence (%)
  - 0.79
  - 1.97
  - RI 37.9%

- [8-20] Prevalence of severe asthma (%)
  - 0.80
  - 2.00
  - RI 39.9%

- [15-48] Prevalence of SAFS in severe asthma (%)
  - 0.45
  - 1.45
  - RI 21.8%

- [86.8m-92.3m] Population
  - 0.96
  - 1.02
  - RI 0.3%

Figure 5-15 Tornado chart: one-way sensitivity analysis for number of cases of chronic pulmonary aspergillosis, Vietnam, 2012 (range 9,162-127,519)

- [10-49.5] Incidence of cavitation in TB (%)
  - 0.39
  - 1.17
  - RI 24.6%

- [99k-170k] Annual cases of TB
  - 0.76
  - 1.31
  - RI 25.7%

- [63-91] CPA cases related to TB (%)
  - 0.83
  - 1.18
  - RI 15.8%

- [17.6-26.4] Incidence of CPA in cavitatory TB (%)
  - 0.82
  - 1.18
  - RI 17.8%

- [1-4] Incidence of CPA in non-cavitatory TB (%)
  - 0.94
  - 1.12
  - RI 16.1%
Figure 5-16 Tornado chart: one-way sensitivity analysis for number of cases of fungal keratitis, Vietnam, 2012 (range 4859-7751)

- **Incidence of fungal keratitis /100k**
  - Range: 0.80 - 1.20
  - RI: 97.6%

- **Population**
  - Range: 0.96 - 1.02
  - RI: 2.4%

Figure 5-17 Tornado chart: one-way sensitivity analysis for number of cases of recurrent vaginal candidiasis, Vietnam, 2012 (range 1,194,070-3,229,512)

- **Incidence recurrent vaginal candidiasis (%)**
  - Range: 0.80 - 1.60
  - RI: 67.7%

- **Proportion adult (%)**
  - Range: 0.94 - 1.06
  - RI: 12.6%

- **Proportion women (%)**
  - Range: 0.94 - 1.06
  - RI: 12.6%

- **Population**
  - Range: 0.96 - 1.02
  - RI: 7.1
5.5 Discussion

All serious mycoses are associated with high case fatality ratios and substantial morbidity. The treatment of serious fungal infections often requires long hospital admissions and prolonged courses of anti-fungal drugs. I estimated the 2012 incidence or prevalence of major fungal infections in Vietnam, and found that 2.47 million individuals were affected by fungal infections and 291,347 of those were ‘serious’ fungal infections.

The health economic implications of serious fungal infections are poorly described in settings such as Vietnam, but are likely to be considerable. Cost of disease estimates are urgently required for proper healthcare planning, and to project resource requirements as economic development leads to a rise in iatrogenic risk factors. My description of the context of serious mycoses in Vietnam suggests the major drivers of the most serious fungal infections are the high incidence of TB (leading to Aspergillus related disease) and the HIV epidemic (leading to some candidal infections, PCP, penicilliosis and CM). Although the prevalence of HIV is not high in Vietnam, the country’s large population means that there are many individuals at risk for serious conditions such as cryptococcal meningitis. The estimated incidence of non-HIV associated candidal disease is also of concern.
Non-serious but chronic mycoses such as recurrent vulvovaginal candidiasis and tinea capitis are not only inconvenient, but can be stigmatizing - further work is required to delineate the problem and ensure access to therapy is available. The incidence of sight-threatening fungal keratitis is high; identifying and mitigating risk factors should be a priority.

Of interest, the incidence of 5/100,000 for AML used in my analysis is higher than other estimates. For example, registry data from Nanjing suggests a rate of 1.66/100,000 (258) whilst the Asia / Pacific Islander ethnic group in the USA’s Survival Epidemiology and End-Results Programme (SEER) registry would suggest an incidence of 2.6/100,000 (259). However, speculation that Vietnam has an above average incidence of AML is widespread, lending some credibility to the local estimate.

There are major limitations to my actuarial approach to describing the incidence and prevalence of serious mycoses. The modeling used only allows crudely informed estimates to be made. Some of the calculations are likely to underestimate the true extent of the problem. For example, no attempt has been made to consider the impact of corticosteroid use on fungal infections. Furthermore, HIV uninfected persons with cryptococcal meningitis and oral candidiasis are not counted. In addition to these limitations is the concern that the approach has not been fully validated against population-based or sentinel surveillance, and certainly not in a tropical setting where mycoses may be more common. Another problem is that without better health economic data it is not possible to make a full estimate of the burden of disease. As data are produced on the clinical outcomes from serious mycoses in settings such as Vietnam it will become possible to enrich the estimates presented here with estimates of impact on disability adjusted life years (DALYs).

The ranges I present for some conditions are very wide reflecting uncertainty driven by the paucity of data. Because there was no rational basis for deciding on the distribution of the data, I didn’t calculate confidence intervals with a Monte Carlo-type simulation. However,
knowing which variables are the main drivers of uncertainty will help to focus resources when deciding in which areas data quality should be improved. The sensitivity analyses will ensure that the most valuable data is collected to inform future estimates.

Notwithstanding the limitations, this is the first attempt to describe serious mycoses in Vietnam, or South East Asia, and provides a starting point from which to better understand the extent of the problem. The data generated should stimulate interest in surveillance of these conditions and will contribute to a growing global effort to raise the profile of these neglected conditions.

During the process of developing this part of my thesis, with an awareness of the limitations of actuarial approaches, I further developed a novel sentinel surveillance design for use in Vietnam. This approach could be the basis of future work to generate data which would be able to validate or refute the actuarial estimates. The study outline is described in the following section.
5.6  *Future Directions: Estimating the Incidence of Serious Fungal Infections in Ho Chi Minh City, Vietnam: Study Concept*

5.6.1  *Overview*

While a useful starting point, actuarial estimates cannot replace surveillance because many assumptions are derived from dissimilar, mostly Western, populations. This study aims to describe the pattern of serious fungal infections in Ho Chi Minh City (HCMC) more clearly. We will use a sentinel surveillance approach, combined with standard treatment costs and published outcome data, where available, to estimate the burden of cryptococcosis, pneumocystis pneumonia, penicilliosis, invasive candidiasis, oesophageal candidiasis, invasive aspergillosis, allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) and fungal keratitis.

5.6.2  *Sites*

There are ten main hospitals in HCMC (Figure 5-19) where complicated patients are managed, and adequate microbiology lab diagnostics are available. These are Hospital for Tropical Diseases, Cho Ray Hospital, Pham Ngoc Thach Hospital, Heart Hospital, Haematology Hospital, Cancer Hospital, Children’s Hospitals 1 and 2, Ophthalmology Hospital, ENT Hospital and People’s Hospital 115.
We will approach these hospitals to discuss their participation and aim to collect prospective data for a one year period at each, counting individual episodes of lab-confirmed or discharge-coded cases of the selected serious fungal infections (Table 5-3).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Source of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcosis</td>
<td>Positive blood culture, positive CSF culture, positive CSF CrAg, coded discharge diagnosis (B45.0-45.9)</td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>Positive lower respiratory sample smear, coded discharge diagnosis (J17.3, B59)</td>
</tr>
<tr>
<td>Penicilliosis</td>
<td>Positive culture, positive microscopy, coded discharge diagnosis (B48.4)</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>Positive blood culture, positive peritoneal fluid culture, coded discharge diagnosis (B37.1, B37.5-37.9)</td>
</tr>
<tr>
<td>Oesophageal candidiasis (HIV)</td>
<td>Coded discharge diagnosis (B20.4)</td>
</tr>
<tr>
<td>Invasive aspergillosis</td>
<td>Coded discharge diagnosis (B44.0)</td>
</tr>
<tr>
<td>ABPA</td>
<td>Coded discharge diagnosis</td>
</tr>
<tr>
<td>Chronic pulmonary aspergillosis</td>
<td>Coded discharge diagnosis</td>
</tr>
<tr>
<td>Fungal keratitis</td>
<td>Positive microscopy corneal samples, coded discharge diagnosis (discuss with ophthalmology hospital)</td>
</tr>
</tbody>
</table>

Table 5-3 proposed conditions to be included in surveillance and corresponding source of cases
5.6.3 Surveillance approach

We will use a sentinel surveillance approach. We will approach key sites selected for the availability of diagnostic microbiology facilities and their willingness to collaborate. We will identify patients with the conditions of interest at these sites, with the recognition that those patients will only represent a proportion of the total population of patients with the conditions of interest in the catchment area of the surveillance sites. This is illustrated by the ‘surveillance pyramid’ (Figure 5-20) showing patients being lost at every stage from developing the condition to being reported to the surveillance programme. By estimating the proportion of patients lost at each stage, it is possible to develop a ‘multiplier’ which will convert the number of patients identified in the surveillance programme to an estimate of disease burden in the catchment area of the surveillance sites.

This will be a pragmatic study, and we don’t plan to alter the usual diagnostic procedures at surveillance sites. This may lead to some under-reporting, which will need to be accounted for in the multiplier. Because the surveillance will be based on laboratory diagnoses and discharge coding data, we will correct for losses at every stage of the surveillance pyramid (Figure 5-20).
Figure 5-20) and, conversely, take account of overestimates in the case of coded
diagnoses. We will carry out these calculations based on methods reported elsewhere (16–
20), although specific multipliers will be determined for each condition of interest taking into
account patient health-seeking behaviour, clinician referral patterns, and frequency /
availability of testing. In order to bridge sentinel site incidence to population incidence we will
conduct a community-based survey of health-seeking behaviour, analyse patient flows
through the healthcare systems of Ho Chi Minh City and establish the catchment areas of the
surveillance sites.

5.6.4 Case ascertainment and multipliers

For all conditions, we will count cases occurring at sentinel sites during the 12 months
period. Because the sentinel sites are also amongst the highest tier hospitals in HCMC, we
would expect a seriously-ill patient’s journey to end at one of them – especially if there was
any diagnostic uncertainty. We will make assumptions about the speed of this journey, in
consultation with local clinicians, to determine how many patients may be lost during the
referral and transfer process for each condition. We will avoid double counting by paying
careful attention to record numbers, names and dates of birth.

Cryptococcosis

We will identify patients through a review of microbiology records and discharge coded
diagnoses at each of the participating sites. We will count cases where there is a positive
blood or CSF culture, a positive serum cryptococcal antigen test (CrAg) or a discharge code
B45.0-B45.9, for an individual patient’s admission. Because cryptococcal diagnostics are
sensitive, the most important multipliers will be related to patient and clinician behaviour –
i.e., what proportion of patients at risk would present to a hospital with the facilities to make
the diagnosis, and then receive the correct diagnostic tests?
**Pneumocystis pneumonia**

Pneumocystis is infrequently identified in the laboratory, so we will rely on discharge diagnoses coded J17.3 or B59 for case identification. The most important multipliers will relate to patient and clinician behaviour, and for cases counted at sentinel sites we will make an estimate about the accuracy of clinical diagnosis, based on international reports.

**Penicilliosis**

We will identify patients through a review of microbiology records and discharge coded diagnoses at each of the participating sites. We will count cases where there is a positive skin smear, blood culture, or a discharge code B48.4, for an individual patient’s admission. As for cryptococcal cases, we will base multipliers on patient and clinician behaviours.

**Oesophageal candidiasis**

The diagnosis of oesophageal candidiasis is most often clinical, and is likeliest to occur in the out-patient setting. We will identify cases at the out-patient clinic of Hospital for Tropical Diseases, which has a stable HIV population, and generate a figure for cases per patient year of follow-up, which we can generalize to the HIV population under follow-up in HCMC.

**Invasive aspergillosis, Chronic pulmonary aspergillosis (CPA), Allergic bronchopulmonary aspergillosis (ABPA)**

We will discuss case ascertainment with colleagues at Pham Ngoc Thach – the majority of diagnoses are likely to be clinical, and so we will identify them from discharge codes. Patients with invasive aspergillosis are likely to be diagnosed as in-patients, and we will identify patients using discharge codes from Pham Ngoc Thach, and the Haematology and Cancer Hospitals. CPA and ABPA on the other hand will frequently be diagnosed in the outpatient
department; we will calculate a separate catchment area for the Pham Ngoc Thach out-patient department to allow calculation of incidence.

5.6.5 Calculating catchment areas

We will estimate catchment areas for each of the participating hospitals according to where the majority of patients are drawn from, based on a random selection of addresses for all admissions in a 12-month period. A patient flow method (18,21) will be used where the top rank-ordered, population-weighted geographic units (or ‘Wards’, in HCMC) accounting for 50-80% of admissions to each hospital are considered to approximate its catchment area. Using this method, maps are drawn at various percentages and the most ‘natural fit’ selected. Another approach will be used, where all admission addresses are plotted onto a map and any Ward contributing more than 0.5-5% of patients to a hospital is considered to be part of that hospital’s catchment area – the population contained within these boundaries becomes the catchment population for that hospital. Using both methods will provide a ‘double-look’, enhancing accuracy.

5.6.6 Health care utilization survey

We will conduct a community based survey to establish the standard pattern of health seeking behaviour amongst the citizens of Ho Chi Minh. Sampling will be designed to maximize the survey’s ‘representativeness’ in terms of geography, demographics and relevance to the conditions of interest. Identifying the correct participants for a health care utilization survey in an urban area with multiple health-care options can be challenging, but once a catchment area has been correctly described, a simple random geographical selection of households can be used (20,270). In this study, we will divide the catchment area covered by the sentinel sites into 5 second latitude by 5 second longitude rectangles – we will randomly select 100 of these rectangles, and invite any household with a front door inside the rectangle to participate in
the survey. Field staff will be equipped with global positioning system devices to facilitate this process. We anticipate that this approach will yield approximately 10,000 household surveys\(^{(270)}\). Although this sample size is arbitrary it is similar to one used in another large Asian city\(^{(270)}\), and when increasing sample size is plotted against the resultant percentage inaccuracies, by confidence interval (as in Figure 5-21), it can be shown to be justified - little further benefit would result from us increasing the sample size.

Figure 5-21 Percentage accuracy against sample size, by varying levels of confidence

We will ask participants about their health seeking behaviors for specific health syndromes (e.g., headache, fever, cough) at various levels of severity (e.g., mild illness, very unwell, fears for life), focusing on which level of care they would initially seek care (e.g., pharmacy, local health clinic, referral hospital). The community survey will also provide an opportunity for us to enquire about specific risk factors for conditions of interest to the Oxford University Clinical Research Unit.
The catchment area and health care utilization information will provide an essential multiplier for estimating population incidence from lab-based and healthcare-based data.

5.6.7 Value of this project

The catchment area data collected will be of great use to anyone doing hospital based research in HCMC, and will provide essential denominator information for estimating the burden of disease. The community survey will also provide an opportunity to enquire about specific risk factors for conditions of interest to the Unit, and will help to direct future surveillance approaches.

In this study, we will attempt to understand catchment populations and referral patterns for a range of relevant infectious disease syndromes. It will provide an opportunity to estimate disease incidence for multiple conditions and help to build the burden-of-disease case for new projects, as well as demonstrating the suitability of the area for public health intervention studies.
5.7 **Statement of contribution**

The model used here to estimate the incidence and prevalence and serious mycoses was originally designed by Prof David Denning and colleagues. They made the model available to colleagues around the world in order to work towards a global estimate. I searched the literature for local demographic and epidemiological data with which to populate the model, and modified the model wherever local data supported modifications. All coded and performed all statistical analyses myself. The outline proposal for sentinel surveillance is also my own work.

I was first author on a poster of the results, which was presented at ECCMID, and a manuscript which was published in Mycoses.
6. Adjunctive Corticosteroids in HIV-associated Cryptococcal Meningitis: A Randomized Controlled Trial in African and Southeast Asian Countries
6.1 Summary

Introduction HIV-associated cryptococcal meningitis causes >600,000 deaths yearly. Treatment has changed little in 20 years, and there are no imminent novel agents. Adjuvant corticosteroids reduce mortality in other forms of meningitis in some populations, but are untested in cryptococcal meningitis. We performed a double-blind randomized controlled trial to determine whether adjunctive treatment with dexamethasone reduces mortality in HIV-associated cryptococcal meningitis.

Methods We recruited adult patients in Vietnam, Thailand, Indonesia, Laos, Uganda and Malawi. All patients received dexamethasone or placebo for 6 weeks, and combination antifungal therapy with amphotericin B and fluconazole.

Results The trial was stopped for safety concerns following enrolment of 451 patients. Mortality by 10 weeks was 47% for dexamethasone vs 41% for placebo (hazard ratio (HR) of time to death 1.11 (95% CI 0.84 to 1.47); P=0.45) and 57% vs 49% by 6 months (HR 1.18 (95% CI 0.91 to 1.53); P=0.20). Disability at 10 weeks was more frequent in patients receiving dexamethasone (‘good’ outcome 13% versus 25% with placebo, odds ratio 0.42 (95% CI 0.25 to 0.69) P<0.001). Clinical adverse events were more common in the dexamethasone group (total number of events = 667 vs 494, P=0.01), including grade 3 or 4 infectious (48 vs 25 patients, P=0.003), renal (22 vs 7, P=0.004) and cardiac events (8 vs 0, P=0.004). Cerebrospinal fluid fungal clearance was slower in the dexamethasone group. Results were consistent across Asian and African sites.

Conclusion Dexamethasone did not reduce mortality in HIV-associated cryptococcal meningitis and was associated with more adverse events and disability.
6.2 Background

A detailed introduction to HIV-associated cryptococcal meningitis is provided in chapter 4. In brief summary, the condition is estimated to cause >180,000 deaths each year, the vast majority in Sub-Saharan Africa, South and South East Asia (271). Mortality on combination therapy remains over 30% at 10 weeks; survivors often have significant disability (3,197). There is a pressing need to improve outcomes. However, no novel anti-cryptococcal agents are close to clinical use; innovative strategies are needed.

6.2.1 Treatment of cryptococcal meningitis

Current guidelines for anti-fungal therapy are based on combination therapy, which is delivered in three phases: induction, consolidation, and maintenance. Clinically and microbiologically, the most effective treatment option for induction therapy is two weeks of amphotericin B (1mg/kg/day) in combination with flucytosine (100mg/kg/day). However, in developing settings, where flucytosine is generally unavailable (198,199), the WHO and the Infectious Disease Society of America (IDSA) recommend induction therapy with two weeks of amphotericin B (1mg/kg/day) combined with fluconazole 800mg/day. Induction is followed by consolidation therapy with fluconazole 800mg/day for a further 8 weeks before switching to maintenance, or secondary prophylaxis, with 200mg/day of fluconazole (186,187).

As described in chapter 4, recommendations for the initiation of ART now favour delayed initiation. The Cryptococcal Optimal ART Timing (COAT) trial in 2014 (210) confirmed the suggestion from several earlier studies that delaying ART for 5-6 weeks was associated with better outcomes than starting 1-2 weeks after the diagnosis of cryptococcal meningitis (26-week mortality 30% vs 45%, a mortality hazard ratio of 1.73 p=0.03).

Managing the complications of cryptococcal meningitis (such as raised intracranial pressure, IRIS, or cryptococcomas) is a significant clinical challenge, and there is little evidence to guide physicians. Whilst it is well established that raised intracranial pressure is associated
with poor outcomes (215), and its control with regular lumbar puncture is recommended in
the IDSA guidelines, no trial evidence exists to prove management improves outcomes.
Indeed, a pharmacological approach to control raised intracranial with acetazolamide was
associated with poorer outcomes, and terminated early (272). As seen above, the incidence of
IRIS can be reduced by properly timed ART. Cerebral cryptococcomas are relatively
uncommon in HIV associated cryptococcal meningitis, but if they do occur IDSA guidelines
recommend prolonged antifungal therapy and careful management of intracranial pressure.

Unfortunately, even when these aspects of clinical care are managed well, case mortality
from cryptococcal meningitis remains unacceptably high, making the search for adjuvant
therapies critical. Adjunctive treatments, such as corticosteroids, have proven beneficial in
central nervous system (CNS) infections in some settings. Dexamethasone reduced mortality
from acute bacterial meningitis in adults in Europe and in adults with microbiologically
confirmed disease in Vietnam (273,274). Dexamethasone reduced mortality in a mixed cohort
of HIV-infected and uninfected adults with tuberculous meningitis (TBM) in Vietnam, but was
not powered to show an effect in HIV patients alone (275). Cryptococcal meningitis shares
pathophysiological features with TBM, including vasculitis, cerebral edema, and raised
intracranial pressure (44), all potentially modifiable by corticosteroids.

Corticosteroids are cheap and readily available where the burden of CM is highest; low
rates of adverse events have been observed in patients with CNS infections (274–276).
Retrospective data suggest they may reduce the risk of blindness in HIV uninfected patients
with CM (277), and animal studies suggest they do not reduce the sterilizing power of
amphotericin or fluconazole and improve survival even in the absence of antifungal therapy
(181,278). Corticosteroids are widely-used in clinical practice for CM in high-burden settings,
particularly Asia, and international guidelines recommend their use in some circumstances
However, data from controlled trials are lacking. For all of the above reasons, we focused our research on this approach.

6.2.2 Potential mechanisms for benefit or risk of dexamethasone therapy in cryptococcal meningitis

The severity of disease arising from infections is determined by a combination of the pathogen and the host immune response (and, unfortunately, sometimes exacerbated by the treatment). The concept underlying the use of corticosteroids in infectious diseases is that they can dampen potentially harmful inflammation, provided the pathogen is controlled by suitable anti-microbial agents. We postulated that dexamethasone therapy may benefit cryptococcal meningitis patients in three main ways: reducing intracranial pressure, reducing inflammation and vasculitis, and reducing cerebral oedema.

Cerebral inflammation leads to raised intracranial pressure and corticosteroids are commonly used, especially in oncology, to address this problem. In cryptococcal meningitis, the pressure effect of inflammation may be exacerbated by CSF outflow obstruction (279). The impacts of corticosteroids on inflammation in cryptococcal meningitis have been well studied in the mouse model. This model of cryptococcal meningitis is the most popular experimental model, and the mouse’s cytokine response bears similarities to that observed in humans with HIV-associated cryptococcal meningitis (280). In this model, reducing inflammation with both corticosteroids (181) and monoclonal antibodies against fungal glucosylceramides (281) led to prolonged survival.

The capsule of Cryptococcus spp. is an important virulence factor (44). One mechanism underlying the capsule’s virulence is that it induces production of vascular endothelial growth factor (VEGF), leading to cerebral oedema (282). Dexamethasone’s ability to downregulate VEGF production is postulated to account for dexamethasone’s beneficial effects in TB meningitis, and may offer similar benefits for cryptococcal meningitis patients (283).
Finally, vasculitis is described in cryptococcal meningitis and leads to significant brain injury (284,285). Corticosteroids are the main treatment option for primary cerebral vasculitic diseases, and preventing cases of vasculitis would be expected to benefit the patient population.

There are some general risks from the use of dexamethasone. Corticosteroid therapy causes immunosuppression, which may predispose to other infections. Other well-established side-effects include hyperglycaemia, secondary hypoadrenalism, Cushing’s-like syndrome, gastrointestinal bleeding, proximal myopathy, development of cataracts, and psychiatric disturbances (286). Reassuringly, in a large randomised placebo controlled trial of dexamethasone for the treatment of TB meningitis, adverse events we not more frequent in the dexamethasone arm (275).

However, there are some specific arguments against the use of corticosteroids in HIV-associated cryptococcal meningitis. Primary amongst these is the belief that since inflammatory responses are dampened in AIDS patients, little will be observed in terms of the proposed anti-inflammatory benefits of corticosteroids. This could potentially leave patients exposed to the harmful effects of corticosteroids, whilst gaining no clinical benefit. However, although the granulomatous reactions seen in non-HIV associated cryptococcal meningitis are generally absent in HIV associated cases, inflammation and oedema remain notable features (287). Furthermore, inflammation is more widely disseminated through the brain parenchyma in HIV-positive than HIV-negative cases (where disease is usually limited to the meninges). Therefore, even immune-deficient patients may benefit from anti-inflammatory therapy.

A second cryptococcal meningitis-specific risk is that a brisk inflammatory response may actually be beneficial in cryptococcal meningitis. Higher baseline levels of the pro-inflammatory cytokines interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and interleukin (IL)-12 have been demonstrated to have benefits for microbiological and clinical outcomes in
cryptococcal meningitis (178,182,214,288). Exogenous administration of IFN-γ was proposed as an adjunctive therapy in 2004 and was shown to improve the rate of fungal clearance in 2012, although no effect on mortality was detected (183,204). Dexamethasone reduces the concentration of pro-inflammatory cytokines in some patients (289,290), which could be a risk of this therapy. However, the role of cytokines beyond baseline, ie. once patients are receiving effective anti-fungal therapy, has not been established. Furthermore, in 2005, Simmons et al detected no measurable effect of dexamethasone on cytokine profiles in TB meningitis (291). Our understanding of the cerebral immune response in serious brain infections remains incomplete, and it is not clear how patients will respond to immune-modulating therapies.

Having considered the potential benefits and risks of adjunctive corticosteroid therapy for adults with HIV-associated cryptococcal meningitis, in high-burden settings where outcomes remain appalling, we conducted a double-blind randomised placebo controlled trial of dexamethasone. Our main goal was to determine whether adjunctive dexamethasone could reduce 10-week mortality in these patients.

6.3 Study Aims

6.3.1 Primary Aim

To determine the effect of dexamethasone adjunctive therapy on survival until 10-weeks, in adult patients with HIV-associated cryptococcal meningitis.

6.3.2 Secondary Aims

To determine the effect of adjuvant dexamethasone on:

- Survival until six months.

- Disability at ten weeks and six months.

- The prevalence of visual deficits at 10 weeks.
- Early fungicidal activity (EFA).

- Intracranial pressure

- The frequency of adverse events.

- The incidence of IRIS, other AIDS-defining infections, recurrence of cryptococcal meningitis, and new neurological events.

6.4 Methods

6.4.1 Study design and participants

The basic outline of the trial design is depicted in Figure 6-1.

![Figure 6-1 Study outline for 2016 CryptoDex trial](image)

We recruited adults (≥18 years) in 13 hospitals in Indonesia, Laos, Thailand, Vietnam, Malawi and Uganda. Eligible patients had HIV, a syndrome consistent with CM, and one or more of: positive cerebrospinal fluid (CSF) India ink stain, positive CSF or blood culture for Cryptococcus species, or positive CSF cryptococcal antigen (IMMY CrAg LFA, Immuno-Mycologics, USA). We excluded patients who were pregnant, had renal failure, had gastrointestinal bleeding, had received more than 7 days of anti-cryptococcal antifungal therapy, were already taking corticosteroids, or required corticosteroid therapy for co-existing
conditions. Informed consent was obtained from all patients, or if incapacitated, their representative. The full inclusion / exclusion criteria are presented in Table 6-1.

Study staff only approached patients for consent if there were no obvious conflicts with the inclusion / exclusion criteria on pre-screening, such as age below 18 or pregnancy. In the absence of such conflicts, a clinical member of the study staff would approach the patient, explain the study, and provide time for reflection before obtaining consent, which was required before any study specific screening tests were performed.

### Inclusion Criteria
- Age ≥18 years
- HIV antibody positive
- Cryptococcal meningitis defined as a syndrome consistent with CM and one or more of:
  - positive CSF India ink (budding encapsulated yeasts)
  - *C. neoformans* cultured from CSF or blood
  - positive cryptococcal antigen Lateral Flow Antigen Test (LFA) in CSF
- Informed consent to participate given by patient or acceptable representative

### Exclusion Criteria
- Pregnancy
- Active gastrointestinal bleeding vomiting blood or melaena stool in the previous week
- Currently receiving treatment for CM and having received ≥1 week of anti-CM therapy
- Known allergy to dexamethasone
- Current corticosteroid use defined as:
  - currently receiving the equivalent of prednisolone 40 mg/day or more
  - currently receiving corticosteroid therapy (any dose) for more than 3 weeks (except topical corticosteroids, which are permitted)
- Concurrent condition for which corticosteroids are indicated because of proven benefit (such as severe *Pneumocystis* pneumonia (pO2 < 70 mm Hg) or tuberculous meningitis)
- Renal failure (defined as creatinine >3 × ULN, despite adequate hydration)

Table 6-1 Inclusion and exclusion criteria for the 2016 CryptoDex trial

Study specific screening tests included a directed clinical history and examination, pregnancy testing, serum creatinine, and confirmatory HIV testing (unless a properly documented result was already available). In most cases, we performed lumbar puncture as
part of screening, although this could be avoided for patients who had had a lumbar puncture within the preceding 48 hrs and who did not require another one for clinical care.

### 6.4.2 Randomization, concealment, and blinding

We randomized patients (1:1), stratified by site based on variable block sizes of lengths 4 and 6. This stratified randomization was to reduce any impact of differing patient populations or healthcare settings. The computer-generated randomization list was accessible only to the central study pharmacists in Vietnam who used it to prepare blinded, sealed treatment packs containing dexamethasone or identical placebos, and distributed them to the sites. We used site specific enrolment logs to assign patients to the next available sequential patient number and corresponding treatment pack. It was agreed prior to the study that the randomization list would only be accessed if study staff needed a specific patient’s treatment to be unblinded, or once the trial was completed.

### 6.4.3 Laboratory investigations

Figure 6-2 shows the laboratory investigation and clinical assessment schedule. Study staff performed lumbar punctures on study days 1, 3, 7 and 14 and more frequently if clinically indicated; we determined quantitative fungal counts and opening CSF pressures every time a lumbar puncture was performed. All results were recorded in a laboratory CRF.

### 6.4.4 Radiology

We arranged chest x-ray at baseline, unless one had already been performed during the current admission. The findings of the chest x-ray were recorded on a specific CRF. Because several sites did not have brain imaging by CT or MRI available, they were not mandated – whenever they were performed, though, we collected the results on a specific CRF.
6.4.5 Treatment

We gave patients either dexamethasone or identical placebo (FKQ, Qui Nhon, Vietnam) for six weeks. For the first two weeks, we gave intravenous therapy at 0.3mg/kg/day for week 1, and 0.2mg/kg/day for week 2. Thereafter, we switched to tapering oral therapy at 0.1mg/kg/day for week 3, 3mg/day for week 4, 2mg/day for week 5, 1mg/day for week 6, then nothing. This is illustrated for a 70kg individual in Figure 6-3. We provided guidance on tapering the dose in case patients had to discontinue study drug for any reason.
Figure 6-2 Lab sampling and clinical assessment schedule for 2016 CryptoDex trial

<table>
<thead>
<tr>
<th>Lab schedule</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 11</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
<th>Day 42</th>
<th>Day 70</th>
<th>Day 182</th>
</tr>
</thead>
<tbody>
<tr>
<td>Take informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Assessment**</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBC (Hb, WCC, plt) 1mL</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na, K, Urea, creat, glu 2 mL</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 / CD8 count 2mL</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV antibody 2mL</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood cultures 5mL</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF Opening pressure</td>
<td>X</td>
<td>X</td>
<td></td>
<td>If indicated</td>
<td>If indicated</td>
<td>If indicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral Flow Antigen on CSF</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF Gram stain, India Ink 0.5mL</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF cell count, protein, glucose 1mL</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF TB smear 6mL***</td>
<td>X</td>
<td></td>
<td></td>
<td>If indicated</td>
<td>If indicated</td>
<td>If indicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF Yeast Quant Count 1mL</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store C. neoformans isolate****</td>
<td>X</td>
<td></td>
<td></td>
<td>If indicated</td>
<td>If indicated</td>
<td>If indicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store CSF supernatant and pellet</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum TB smear*****</td>
<td>X</td>
<td></td>
<td></td>
<td>If indicated</td>
<td>If indicated</td>
<td>If indicated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest X-ray***</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store blood plasma 4.5mL</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store blood cell pellet</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Approximate blood volume mL</td>
<td>16.5</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Approximate CSF volume mL</td>
<td>8.5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
</tr>
</tbody>
</table>

** Glasgow coma score (GCS) Assessment is daily while an in-patient. When outpatient assessment can take place at the scheduled time + up to 5 days (eg 4 week assessment on day 28-33). Day 182 assessment may be by telephone.

***Optional if local resources are unavailable

****Also store any isolate where the quantitative culture assessment is higher than the previous assessment or relapse case

*****Perform sputum smear if patient can produce a sample

NB: Blood volumes are estimates
Anti-fungal induction therapy consisted of amphotericin B deoxycholate 1mg/kg/day (Bharat Pharmaceuticals, India) and fluconazole 800mg/day (Ranbaxy, India) for two weeks, followed by consolidation (fluconazole 800mg/day for 8 weeks) then maintenance (fluconazole 200mg/day), as recommended in international guidelines(186).

Although medical management was left at the discretion of the treating physician, the protocol did initially recommended antiretroviral therapy (ART) to begin 2 to 4 weeks after starting antifungal treatment. After the publication of the COAT trial (210), we updated this advice and recommended that ART should not be commenced before 5 weeks. All patients received daily co-trimoxazole prophylaxis.

### 6.4.6 Assessment of Primary Endpoint

We collected data on survival until 10 weeks after randomisation.
6.4.7 Assessment of Secondary Endpoints

We collected data on survival until six months after randomisation, to make certain that any early benefit of corticosteroid therapy is not subsequently lost through, for example, an increased incidence of other infections or recurrence of cryptococcal meningitis.

We used the modified Rankin score and the Two Simple Questions to measure disability outcomes. These measures have been well-validated in describing outcomes for stroke patients, and show little inter-observer variability (292,293). Thwaites et al also used this approach in their 2004 TB meningitis study – our decision to use the same scoring system was to make direct comparison of outcomes possible. Both the modified Rankin score and the Two Simple Questions look at the post-recovery degree of dependence, which is a clinically meaningful measure. The Two Simple Questions are: ‘do you require help from anybody for everyday activities? E.g. washing, eating, drinking, going to the toilet’ and, if the answer is no, ‘Has your illness left you with any other problems?’. The modified Rankin scale categorises patients according to the following criteria: 0 = no symptoms; 1 = some symptoms, but no significant disability; 2 = Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities; 3 = Moderate disability. Requires some help, but can walk unassisted; 4 = Moderate severe disability. Unable to attend to own bodily needs without assistance, and unable to walk unassisted; 5 = Severe disability. Requires constant nursing care and attention. Bedridden and incontinent. We divided both outcome measures in ‘good’, ‘intermediate’, and ‘severe’ disability, based on their worst scores, at 10 weeks and 6 months, according to Table 6-2.
Table 6-2 Disability outcome categorised according to Modified Rankin and Two Simple Question tools

Visual function was assessed using a simple six point scale, to ensure that assessments could be carried out uniformly across variably equipped sites. The scoring system is shown in Table 6-3, and results were recorded for right eye, left eye and both eyes.

<table>
<thead>
<tr>
<th>Level of Visual Function</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
</tr>
<tr>
<td>Blurred</td>
<td>2</td>
</tr>
<tr>
<td>Finger counting</td>
<td>3</td>
</tr>
<tr>
<td>Movement perception</td>
<td>4</td>
</tr>
<tr>
<td>Light perception</td>
<td>5</td>
</tr>
<tr>
<td>No Light perception</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 6-3 Visual assessment: level of function and score

Early fungicidal activity was measured over the first two weeks of therapy by counting the number of cryptococcal colony forming units per ml of cerebrospinal fluid (CSF) every time a lumbar puncture was undertaken, as described by Brouwer et al in a 2004 clinical trial (191). This metric is an indicator of effectiveness of anti-fungal therapy, although a recent meta-analysis did not demonstrate any role for it as a surrogate marker for clinical outcome (294).
Similar to the assessment of EFA, every time a lumbar puncture was performed we recorded the opening and closing pressure of CSF in cm of CSF, using manometers.

We collected information on the following adverse events until week 10: any grade 3 or 4 adverse events, all episodes of IRIS, new AIDS-defining illnesses, recurrence of cryptococcal meningitis, or new neurological events. A grade 3 adverse event is one requiring / prolonging hospital admission, or limiting ability for self-care; a grade 4 event is one with immediate life-threatening consequences. We categorized all clinical adverse events according to the Medical Dictionary for Regulatory Activities (MedDRA) system. Only laboratory adverse events meeting these clinical criteria were reported. Paradoxical IRIS was defined in accordance with the 2010 proposal by Haddow et al (295). In brief, this case-definition requires that the patient is taking ART, had a confirmed case of cryptococcal meningitis, and initially responded to anti-fungal therapy – a positive case will show signs of clinical deterioration within 12 months of starting, or changing, ART and will have no evidence of recurrent cryptococcal meningitis or other causes for their symptoms. AIDS-defining illnesses were diagnosed according to CDC classifications (296). A new neurological event was diagnosed if there was a fall in Glasgow coma score (GCS) by ≥2 points for ≥2 days from the highest previous GCS or if any of the following occurred: cerebellar symptoms, coma, hemiplegia, paraplegia, seizures, cerebral herniation, new onset blindness or deafness, or cranial nerve palsy.

6.5 Statistical Methods

6.5.1 Sample size

The trial was powered to detect a hazard ratio of 0.7 in favor of dexamethasone for the primary endpoint of overall survival until 10 weeks with 80% power at the two-sided 5% significance level. Assuming an overall 10-week mortality of at least 30%, this led to a target sample size of 880 subjects. We based our estimate of the effect size on the data from a trial of dexamethasone for TB meningitis, which observed a hazard ratio of 0.69 (297). Because a
major goal of the trial was to produce robust, generalisable and clinically relevant evidence, we planned to recruit roughly equal numbers of patients at Asian and African sites.

### 6.5.2 General

All analyses were defined prior to unblinding and detailed in the published protocol(298) and statistical analysis plan. Statistical analyses were performed with R version 3.1.2(244). We summarized baseline characteristics using median and inter-quartile ranges (IQR) for continuous, or number (n) and percentage (%) for categorical, data.

### 6.5.3 Analysis Populations

The main analysis populations were the intention-to-treat population (ITT) and the per-protocol population. We included all randomised patients in the ITT population, except for any mistakenly enrolled or any who did not receive their allocated treatment because of administration errors. Patients who received no doses of the study treatment, for any reason, were also excluded from the ITT. The per-protocol population contains all patients above, except those who had major protocol violations or received less than one week of study drug, for any reason other than death.

We defined several subgroup of interest *a priori* based on our clinical experience and our understanding of the cryptococcal meningitis literature. The subgroups are presented in Table 6-4.
<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continent</td>
<td>Africa / Asia</td>
</tr>
<tr>
<td>Country</td>
<td>Indonesia / Laos / Thailand / Vietnam / Malawi / Uganda</td>
</tr>
<tr>
<td>Presence of IDSA indications for corticosteroid treatment at baseline</td>
<td>Yes / No</td>
</tr>
<tr>
<td>- Cryptococcoma with mass effect</td>
<td>Yes / No</td>
</tr>
<tr>
<td>- Acute respiratory distress syndrome</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Unmasking IRIS at baseline</td>
<td>Yes / No</td>
</tr>
<tr>
<td>Glasgow coma score at baseline &lt;15</td>
<td>Yes / No</td>
</tr>
<tr>
<td>On ARV at baseline</td>
<td>no / ≤ 3 months / &gt; 3 months</td>
</tr>
<tr>
<td>Gender</td>
<td>Male / Female</td>
</tr>
<tr>
<td>Quantitative fungal count at baseline</td>
<td>&lt;10^5 cells/ml / ≥10^5 cells/ml</td>
</tr>
<tr>
<td>Opening pressure at baseline &gt; 18cmCSF</td>
<td>Yes / No</td>
</tr>
<tr>
<td>CSF white cell count at baseline &lt;5</td>
<td>Yes / No</td>
</tr>
</tbody>
</table>

Table 6-4 Predefined subgroups for analysis in the 2016 CryptoDex trial

### 6.5.4 Primary endpoint analysis

Survival was analyzed with a Cox proportional hazards model with stratification by continent in the intention-to-treat population and pre-defined subgroups. A major benefit of using a Cox model over log-rank testing to compare the treatment arms is that the Cox model, as implemented in R, automatically provides estimates of the effect size, confidence intervals and p-values. Non-informative censoring and proportionality of hazards are the key assumptions for using Cox regression models. This study was designed with an emphasis on complete and careful follow-up to satisfy the first, and we formally tested the second assumption based on scaled Schoenfeld residuals. We used the Kaplan-Meier method to estimate survival curves by treatment arm, both overall and by continent, with numeric estimates of survival at 10 weeks and 6 months. In the event of non-proportional hazards being identified, we planned to formally compare 10-week (and 6-month) survival probabilities between the two groups based on Kaplan-Meier estimation and Greenwood’s formula to approximate variance.

### 6.5.5 Secondary endpoint analysis

Survival until six months was analysed in the same way as the primary analysis.
We compared the probability of a ‘good’ disability outcome at 10 weeks and 6 months between the two arms with a logistic regression model adjusted for continent (in addition to treatment arm).

For visual deficit at 10 weeks we analysed the odds of having ‘normal acuity’ at 10 weeks between the arms, acknowledging that there is a potential for bias in that assessments could only be performed on survivors.

Using a linear mixed effects model, we compared early fungicidal activity (ie. longitudinal log_{10}-quantitative fungal counts) between the arms, and by continent, treating negative fungal cultures as left-censored.

The statistical analysis of longitudinal intracranial pressure was the same as the analysis of early fungicidal activity, except that there is no lower limit of detection for intracranial pressure.

Our approach to analyzing adverse events was to summarise their frequency by treatment arm, but also by continent and timing. We took a similar approach for incidence of IRIS, other AIDS-defining infections, recurrence of cryptococcal meningitis, and new neurological events, but including a stratified Cox regression time to event analysis.

### 6.6 Ethics

The study protocol was approved by institutional review boards and regulatory authorities for each site, and the Oxford University Tropical Research Ethics Committee. An independent Data Monitoring and Ethics Committee oversaw trial safety, analyzing unblinded data after every 50 deaths, according to their charter. A trial steering committee consisting of 3 independent members, 2 study investigators and an observer advised on the running of the trial. The trial was registered at www.controlled-trials.com (ISRCTN59144167). The funding
bodies and drug manufacturers played no part in study design, implementation, analysis, or decision to publish the results.

6.7 Results

Recruitment began in February 2013. As per their charter, the Data Monitoring and Ethics Committee (DMEC) reviewed unblinded results after approximately every 50 deaths. Their third analysis began on the 15th August 2014, including 172 deaths out of 411 subjects enrolled. On the 29th August 2014, we received a letter from the DMEC, recommending that the trial be stopped. The full details and implications of this recommendation are explored in Chapter 7 but, in summary, it was not based on crossing a pre-defined stopping boundary with respect to the primary endpoint of ten-week mortality; rather, it was based on the clinical judgment that dexamethasone was causing harm across key endpoints including fungal clearance, adverse events and disability outcomes. We suspended recruitment the same day and convened a meeting of the trial steering committee on the 2nd September 2014. At this meeting, it was agreed that the trial should be stopped, and all patients currently receiving study drug (i.e. patients in the first six weeks of enrolment) should undergo a rapid treatment taper, although it was agreed that unblinding was unnecessary. Investigators at all sites were informed of this plan, and it was implemented immediately. The trial was formally stopped on 12th September 2014, and all participants completed 6 months of follow-up as planned.

By the time of suspension we had screened 823 patients and enrolled 451; 227 randomized to placebo and 224 to dexamethasone. We excluded one patient in the placebo arm from the intention-to-treat analysis who never received the allocated intervention due to a drug administration error. We excluded 24 patients from the per-protocol analysis. Forty-two patients failed screening because they had already received over 24 hours of corticosteroid therapy, 41 of these patients were in Asia. The full details of recruitment, drug and analysis population allocations are depicted in the Consort flowchart in Figure 6-4.
Figure 6-4 Consort flowchart for the 2016 CryptoDex trial. *One participant may have multiple reasons; ** Less than 1 week of amphotericin B antifungal therapy after randomization for reasons other than death; *** Less than 1 week of study drug after randomization for reasons other than death

6.7.1 Baseline characteristics

Baseline characteristics were well balanced between treatment arms as is shown in Table 6-5. There were clear differences between Asian and African patients, including prevalence of drug use (18% vs 0%, P<0.001), cranial nerve palsies (19% vs 6%, P<0.001), visual impairment (21% vs 12%, P=0.02), CSF fungal load (median 4.80 vs 3.83 log_{10} colony forming units
(CFU)/ml, P<0.001), and CD4 count (median 16 vs 26 cells/mm³, P=0.04) respectively. Asian participants were more likely to be ART-naïve at baseline than African participants (77% vs 45%, P<0.001), as is further depicted in Figure 6-5.

Figure 6-5 Anti-retroviral therapy (ART) usage at 2016 CryptoDex study entry, by continent
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (N=226)</th>
<th>Dexamethasone (N=224)</th>
<th>Africa (N=246)</th>
<th>Asia (N=204)</th>
<th>Comparison between continents (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summary statistic</td>
<td>Summary statistic</td>
<td>Summary statistic</td>
<td>Summary statistic</td>
<td></td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td>124/226 (55%)</td>
<td>122/224 (54%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>132/226 (58%)</td>
<td>147/224 (66%)</td>
<td>144/246 (59%)</td>
<td>135/204 (66%)</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>History of IV drug use</strong></td>
<td>35 (30,40)</td>
<td>35 (31,41)</td>
<td>35 (30,41)</td>
<td>35 (31,40)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>History of IV drug use</strong></td>
<td>18/215 (8%)</td>
<td>17/215 (8%)</td>
<td>0/234 (0%)</td>
<td>35/196 (18%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Current ART status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- Not on ART</td>
<td>133/226 (59%)</td>
<td>135/224 (60%)</td>
<td>111/246 (45%)</td>
<td>157/204 (77%)</td>
<td></td>
</tr>
<tr>
<td>- On ART &lt;=3 months</td>
<td>46/226 (20%)</td>
<td>41/224 (18%)</td>
<td>54/246 (22%)</td>
<td>33/204 (16%)</td>
<td></td>
</tr>
<tr>
<td>- On ART &gt;3 months</td>
<td>47/226 (21%)</td>
<td>48/224 (21%)</td>
<td>81/246 (33%)</td>
<td>14/204 (7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Illness duration - days</strong></td>
<td></td>
<td></td>
<td>14 (7,30) [n=241]</td>
<td>13 (7,21) [n=204]</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Headache</strong></td>
<td></td>
<td></td>
<td>212/226 (94%)</td>
<td>217/224 (97%)</td>
<td></td>
</tr>
<tr>
<td><strong>Fever</strong></td>
<td></td>
<td></td>
<td>134/223 (60%)</td>
<td>147/222 (66%)</td>
<td></td>
</tr>
<tr>
<td><strong>History of neck stiffness</strong></td>
<td></td>
<td></td>
<td>103/219 (47%)</td>
<td>106/222 (48%)</td>
<td></td>
</tr>
<tr>
<td><strong>History of fits</strong></td>
<td></td>
<td></td>
<td>43/225 (19%)</td>
<td>35/223 (16%)</td>
<td></td>
</tr>
<tr>
<td><strong>Glasgow Coma Score</strong></td>
<td></td>
<td></td>
<td>9/226 (4%)</td>
<td>5/223 (2%)</td>
<td>0.21</td>
</tr>
<tr>
<td>- 10 or lower</td>
<td></td>
<td></td>
<td>10/246 (4%)</td>
<td>4/203 (2%)</td>
<td></td>
</tr>
<tr>
<td>- 11 to 14</td>
<td></td>
<td></td>
<td>44/246 (18%)</td>
<td>28/203 (14%)</td>
<td></td>
</tr>
<tr>
<td>- 15</td>
<td></td>
<td></td>
<td>192/226 (84%)</td>
<td>187/223 (84%)</td>
<td></td>
</tr>
<tr>
<td><strong>Cranial nerve palsies</strong></td>
<td></td>
<td></td>
<td>11/215 (5%)</td>
<td>10/221 (5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>- CN 6</td>
<td></td>
<td></td>
<td>19/215 (9%)</td>
<td>12/221 (5%)</td>
<td></td>
</tr>
<tr>
<td>- Other CN</td>
<td></td>
<td></td>
<td>185/215 (86%)</td>
<td>199/221 (90%)</td>
<td></td>
</tr>
<tr>
<td><strong>Abnormal visual acuity</strong></td>
<td></td>
<td></td>
<td>32/205 (16%)</td>
<td>34/208 (16%)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Papilloedema</strong></td>
<td></td>
<td></td>
<td>23/195 (12%)</td>
<td>26/195 (13%)</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Opening pressure (cmCSF)</strong></td>
<td>24 (16,35) [n=203]</td>
<td>22(15,32) [n=200]</td>
<td>21 (16,31) [n=213]</td>
<td>25 (15,35) [n=190]</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Opening pressure &gt;18cm CSF</strong></td>
<td>135/203 (67%)</td>
<td>129/200 (64%)</td>
<td>139/213 (65%)</td>
<td>125/190 (66%)</td>
<td>0.92</td>
</tr>
</tbody>
</table>
Table 6-5 Baseline patient characteristics by treatment arm and continent for 2016 CryptoDex trial. Continuous data variables are presented as median (interquartile range). There were no significant between-treatment group differences at baseline (all P>0.1) according to Fisher’s exact test (categorical data) or the Wilcoxon rank-sum test (continuous data). P-values for between-continent differences are listed.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median (IQR) [n]</th>
<th>Median (IQR) [n]</th>
<th>Median (IQR) [n]</th>
<th>Median (IQR) [n]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF white cell count (cells/µL)</td>
<td>19(5,55) [212]</td>
<td>20(5,60) [213]</td>
<td>15(5,40) [224]</td>
<td>30(8,92) [201]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF glucose (mmol/L)</td>
<td>2.34(1.57,2.93)</td>
<td>2.27(1.54,2.87)</td>
<td>2.43(1.75,2.96)</td>
<td>2.11(1.39,2.82)</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood glucose (mmol/L)</td>
<td>5.60(4.92,6.76)</td>
<td>5.70(4.99,6.60)</td>
<td>5.50(4.80,6.27)</td>
<td>6.05(5.09,7.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSF:blood glucose ratio</td>
<td>0.39(0.27,0.50)</td>
<td>0.37(0.24,0.48)</td>
<td>0.42(0.27,0.52)</td>
<td>0.34(0.24,0.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log_{10} CSF fungal count</td>
<td>4.37(2.56,5.55)</td>
<td>4.25(2.07,5.36)</td>
<td>3.83(1.60,5.04)</td>
<td>4.80(3.16,5.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4 (cells/µL)</td>
<td>20 (7,52) [214]</td>
<td>18(7,52) [212]</td>
<td>26 (7,71) [234]</td>
<td>16(8,40) [192]</td>
<td>0.04</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.73(0.58,0.92)</td>
<td>0.72(0.59,0.88)</td>
<td>0.70(0.57,0.92)</td>
<td>0.74(0.60,0.89)</td>
<td>0.41</td>
</tr>
</tbody>
</table>
6.7.2 Primary outcome – Mortality by 10 weeks

Key study outcomes, including the primary outcome, are summarized in Table 2. Kaplan-Meier survival curves for the whole study population, and by continent, are shown in Figure 6-6, Figure 6-7, and Figure 6-8. By week 10, 106 (47%) patients had died in the dexamethasone arm vs. 93 (41%) in the placebo arm. The intention-to-treat analysis showed no statistically significant differences in survival by 10 weeks (Hazard Ratio (HR) (95% confidence interval (CI)) 1.11 (0.84 to 1.47); P=0.45).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Placebo (n=226)</th>
<th>Dexamethasone (n=224)</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Outcome: Deaths by week 10 - events (risk)</td>
<td></td>
<td></td>
<td>Hazard ratio:</td>
</tr>
<tr>
<td>- Intention-to-treat population (ITT)</td>
<td>93/226 (41%)</td>
<td>106/224 (47%)</td>
<td>1.11 (0.84-1.47); p=0.45a</td>
</tr>
<tr>
<td>- Per protocol population</td>
<td>87/213 (41%)</td>
<td>103/213 (49%)</td>
<td>1.16 (0.87-1.54); p=0.31b</td>
</tr>
<tr>
<td>- African patients</td>
<td>51/124 (42%)</td>
<td>63/122 (52%)</td>
<td>1.26 (0.87-1.82); p=0.23c</td>
</tr>
<tr>
<td>- Asian patients</td>
<td>42/102 (41%)</td>
<td>43/102 (42%)</td>
<td>0.95 (0.62-1.45); p=0.80d</td>
</tr>
<tr>
<td>- Glasgow Coma Score 15</td>
<td>60/176 (34%)</td>
<td>82/187 (44%)</td>
<td>1.29(0.93-1.80); p=0.13</td>
</tr>
<tr>
<td>- Glasgow Coma Score &lt;15</td>
<td>33/50 (66%)</td>
<td>23/36 (64%)</td>
<td>0.86 (0.51-1.48); p=0.60</td>
</tr>
<tr>
<td>- ART naive at enrolment</td>
<td>57/133 (43%)</td>
<td>68/135 (50%)</td>
<td>1.15 (0.81-1.63); p=0.45</td>
</tr>
<tr>
<td>- On ART &lt;3months at enrolment</td>
<td>16/46 (35%)</td>
<td>21/41 (51%)</td>
<td>1.49 (0.77-2.87); p=0.23</td>
</tr>
<tr>
<td>- On ART &gt;3months at enrolment</td>
<td>20/47 (43%)</td>
<td>17/48 (36%)</td>
<td>0.77 (0.40-1.47); p=0.43</td>
</tr>
<tr>
<td>- Quantitative fungal count &gt;10^5 CFU/ml</td>
<td>42/81 (53%)</td>
<td>35/63 (56%)</td>
<td>0.99 (0.63-1.56); p=0.98</td>
</tr>
<tr>
<td>- Quantitative fungal count ≤10^5 CFU/ml</td>
<td>47/131 (36%)</td>
<td>63/141 (45%)</td>
<td>1.24(0.85-1.81); p=0.26</td>
</tr>
<tr>
<td>- CSF opening pressure &gt;18cm CSF</td>
<td>57/135 (43%)</td>
<td>64/129 (50%)</td>
<td>1.14 (0.80-1.63); p=0.47</td>
</tr>
<tr>
<td>- CSF white cell count &lt;5cells/µl</td>
<td>12/17 (71%)</td>
<td>11/25 (44%)</td>
<td>0.53 (0.23-1.21); p=0.13</td>
</tr>
<tr>
<td>Deaths by month 6 – events (risk)</td>
<td></td>
<td></td>
<td>Hazard ratio:</td>
</tr>
<tr>
<td>- ITT</td>
<td>109/226 (49%)</td>
<td>128/224 (57%)</td>
<td>1.18 (0.91-1.53); p=0.20a</td>
</tr>
<tr>
<td>- Per protocol population</td>
<td>103/213 (48%)</td>
<td>125/213 (59%)</td>
<td>1.23 (0.95-1.60); p=0.12b</td>
</tr>
<tr>
<td>- African patients</td>
<td>62/124 (51%)</td>
<td>75/122 (62%)</td>
<td>1.28 (0.91-1.79); p=0.16c</td>
</tr>
<tr>
<td>- Asian patients</td>
<td>47/102 (46%)</td>
<td>53/102 (52%)</td>
<td>1.06 (0.72-1.58); p=0.76d</td>
</tr>
<tr>
<td>Disability at 10 weeks - ITT (n=220a)</td>
<td></td>
<td></td>
<td>Odds ratios of status “good”:</td>
</tr>
<tr>
<td>- Good</td>
<td>55 (25%)</td>
<td>28 (13%)</td>
<td></td>
</tr>
<tr>
<td>- Intermediate</td>
<td>46 (21%)</td>
<td>53 (24%)</td>
<td>ITT: 0.42 (0.25-0.69); p&lt;0.001</td>
</tr>
<tr>
<td>- Severe disability</td>
<td>26 (12%)</td>
<td>35 (16%)</td>
<td>Africa: 0.43 (0.18-0.97); p=0.04</td>
</tr>
<tr>
<td>- Death</td>
<td>93 (42%)</td>
<td>106 (48%)</td>
<td>Asia: 0.41 (0.21-0.77); p=0.005</td>
</tr>
<tr>
<td>Disability at 6 month - ITT (n=223a)</td>
<td></td>
<td></td>
<td>Odds ratios of status “good”:</td>
</tr>
<tr>
<td>- Good</td>
<td>68 (30%)</td>
<td>40 (18%)</td>
<td></td>
</tr>
<tr>
<td>- Intermediate</td>
<td>34 (15%)</td>
<td>40 (18%)</td>
<td>ITT: 0.49 (0.31-0.77); p=0.002</td>
</tr>
<tr>
<td>- Severe disability</td>
<td>12 (5%)</td>
<td>15 (7%)</td>
<td>Africa: 0.63 (0.32-1.19); p=0.15</td>
</tr>
<tr>
<td>- Death</td>
<td>109 (49%)</td>
<td>128 (57%)</td>
<td>Asia: 0.40 (0.21-0.73); p=0.003</td>
</tr>
<tr>
<td>Visual acuity at 10 weeks – probability of a “normal” visual acuity – events (%)</td>
<td></td>
<td></td>
<td>Odds ratios of acuity “normal”:</td>
</tr>
<tr>
<td>- ITT</td>
<td>122/127 (96%)</td>
<td>94/107 (88%)</td>
<td>0.30 (0.09,0.84); p=0.02</td>
</tr>
</tbody>
</table>
- Normal acuity at baseline
  - 105/108 (97%) vs 84/89 (94%): 0.51 (0.10-2.20); p=0.37
- African patients
  - 68/69 (99%) vs 52/54 (96%): 0.38 (0.02-4.10); p=0.42
- Asian patients
  - 54/58 (93%) vs 42/53 (79%): 0.28 (0.07-0.89); p=0.03

**CSF fungal decline, first 14 days -**

<table>
<thead>
<tr>
<th>Group</th>
<th>Estimated change (95% CI) [log_{10} CFU/ml of CSF per day]</th>
<th>Difference in estimated change:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- ITT</td>
<td>-0.30 (-0.33,-0.27) -0.21 (-0.23,-0.18)</td>
<td>0.09 (0.06,0.13); p &lt;0.001</td>
</tr>
<tr>
<td>- African patients</td>
<td>-0.26 (-0.30,-0.22) -0.19 (-0.23,-0.16)</td>
<td>0.07 (0.02,0.12); p=0.005</td>
</tr>
<tr>
<td>- Asian patients</td>
<td>-0.35 (-0.40,-0.30) -0.22 (-0.26,-0.19)</td>
<td>0.12 (0.07,0.18); p &lt;0.001</td>
</tr>
</tbody>
</table>

**CSF opening pressure, first 14 days -**

<table>
<thead>
<tr>
<th>Group</th>
<th>Estimated absolute change (95% CI) [cm CSF over 14 days]</th>
<th>Difference in estimated change:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- ITT</td>
<td>-3.2 (-5.8,-0.5) -9.2 (-11.9,-6.5)</td>
<td>-6.1 (-9.4,-2.7); p &lt;0.001</td>
</tr>
<tr>
<td>- African patients</td>
<td>-5.5 (-9.0,-2.1) -10.7 (-14.3,-7.0)</td>
<td>-5.1 (-9.4,-0.8); p=0.02</td>
</tr>
<tr>
<td>- Asian patients</td>
<td>0.1 (-4.1,4.2) -7.7 (-11.7,-3.8)</td>
<td>-7.8 (-12.9,-2.6); p=0.003</td>
</tr>
</tbody>
</table>

Table 6-6 Key outcomes from 2016 CryptoDex trial by treatment arm. Risks were estimated with the Kaplan-Meier method. CI=confidence interval. a Test for proportional hazards: p<0.001 (week 10), p=0.001 (month 6), estimated absolute risk difference: 6.01% (95% CI -3.19% to 15.20%); p=0.20 (week 10), 8.68% (-0.54% to 17.90%); p=0.07 (month 6). b Test for proportional hazards: p<0.001 (week 10), p=0.001 (month 6), estimated absolute risk difference: 7.61% (-1.82% to 17.05%); p=0.11 (week 10), 10.50% (1.07% to 19.94%); p=0.03 (month 6). c Test for proportional hazards: p=0.03 (week 10), p=0.08 (month 6), estimated absolute risk difference: 10.23% (-2.25% to 22.71%); p=0.11 (week 10), 11.13% (-1.27% to 23.52%); p=0.08 (month 6). d Test for proportional hazards: p=0.01 (week 10), p=0.004 (month 6), estimated absolute risk difference: 0.98% (-12.55% to 14.51%); p=0.89 (week 10), 5.80% (-7.91% to 19.51%); p=0.41 (month 6). e n= number of participants with completed assessments.
Figure 6-6 Kaplan-Meier survival curve for all patients in 2016 CryptoDex trial, dexamethasone vs placebo

Figure 6-7 Kaplan-Meier survival curve for patients from African sites in 2016 CryptoDex trial, dexamethasone vs placebo
6.7.3 Exploratory analyses around the primary endpoint

Because tests for non-proportional hazards based on weighted Schoenfeld residuals provided clear evidence that the hazards were not proportional, we also formally compared 10-week (and 6-month) survival probabilities between the two groups. These results, presented in Table 6-7, did not show a statistically significant difference in mortality between the arms at 10 weeks.
Furthermore, given the suggestion that the effect of dexamethasone might change over time, we performed two exploratory analyses to demonstrate how mortality risk varied over time, and to determine hazard ratios at three discrete time-periods in the 10 weeks after randomization. Figure 6-9 shows how the observed hazards changed over time by continent, and Table 6.8 contains full results of the discrete time-period hazard ratio estimates: which gave, in brief, hazard ratios (HR) of 0.77 (95%CI 0.54 to 1.09; p=0.14) for days 1-22, 1.94 (0.97 to 3.88; p=0.06) for days 23-43 and 2.50 (1.23 to 5.05; p=0.01) for days 44-71. The results of these exploratory analyses are further examined in Figure 6-10 which visually combines the two analyses.

<table>
<thead>
<tr>
<th></th>
<th><strong>Placebo (n=226)</strong></th>
<th><strong>Dexamethasone (n=224)</strong></th>
<th><strong>Comparison</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10 Weeks</strong></td>
<td></td>
<td></td>
<td>Absolute risk difference (95%CI); p-value</td>
</tr>
<tr>
<td><strong>ITT</strong></td>
<td>93/226 (41%)</td>
<td>106/224 (47%)</td>
<td>6% (-3.19 to 15.20); p=0.20</td>
</tr>
<tr>
<td><strong>Per protocol</strong></td>
<td>87/213 (41%)</td>
<td>103/213 (49%)</td>
<td>8% (-1.82 to 17.05); p=0.11</td>
</tr>
<tr>
<td><strong>African patients</strong></td>
<td>51/124 (42%)</td>
<td>63/122 (52%)</td>
<td>10% (-2.25 to 22.71); p=0.11</td>
</tr>
<tr>
<td><strong>Asian patients</strong></td>
<td>42/102 (41%)</td>
<td>43/102 (42%)</td>
<td>1% (-12.55 to 14.51); p=0.89</td>
</tr>
<tr>
<td><strong>6 Months</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ITT</strong></td>
<td>109/226 (49%)</td>
<td>128/224 (57%)</td>
<td>9% (-0.54 to 17.90); p=0.07</td>
</tr>
<tr>
<td><strong>Per protocol</strong></td>
<td>103/213 (48%)</td>
<td>125/213 (59%)</td>
<td>11% (1.07 to 19.94); p=0.03</td>
</tr>
<tr>
<td><strong>African patients</strong></td>
<td>62/124 (51%)</td>
<td>75/122 (62%)</td>
<td>11% (-1.27 to 23.52); p=0.08</td>
</tr>
<tr>
<td><strong>Asian patients</strong></td>
<td>47/102 (46%)</td>
<td>53/102 (52%)</td>
<td>6% (-7.91 to 19.51); p=0.41</td>
</tr>
</tbody>
</table>

Table 6-7 Results of formal comparison of risk of mortality between dexamethasone and placebo arms of the 2016 CryptoDex trial at 10 weeks and 6 months after enrollment. Also shown, a measure of the absolute difference in risk.
Figure 6-9 Observed difference in the absolute risk of death between dexamethasone and placebo over time (black lines), estimates±/standard error (dark grey areas), and point-wise 95% confidence intervals (light grey areas).

<table>
<thead>
<tr>
<th>Time period</th>
<th>Placebo (n=226)</th>
<th>Dexamethasone (n=224)</th>
<th>Comparison</th>
<th>Test for proportional hazards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>events/n (%)</td>
<td>events/n (%)</td>
<td>HR (95%CI); p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Days 1-22</td>
<td>70/226 (31)</td>
<td>56/224 (25)</td>
<td>0.77 (0.54-1.09); p=0.14</td>
<td>0.35</td>
</tr>
<tr>
<td>Days 23-43</td>
<td>12/154 (8)</td>
<td>24/167 (14)</td>
<td>1.94 (0.97-3.88); p=0.06</td>
<td>0.14</td>
</tr>
<tr>
<td>Days 44-71</td>
<td>11/142 (8)</td>
<td>26/143 (18)</td>
<td>2.50 (1.23-5.05); p=0.01</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table 6-8 Hazard ratios for mortality during days 1-22, 23-43, and 44-71. This division splits the time axis into the first half of the study treatment period, the second half, and the time remaining up until 10 weeks.
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Figure 6-10 Visualisation of proportional hazard exploratory analyses, showing 10 week timeline with hazard ratios for days 1-22, 23-43, and 44-70, in the context of differences in mortality over time for the full 6 months of the 2016 CryptoDex trial

6.7.4 Mortality by 6 months

By 6 months, 128 (57%) patients had died in the dexamethasone arm versus 109 (49%) assigned to placebo. The pre-defined Cox regression analysis of time to death did not reach statistical significance (HR 1.18 (95%CI 0.91 to 1.53); p=0.20). However, a formal comparison of the risk of death at 6 months showed a trend towards harm in the dexamethasone arm, with an absolute risk increase of 9% (-1% to 18%; p=0.07) in the intention to treat population and of 11% (1% to 20%; p=0.03) in the per-protocol population (see Table 6-7).

6.7.5 Disability including visual acuity

Dexamethasone was associated with a significantly increased risk of death or disability at 10 weeks and 6 months; odds ratios (OR) for a ‘good’ outcome were 0.42 (95%CI 0.25 to 0.69); P<0.001 at 10 weeks and 0.49 (0.31 to 0.77); P=0.002 at 6 months. Results were consistent across continents as seen in Table 6-6, a forest plot of odds ratios for a ‘good’ disability outcome by continent at 10 weeks and 6 months in Figure 6-11. The predefined visual impairment analyses in survivors at 10 weeks (n=234), Table 6-6, indicated that ‘normal’ visual
acuity was less common in those receiving dexamethasone (88% vs. 96%; OR 0.30 (95%CI 0.09 to 0.84); p=0.02). The effect in African patients was not statistically significant (96% vs. 99%; OR 0.38 (0.02-4.10); p=0.42), but it was in Asian patients (79% vs. 93%; OR 0.28 (0.07-0.89); p=0.03). However, an exploratory analysis of the overall population, excluding those with baseline visual abnormalities, (n=197) showed no statistically significant difference (94% vs. 97%; OR 0.51 (0.10 to 2.20); p=0.37).

6.7.6 Early fungicidal activity

Dexamethasone was associated with significantly slower rates of decline of cryptococcal CFU in CSF over the first 2 weeks of treatment, and this is visually depicted in Figure 6-12. The rate of decline (log_{10} CFU/mL of CSF per day (95%CI)) in the dexamethasone arm was -0.21 (-0.24 to -0.19) vs -0.31 (-0.34 to -0.28) in the placebo arm, P<0.001 Table 6-6. Cases of relapse were rare and similarly frequent in both groups (5 in the dexamethasone arm, 7 in the placebo arm).
Figure 6-12 Early fungicidal activity graph showing log_{10}CFU/ml of CSF over first 15 days of the 2016 CryptoDex trial. Light grey lines are all measurements for individual patients and the bold lines are LOESS smoothers calculated with the use of local regression. Placebo is blue and dexamethasone is red. CSF fungal decline over the first 14 days was significantly slower in patients receiving dexamethasone than patients receiving placebo (estimated change (95% CI) in log_{10} CFU/mL of CSF per day -0.21 (-0.23, -0.18) vs -0.30 (-0.33 to -0.27) P<0.001). (CFU = colony-forming units).

6.7.7 CSF opening Pressure

Dexamethasone was associated with a larger reduction in CSF opening pressure over the first two weeks, as visually depicted in Figure 6-13. The estimated rate of change was -9.2 cmCSF (95%CI -11.9, -6.5) in the dexamethasone arm vs. -3.2 cmCSF (95%CI -5.8, -0.5) in the placebo arm (p<0.001) Table 6-6 over those two weeks. This effect was consistent across continents, although the magnitude of effect was larger in Asia than Africa. The starting point didn’t vary by continent, 21cmCSF (16, 31) in Asia vs. 25cmCSF (15, 35) in Africa, p=0.28. For Asian patients only, the rate of decline in the dexamethasone arm was -7.7cmCSF (-11.7, -3.8) vs. 0.1cmCSF (-4.1, 4.2) in the placebo arm (difference of -7.8cmCSF (-12.9, -2.6); p=0.003). The same results for African patients only were -10.7cmCSF (-14.3, -7.0) vs. -5.5cmCSF (-9.0, -2.1) (difference of -5.1cmCSF (-9.4, -0.8); p=0.02).
Figure 6-13 Graph of dexamethasone’s impact on intracranial pressure over the first 15 days of the 2016 CryptoDex trial. Light grey lines are all pressure measurements (in cmCSF) for individual patients and the bold lines are LOESS smoothers calculated with the use of local regression for placebo (blue) and dexamethasone (red). The rate of decline in opening pressure of CSF over the first 15 days was significantly greater in patients receiving dexamethasone than patients receiving placebo (-9.2 (-11.9,-6.5) vs. -3.2 (-5.8,-0.5); p <0.001).

6.7.8 Clinical adverse events

There were more clinical adverse events in the dexamethasone arm than the placebo arm: 667 vs 494 (P=0.01). The adverse events observed are documented in Table 6-9 where we summarized adverse events by MedDRA category.
### Clinical Adverse Events

<table>
<thead>
<tr>
<th>Event Category</th>
<th>Placebo (n=226)</th>
<th>Dexamethasone (n=224)</th>
<th>Comparison (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of adverse events</td>
<td>494</td>
<td>667</td>
<td>0.01</td>
</tr>
<tr>
<td>Number of patients with at least one event</td>
<td>191 (85%)</td>
<td>193 (86%)</td>
<td>0.69</td>
</tr>
<tr>
<td>New neurological event (NNE)</td>
<td>59 (26%)</td>
<td>61 (27%)</td>
<td>0.83</td>
</tr>
<tr>
<td>New AIDS defining illness (NADI)</td>
<td>87 (39%)</td>
<td>87 (39%)</td>
<td>1</td>
</tr>
<tr>
<td>Immune reconstitution inflammatory syndrome (IRIS)</td>
<td>6 (3%)</td>
<td>7 (3%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>85 (38%)</td>
<td>78 (35%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>83 (37%)</td>
<td>96 (43%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>25 (11%)</td>
<td>48 (21%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>16 (7%)</td>
<td>29 (13%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Renal and urinary disorders</td>
<td>7 (3%)</td>
<td>22 (10%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders</td>
<td>14 (6%)</td>
<td>9 (4%)</td>
<td>0.39</td>
</tr>
<tr>
<td>Hepatobiliary disorders</td>
<td>3 (1%)</td>
<td>10 (4%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Vascular disorders</td>
<td>4 (2%)</td>
<td>9 (4%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>3 (1%)</td>
<td>6 (3%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>0 (0%)</td>
<td>8 (4%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Endocrine disorders</td>
<td>3 (1%)</td>
<td>3 (1%)</td>
<td>1</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>1 (0.4%)</td>
<td>3 (1%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Immune system disorders</td>
<td>1 (0.4%)</td>
<td>1 (0.5%)</td>
<td>1</td>
</tr>
<tr>
<td>Injury, poisoning and procedural complications</td>
<td>1 (0.4%)</td>
<td>2 (0.9%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Reproductive system and breast disorders</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Pregnancy, puerperium and perinatal conditions</td>
<td>1 (0.4%)</td>
<td>0 (0%)</td>
<td>1</td>
</tr>
<tr>
<td>Systemic disorders</td>
<td>1 (0.4%)</td>
<td>0 (0%)</td>
<td>1</td>
</tr>
</tbody>
</table>

### Grade 3 and 4 Laboratory Adverse Events

<table>
<thead>
<tr>
<th>Event Category</th>
<th>Placebo (n=226)</th>
<th>Dexamethasone (n=224)</th>
<th>Comparison (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of adverse events</td>
<td>835</td>
<td>1023</td>
<td>0.02</td>
</tr>
<tr>
<td>Number of individuals with any event (% of n)</td>
<td>192 (85%)</td>
<td>202 (90%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Anaemia</td>
<td>112 (50%)</td>
<td>120 (54%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Leucocytopaenia</td>
<td>41 (18%)</td>
<td>36 (16%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Neutropaenia</td>
<td>59 (26%)</td>
<td>42 (19%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Thrombocytopaenia</td>
<td>25 (11%)</td>
<td>33 (15%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Elevated ALT</td>
<td>3 (1%)</td>
<td>10 (5%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Elevated AST</td>
<td>11 (5%)</td>
<td>14 (6%)</td>
<td>0.54</td>
</tr>
<tr>
<td>Hyperglycaemia</td>
<td>6 (3%)</td>
<td>32 (14%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypoglycaemia</td>
<td>6 (3%)</td>
<td>5 (2%)</td>
<td>1</td>
</tr>
<tr>
<td>Hypercreatinemia</td>
<td>50 (22%)</td>
<td>79 (35%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Hyperkalaemia</td>
<td>19 (8%)</td>
<td>52 (23%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypokalaemia</td>
<td>132 (58%)</td>
<td>108 (48%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Hypernatraemia</td>
<td>7 (3%)</td>
<td>2 (1%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Hyponatraemia</td>
<td>75 (33%)</td>
<td>114 (51%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6-9: Adverse events by treatment for the 2016 CryptoDex trial. Unless otherwise stated, figures refer to the number of patients with at least one adverse event of the respective type. All comparisons are based on Fisher’s exact test apart from the total number of adverse events for which the Wilcoxon rank sum test was used to compare the number of events per patient.
87 patients in each arm experienced new AIDS defining illnesses; however, the rate of the combined end-point of new AIDS defining illnesses or death by six months was higher in the dexamethasone arm (HR 1.26; 95%CI 1.00 to 1.58; p=0.05). Adverse events categorized as ‘infections/infestations’ occurred in 48 (21%) participants in the dexamethasone arm vs 25 (11%) participants in the placebo arm (p=0.003). There were more gastrointestinal, renal/urinary, and cardiac disorders in the dexamethasone arm (respectively, 29(13%) vs 16(7%) p=0.04; 22(10%) vs 7(3%), p=0.004 and 8(4%) vs 0(0%), p=0.004). Gastrointestinal bleeding was equally rare in both arms. There were 19 cases of acute renal failure in the dexamethasone arm (7 in the placebo arm). Fifteen (79%) of these occurred in the setting of an infectious episode. The rates of paradoxical IRIS at 10 weeks and 6 months were similar in both treatment arms Table. Median time to starting ART from study entry was 42 days in the placebo group and 46 days in the dexamethasone group.

6.7.9 Laboratory adverse events

There were 1023 grade 3 or 4 laboratory adverse events in the dexamethasone arm, compared to 835 in the placebo arm (p=0.02). Hypercreatininaemia, hyperkalaemia, hypokalaemia, hyponatraemia and hyperglycaemia all occurred significantly more frequently in patients receiving dexamethasone Table 6-9.

6.7.10 Sub group analyses

There were no differences in 10 week or 6 month mortality between treatment arms in any of the predefined sub-group analyses: continent, country, gender, baseline Glasgow Coma Score, ART status, age, fungal burden, CD4 count, baseline CSF opening pressure and CSF white cell count greater or <5 cells/µl (Table 6-10). The data for 10-week mortality outcome by subgroup is also shown in a forest plot of hazard ratios in Figure 6-14. No evidence of heterogeneity of effect was seen.
<table>
<thead>
<tr>
<th>*Subgroup</th>
<th>Placebo (n=226)</th>
<th>Dexamethasone (n=224)</th>
<th>Comparison Estimate (95% CI); p-value</th>
<th>**Test for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deaths by week 10</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT</td>
<td>93/226 (41%)</td>
<td>106/224 (47%)</td>
<td>1.11(0.84-1.47); p=0.45</td>
<td></td>
</tr>
<tr>
<td>Per Protocol</td>
<td>87/213 (41%)</td>
<td>103/213 (49%)</td>
<td>1.16(0.87-1.54); p=0.31</td>
<td></td>
</tr>
<tr>
<td><strong>Continent</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.32</td>
</tr>
<tr>
<td>Africa</td>
<td>51/124 (42%)</td>
<td>63/122 (52%)</td>
<td>1.26(0.87-1.82); p=0.23</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>42/102 (41%)</td>
<td>43/102 (42%)</td>
<td>0.95(0.62-1.45); p=0.80</td>
<td></td>
</tr>
<tr>
<td><strong>GCS</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>-15</td>
<td>60/176 (34%)</td>
<td>82/187 (44%)</td>
<td>1.29(0.93-1.80); p=0.13</td>
<td></td>
</tr>
<tr>
<td>- &lt;15</td>
<td>33/50 (66%)</td>
<td>23/36 (64%)</td>
<td>0.86(0.51-1.48); p=0.60</td>
<td></td>
</tr>
<tr>
<td><strong>ART status</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.35</td>
</tr>
<tr>
<td>- ART naïve</td>
<td>57/133 (43%)</td>
<td>68/135 (50%)</td>
<td>1.15(0.81-1.63); p=0.45</td>
<td></td>
</tr>
<tr>
<td>- On ART &lt;=3 months</td>
<td>16/46 (35%)</td>
<td>21/41 (51%)</td>
<td>1.49(0.77-2.87); p=0.23</td>
<td></td>
</tr>
<tr>
<td>- ART for &gt;3 months</td>
<td>20/47 (43%)</td>
<td>17/48 (36%)</td>
<td>0.77(0.40-1.47); p=0.43</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>- Female</td>
<td>39/94 (42%)</td>
<td>37/77 (48%)</td>
<td>1.15(0.73-1.80); p=0.55</td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>54/132 (41%)</td>
<td>69/147 (47%)</td>
<td>1.09(0.76-1.55); p=0.65</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>- &lt;=35 years</td>
<td>35/118 (30%)</td>
<td>48/117 (41%)</td>
<td>1.47(0.95-2.28); p=0.08</td>
<td></td>
</tr>
<tr>
<td>- &gt;35 years</td>
<td>58/108 (54%)</td>
<td>58/107 (55%)</td>
<td>0.89(0.62-1.28); p=0.54</td>
<td></td>
</tr>
<tr>
<td><strong>CSF quantitative fungal count</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.43</td>
</tr>
<tr>
<td>- &lt;10⁵ CFU/ml</td>
<td>47/131 (36%)</td>
<td>63/141 (45%)</td>
<td>1.24(0.85-1.81); p=0.26</td>
<td></td>
</tr>
<tr>
<td>- &gt;10⁵ CFU/ml</td>
<td>42/81 (53%)</td>
<td>35/63 (56%)</td>
<td>0.99(0.63-1.56); p=0.98</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline CD4 count</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>- &lt;=25 cells/µL</td>
<td>49/117 (43%)</td>
<td>62/122 (51%)</td>
<td>1.24(0.85-1.80); p=0.27</td>
<td></td>
</tr>
<tr>
<td>- &gt;25 cells/µL</td>
<td>39/97 (40%)</td>
<td>38/90 (42%)</td>
<td>0.96(0.61-1.50); p=0.86</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline opening pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>- &lt;=18 cmCSF</td>
<td>29/68 (43%)</td>
<td>32/71 (45%)</td>
<td>1.00(0.60-1.66); p=1.00</td>
<td></td>
</tr>
<tr>
<td>- &gt;18 cmCSF</td>
<td>57/135 (43%)</td>
<td>64/129 (50%)</td>
<td>1.14(0.80-1.63); p=0.47</td>
<td></td>
</tr>
<tr>
<td><strong>Baseline CF white cell count</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>- &lt;5 cells/µl</td>
<td>12/17 (71%)</td>
<td>11/25 (44%)</td>
<td>0.53(0.23-1.21); p=0.13</td>
<td></td>
</tr>
<tr>
<td>- &gt;=5 cells/µl</td>
<td>79/195 (41%)</td>
<td>90/188 (48%)</td>
<td>1.13(0.83-1.53); p=0.43</td>
<td></td>
</tr>
<tr>
<td><strong>Deaths by month 6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT</td>
<td>109/226 (49%)</td>
<td>128/224 (57%)</td>
<td>1.18(0.91-1.53); p=0.20</td>
<td></td>
</tr>
<tr>
<td>Per Protocol</td>
<td>103/213 (48%)</td>
<td>125/213 (59%)</td>
<td>1.23(0.95-1.60); p=0.12</td>
<td></td>
</tr>
<tr>
<td><strong>Continent</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Africa</td>
<td>62/124 (51%)</td>
<td>75/122 (62%)</td>
<td>1.28(0.91-1.79); p=0.16</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>47/102 (46%)</td>
<td>53/102 (52%)</td>
<td>1.06(0.72-1.58); p=0.76</td>
<td></td>
</tr>
<tr>
<td><strong>GCS</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
</tr>
<tr>
<td>-15</td>
<td>73/176 (42%)</td>
<td>101/187 (54%)</td>
<td>1.36(1.00-1.83); p=0.05</td>
<td></td>
</tr>
<tr>
<td>- &lt;15</td>
<td>36/50 (72%)</td>
<td>26/36 (72%)</td>
<td>0.88(0.53-1.46); p=0.62</td>
<td></td>
</tr>
<tr>
<td><strong>ART status</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>- ART naïve</td>
<td>65/133 (49%)</td>
<td>83/135 (61%)</td>
<td>1.27(0.92-1.76); p=0.15</td>
<td></td>
</tr>
</tbody>
</table>
On ART \(\leq 3\) months 21/46 (47%) 25/41 (61%) 1.41(0.79-2.53); \(p=0.24\)
- ART for >3 months 23/47 (50%) 20/48 (42%) 0.79(0.43-1.44); \(p=0.44\)

**Gender** 0.97
- Female 47/94 (51%) 45/77 (59%) 1.18(0.79-1.78); \(p=0.42\)
- Male 62/132 (47%) 83/147 (56%) 1.18(0.85-1.64); \(p=0.33\)

**Age** 0.13
- \(\leq 35\) years 46/118 (40%) 61/117 (52%) 1.48(1.01-2.18); \(p=0.04\)
- >35 years 63/108 (58%) 67/107 (63%) 0.97(0.69-1.37); \(p=0.85\)

**CSF quantitative fungal count** 0.42
- <10^5 CFU/ml 57/131 (44%) 76/141 (54%) 1.28(0.91-1.81); \(p=0.15\)
- >10^5 CFU/ml 46/81 (58%) 39/63 (62%) 1.02(0.67-1.57); \(p=0.91\)

**Baseline CD4 count** 0.80
- \(\leq 25\) cells/µL 61/117 (53%) 74/122 (61%) 1.23(0.87-1.73); \(p=0.24\)
- >25 cells/µL 40/97 (41%) 45/90 (50%) 1.12(0.73-1.72); \(p=0.60\)

**Baseline opening pressure** 0.68
- \(\leq 18\) cmCSF 35/68 (51%) 41/71 (58%) 1.10(0.70-1.74); \(p=0.68\)
- >18 cmCSF 63/135 (47%) 75/129 (58%) 1.23(0.88-1.72); \(p=0.22\)

**Baseline CSF white cell count** 0.07
- <5 cells/µL 12/17 (71%) 12/25 (48%) 0.57(0.25-1.28); \(p=0.17\)
- \(\geq 5\) cells/µL 92/195 (47%) 111/188 (59%) 1.24(0.94-1.64); \(p=0.12\)

Table 6-10 Summary of all pre-specified subgroup analyses for mortality by 10 weeks and 6 months.

*At study entry. In addition to probable unmasking IRIS, subgroup analyses by other IDSA indications for corticosteroid treatment at baseline were also pre-defined. However, numbers were too low (no patients with cryptococcoma with mass effect and only three patients with acute respiratory distress syndrome) to actually perform the respective subgroup analyses.

** Heterogeneity was assessed with likelihood ratio tests for an interaction between treatment assignment and the grouping variable.
Figure 6-14 Hazard ratios and 95% confidence intervals for 10 week mortality by subgroup. Anything to the right of the dotted red line indicates a worse outcome for patients receiving dexamethasone.

6.8 Discussion

We set out to test whether adjunctive treatment with dexamethasone administered at the point of diagnosis is beneficial in HIV-associated CM. We found compelling evidence that at this dose and duration it is harmful, with significantly increased disability and excess severe adverse events including infectious episodes, renal, gastrointestinal and cardiac disorders. The study was stopped early because of consistent evidence of harm across several end-points. Consequently, we lacked power to demonstrate an effect of dexamethasone on death by 10 weeks - the primary endpoint. However, consistent with the evidence of harm, the hazard ratios for survival at 10 weeks and 6 months did not favor dexamethasone, and a formal comparison of risks of death at 6 months was suggestive of harm (p=0.07), reaching statistical significance in the per protocol analysis (p=0.03). Therefore, it is highly unlikely that dexamethasone benefits survival – continuing the trial would not have altered our findings, and would have exposed participants to unacceptable harm. The consistency of findings across Asian and African populations and all predefined subgroups strengthens this conclusion.
Although the overall findings show that dexamethasone is harmful, there were some intriguing signals, with tests for proportional hazards suggesting the effect of dexamethasone may be time-dependent. Our exploratory analyses suggest the effect of dexamethasone may be in the direction of benefit over the first 3 weeks of treatment – possibly reflecting pressure modulation. Although purely speculative, it is possible that a shorter duration of dexamethasone might have resulted in a different overall outcome. It is feasible that any short-term benefit of corticosteroid therapy was negated by side-effects of longer-term usage, including infections, especially in this already immune deplete group.

We hypothesized that dexamethasone would improve outcome through reducing intracranial pressure and inflammatory complications, and decreasing the incidence of IRIS. CSF opening pressure did decline more rapidly in patients receiving dexamethasone, but this didn’t translate into a survival benefit, even for patients with raised pressures at baseline. Unfortunately, we are no closer to understanding the best way to manage raised intracranial pressure in cryptococcal meningitis - although the administration of dexamethasone at the doses and durations used here can be added to acetazolamide as a harmful intervention.

IRIS is a difficult management problem in CM. Current guidelines suggest corticosteroids may be beneficial (186,299). Almost 20% of patients had begun ART in the 3 months prior to study entry, and therefore may have had unmasking IRIS - *a priori* occult infection ‘revealed’ and worsened by ART-induced immune reconstitution (295). Even in this subgroup, we found no suggestion of benefit. Paradoxical IRIS occurred in only 13 patients so we lacked power to detect any effect of dexamethasone on this outcome and cannot comment on the value of dexamethasone for this indication. The number of paradoxical IRIS cases is lower than we expected – a previous prospective study of CM patients identified 13 cases in a cohort of 101 with 6 months follow-up (299). Though unlikely, it is possible that the condition was under-diagnosed. However, predefined sub-group analyses for factors previously associated with
increased risk of paradoxical IRIS (low CD4 count, low CSF cellularity, and high CSF fungal burden(299,300)) failed to identify a beneficial effect of dexamethasone.

It is not clear why dexamethasone was harmful. We chose a dosing schedule routinely used for TB meningitis in Vietnam, in similarly immune-suppressed HIV patients, which actually reduces the incidence of adverse events suffered by patients being treated for TB meningitis (3,209,275,301). Some possible explanations for the poor outcomes in our study may be found in the early fungal clearance results, and the incidence of infectious adverse events. Dexamethasone was associated with slower rates of decline of Cryptococcus counts in CSF, which may be associated with worse clinical outcomes(302).

Higher levels of pro-inflammatory cytokines, such as IFN-γ, at baseline have been associated with faster CSF cryptococcal clearance and improved survival (183,184). At least in healthy individuals, it is known that dexamethasone causes a profound reduction in IFN-γ (180) – it is possible that dexamethasone reduced pro-inflammatory cytokines in our patients and affected their ability to clear infection. Contrary to that reasoning, however, the TB meningitis trial showed no impact of dexamethasone on cytokine concentrations (291). Clearly the impact of corticosteroids on cytokine responses in the CSF, and downstream effects on clinical and mycological outcomes, are incompletely understood. I will address this issue in Chapter 8 of my thesis.

The increased risk of other acute infections in the dexamethasone arm may have contributed to the harm observed. Seventy-nine percent of cases of acute renal failure in this arm were associated with severe infections and are likely a consequence of sepsis rather than dexamethasone, for which renal failure is not an established side-effect.

We tested an adjunctive immune-modulating treatment because of a lack of novel antifungal agents - the poor performance of those currently available was confirmed here.
Mortality at 10 weeks for participants of this trial in the placebo arm were over 40%, despite patients receiving optimal clinical care, and guidelines directed antifungal therapy. With no effective adjunctive therapy yet proven, improving access to the most effective antifungal treatments, including flucytosine, must remain a global priority (3,11,197,303).

There were interesting differences between the patient populations in Asia and Africa. The fact that the majority of patients presenting with cryptococcal meningitis in Asia were naive of their HIV diagnosis suggests that HIV case-identification measures could be improved. On the other hand, one third of patients presenting in Africa had been established on ART for more than 3 months, suggesting the possibility of ART treatment failure. Further epidemiological work to properly describe the problems of failure-to-treat and treatment failure is indicated.

Outcomes also appear to vary by continent. These differences were not a focus of the study, and results are descriptive rather than hypothesis driven. Mostly, the differences were in terms of degree – for example the negative influence of dexamethasone on survival, disability, adverse events, and fungal clearance was more pronounced in African patients. This is also visually apparent from the Kaplan-Meier curves. African participants also experienced slighter reductions in intracranial pressure than Asian participants. However, interestingly, dexamethasone’s negative influence on visual acuity was more pronounced in Asian participants – the probability of a ‘good’ visual acuity outcome for African patients only fell from 99% to 96% when they received corticosteroids, for Asia the fall was from 93% to 79% (an odds ratio of 0.28 (0.07, 0.89); p=0.03). The reasons for these differences remain unclear, but potential differences in inflammatory phenotype and genotype will be examined in Chapter 8.

This pragmatic trial set out to answer a question important to doctors working where CM is most prevalent: does treatment with adjuvant corticosteroids, started at the point of
diagnosis, improve survival in HIV-associated cryptococcal meningitis? The answer is clearly no.

However, while we have shown that a universal approach to dexamethasone prescription is harmful, there may still be a role for corticosteroids. Current guidelines recommend their use where patients have cryptococcomas with mass effect, acute respiratory distress syndrome, or IRIS. These events were infrequent in our study. Therefore, we lacked power to test these particular indications; generating high quality evidence to test these indications will be exceptionally difficult. Using corticosteroids in a different dosing schedule may have lead to different outcomes for this patient population – a clinical trial of short course corticosteroids may be justified. Finally, there may be a role for dexamethasone in patients without HIV.

The results described here went against our hypothesis, but they are still of great value to clinicians working in high CM-burden settings. Anecdotally, corticosteroids are commonly prescribed for CM cases, especially in Asia. Of note, 42 (11%) of all patients who failed study screening failed because they had already been prescribed corticosteroids for their CNS disease. Here we have shown that such use is not justified.
6.9 Statement of contribution

I joined the trial team after the trial protocol had been written and had received its initial ethical approvals. I was involved in all site initiation visits. I delivered protocol training and SOP development workshops at all African sites, and in Indonesia. I was responsible for preparing protocol amendments for review by ethical committees prior to the trial commencing. I also turned the protocol into a manuscript which was published in Trials journal.

Once the trial was underway, I was responsible for its day to day running. I traveled around all sites to provide ongoing support and training, and to share best practice from other sites. I observed recruitment rates, and produced a monthly newsletter to encourage ongoing recruitment. I was also responsible for co-ordinating monitoring visits to all sites, and acted as one of the monitors during site visits to African sites.

I prepared all documents and data required for the DSMB. I co-authored the statistical analysis plan for interim analyses. When the trial was discontinued early, I produced a media strategy, including responses to the types of questions likely to be raised.

I co-authored the final statistical analysis plan. The actual analyses were run by a professional statistician. I wrote the first draft of the manuscript, collated feedback, and submitted the final document to NEJM. I was first author on the final paper.
7. Stopping Trials Early: a Review of the Literature and a Case Report on the CryptoDex Trial
7.1 Background

Ethical frameworks are vitally important in the conduct of clinical research, from conception to completion and especially where decisions about stopping research early must be made. In their seminal 2000 paper, Emmanuel et al reviewed the existing body of statements and declarations for the ethical conduct of clinical trials. They published a list of seven essential criteria (304), by which the ethical conduct of a trial can be assessed. In a 2004 follow-up paper, they added another criterion, specifically for research in developing countries: collaborative partnerships (305). These criteria, presented in Table 7-1, have been adopted by the WHO (http://www.who.int/ethics/Ethics_basic_concepts_ENG.pdf) and the NIH (https://clinicalcenter.nih.gov/recruit/ethics.html).
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Explanation</th>
<th>Ethical justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social or scientific value</td>
<td>Evaluation of a treatment, intervention, or theory that will improve health and well-being or increase knowledge</td>
<td>Scarce resources; non-exploitation</td>
</tr>
<tr>
<td>Scientific validity</td>
<td>Use of accepted scientific principles and methods, including statistical techniques, to produce reliable and valid data</td>
<td>Scarce resources; non-exploitation</td>
</tr>
<tr>
<td>Fair subject selection</td>
<td>Selection of subjects so that stigmatized and vulnerable individuals are not targeted for risky research and the rich and socially powerful not favored for potentially beneficial research</td>
<td>Justice</td>
</tr>
<tr>
<td>Favorable risk-benefit</td>
<td>Minimization of risks; enhancement of potential benefits; risks to the subject are proportionate to the benefits to the subject and society</td>
<td>Nonmaleficence; beneficence; non-exploitation</td>
</tr>
<tr>
<td>Independent review</td>
<td>Review of the design of the research trial, its proposed subject population, and risk-benefit ratio by individuals unaffiliated with the research</td>
<td>Public accountability; minimizing influence of potential conflicts of interest</td>
</tr>
<tr>
<td>Informed consent</td>
<td>Provision of information to subjects about purpose of the research, its procedures, potential risks, benefits, and alternatives, so that the individual understands this information and can make a voluntary decision whether to enroll and continue to participate</td>
<td>Respect for autonomy</td>
</tr>
<tr>
<td>Respect for potential and enrolled subjects</td>
<td>Respect for subjects by (1) permitting withdrawal from the research; (2) protecting privacy through confidentiality; (3) informing subjects of newly discovered risks or benefits; (4) informing subjects of results of clinical research; (5) maintaining welfare of subjects</td>
<td>Respect for subject autonomy; welfare</td>
</tr>
</tbody>
</table>

Table 7-1 Seven criteria for evaluating the ethical conduct of clinical research from Emmanuel et al JAMA 283(20) May 2000

Several of the criteria proposed by Emanuel et al are clearly pertinent to decisions about stopping trials early, and can be used as a framework to assess such decisions. Sponsors and investigators may wish to stop clinical trials early for a variety of logistical, scientific and ethical reasons. Several reasons are considered justifiable (306). The most obvious of these is where overwhelming evidence to answer the hypothesis is accrued earlier than expected – either in terms of benefit or harm from the intervention (306). However, it may also be
justifiable to stop a trial when it becomes apparent that answering the hypothesis will be impossible.

The robustness of decisions to stop trials early is likely to be enhanced, and bias mitigated, by the presence of an independent data safety and monitoring board (DSMB). In their 2006 book - Data Monitoring in Clinical Trials - DeMets, Furberg, and Friedman argue that independent DSMBs are primarily there to ensure trial participants are not unduly harmed, but also to enhance the quality and integrity of clinical trials (306). Their role has also been defined as balancing the interests of individual trial participants with those of society as a whole, during the conduct of clinical trials (307).

### 7.1.1 Stopping rules

#### 7.1.1.1 Stopping early for benefit

The different reasons for stopping require different decision-making approaches. When stopping for benefit, because overwhelming evidence to answer the hypothesis has already been accrued, the statistical basis must be robust enough for the findings to be accepted by the wider medical and research communities. The most common statistical approaches to stopping early for benefit after an interim analysis are those of Pocock (308), O’Brien and Fleming (309), (including the Lan-DeMets modification) and Haybittle-Peto (306). All are designed to correct for the multiple testing inevitable with interim analyses, and to provide a statistical basis for stopping early. The Pocock approach uses one fixed, reduced, p-value at each analysis – including the final analysis. Because the resultant p-value can be difficult to interpret and report, it is often overlooked in favour of the other two. However, it has the benefit of providing the most relaxed stopping boundary early in the trial. The approach of O’Brien and Fleming results in p-values close to 0.05 for determining statistical significance at the end of the trial. The stopping boundary changes with each interim analysis. Early in the trial, the level is very stringent, but this progressively relaxes as the trial approaches
completion. A major drawback of both the Pocock and the O’Brien and Fleming approaches is that the number of interim analyses must be determined in advance of the trial, reducing the flexibility of the DSMB. Lan and De Mets designed a modified version of the O’Brien and Fleming approach, which does allow flexibility in interim analyses (310). The Haybittle-Peto boundary, however, is the most flexible with regards to the number of interim analyses, and is especially easy to use and report. It requires an interim p-value of <0.001 to consider stopping for efficacy, and remains constant throughout the trial. The final 0.05 p-value for determining statistical significance remains unchanged. The main argument against the Haybittle-Peto boundary is that it is excessively conservative (306,311), especially early in the trial. The interim and final p-values for determining statistical significance using these three approaches are shown in Table 7-2, taken from Schultz et al Lancet 2005 (311).

<table>
<thead>
<tr>
<th>Number of Analyses</th>
<th>Pocock</th>
<th>Haybittle-Peto</th>
<th>O’Brien Fleming</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.029</td>
<td>0.001</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>0.029</td>
<td>0.05</td>
<td>0.048</td>
</tr>
<tr>
<td>1</td>
<td>0.022</td>
<td>0.001</td>
<td>0.0005</td>
</tr>
<tr>
<td>2</td>
<td>0.022</td>
<td>0.001</td>
<td>0.014</td>
</tr>
<tr>
<td>3</td>
<td>0.022</td>
<td>0.005</td>
<td>0.045</td>
</tr>
<tr>
<td>1</td>
<td>0.018</td>
<td>0.001</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>0.018</td>
<td>0.001</td>
<td>0.004</td>
</tr>
<tr>
<td>3</td>
<td>0.018</td>
<td>0.001</td>
<td>0.019</td>
</tr>
<tr>
<td>4</td>
<td>0.018</td>
<td>0.05</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Table 7-2 P-values to guide stopping for efficacy according to the number of planned interim analyses, using Pocock, Haybittle-Peto, and O’Brien Fleming corrections for multiple testing, from Schultz et al Lancet 2005.

However, all approaches risk conflict with the ethical principles outlined above. Mueller et al argued that stopping trials early, for apparent benefit, risked violating scientific validity, scientific value, informed consent, and respect for enrolled subject criteria (312). Their main concern was that trials stopped early for benefit often grossly overstate the efficacy of the intervention. This problem has been demonstrated in several meta-analyses, most recently in a 2010 meta-regression of 91 trials stopped early compared to 424 completed trials which showed truncated trials systematically over-estimated the effect size by approximately one third. The phenomenon was even greater in trials with fewer than 500 participants (313).
Importantly, the authors found that the presence of a DSMB with clear stopping rules was insufficient to ameliorate this problem. The exaggerated effect-size bias in truncated trials, combined with the intractable bias in favour of reporting positive results, leads to a biased evidence base for clinical decision-makers and denies the community the benefit of accurate scientific data.

On the other hand, the argument for stopping early for benefit is that it is unethical to continue randomizing patients to the alternative once equipoise has been lost, in line with the favorable risk-benefit criterion. This needs to be balanced carefully with the issues described above.

Another significant complication to the decision to stop early for a primary outcome benefit is the loss of secondary outcome and safety data. DeMets’ “Data Monitoring in Clinical Trials – A Case Studies Approach” (306) provides the example of the CURE study published in the *Eur Heart J*, 2000. This study of clopidogrel in secondary prevention for unstable angina had cardiovascular death, myocardial infarction, stroke, and time to first episode of refractory angina as co-primary outcomes, and bleeding complications as secondary outcomes. There was a trend to benefit in the primary outcome at the first interim analysis, and by the second analysis it met all stopping criteria. However, the DSMB was concerned about an emerging trend towards harm in the secondary outcome and decided to continue the trial to completion. By the time of completion, the trend towards harm had reversed. Had the trial stopped at the second analysis, the uptake of clopidogrel by clinicians may have been substantially less, and society would have suffered from a violation of the social or scientific value and scientific validity criteria.

A similar situation arose as data on intensive control of blood glucose in critically ill patients emerged between 2001 and 2008. The first trial was stopped early, in accordance with its predefined non-stringent stopping boundary of p<0.01 (314). It showed intensive
glycaemic control reduced mortality and these findings rapidly informed international treatment guidelines. Indeed, although subsequent trials and meta-analyses have shown that there is no overall benefit with the risks of hypoglycemia out-weighing any benefits, some guidelines still recommend intensive glycaemic control (315). This example demonstrates how stopping trials early can have enduring repercussions for the scientific and social value criterion.

As described here, decisions to stop early for benefit are complicated. Although having an independent DSMB may not completely prevent bias, it is probably the best way to ensure all ethical criteria are appropriately balanced, in line with the independent review ethical criterion.

7.1.1.2 Stopping early for harm

It is generally not necessary or desirable to provide conclusive evidence of harm from an intervention. Therefore, stopping rules are often asymmetric, with a more relaxed stopping boundary for harm and an acceptance that trends towards harm are sufficient reason to stop (307,311). In illustration, an asymmetric boundary incorporating the Haybittle-Peto approach would use p<0.001 as a stopping guideline with respect to benefit, and p<0.01 for harm. This was the approach we used in the CryptoDex trial. Other approaches described are where the Haybittle-Peto boundary is used for benefit, and the more lenient Pocock stopping rule is used for harm (306,311).

The COAT trial in 2014 is an example of a trial stopped early for harm (210). The hypothesis in this trial was that early anti-retroviral therapy would reduce mortality at 26 weeks. The researchers enrolled patients who had been treated for cryptococcal meningitis for 1 week and compared the survival effect of anti-retroviral therapy within 48 hours, to therapy after 4 weeks. Although full details of the DSMB’s deliberations have not been published, the original paper contains some details of the interim analysis process. The
independent DSMB used a modified version of the O’Brien and Fleming approach, although the boundaries are not stated and it is not clear whether they were asymmetric. Regardless, the trial was stopped after the second interim analysis when higher mortality was detected in the early anti-retroviral therapy group. At this point they had recruited 177 of an anticipated sample of 500. The hazard ratio for death by 26 weeks was 1.73 (95% CI 1.06 to 2.82; p=0.03). This p-value is relatively lenient for an early interim analysis, and may indicate an asymmetric stopping boundary. The COAT trial authors acknowledge the risk of an exaggerated effect-size bias in small trials stopped early, and note that stopping the trial for safety reasons made subgroup analyses impossible. The fact that the DMSB was independent aligns well with the independent review criterion. However, without a detailed case-study it is not possible to speculate further on the DSMB process, or to assess how conduct aligned with the social or scientific value or scientific validity criteria.

Rarely, it is desirable to generate conclusive evidence of harm with regards to a primary end-point. The MERIT-HF trial tested the effect of metoprolol on death and hospitalization in patients with congestive heart failure (316). The DSMB charter for this trial stated that if a trend towards harm emerged, the trial should continue until sufficient data for a statistically robust conclusion had accrued. The rationale for this was that metoprolol is frequently used for patients in the population with other co-morbidities, for reasons other than mortality prevention. Therefore, being able to distinguish neutral and harmful mortality effects would have major implications for clinicians (306). In this example, all of the ethical criteria were well-considered. The most socially sensitive of the ethical criteria is the favorable risk-benefit balance. However, it is clear from the case study that the DSMB carefully ensured that the possible risks to the participants were proportionate to the likely benefits to participants, and the benefits for society at large.
7.1.1.3 Stopping early for futility

Stopping for futility may be justified. Examples of this include situations where recruitment is too slow, where event rates differ greatly from those anticipated, or where even completing the trial would fail to produce clinically meaningful results (306). Conditional power is the method most commonly used to make decisions about futility (311). This method assesses the probability that a benefit of an intervention will eventually be detected, given the data accumulated to date. Most trials begin with a power in excess of 80% for the target hazard ratio. If that power falls to a low level for a range of reasonable assumed treatment effects (including the target hazard ratio), there is little reason to continue the trial because the treatment is unlikely to show benefit. An arbitrary level often adopted to define futility is if the probability of showing a meaningful benefit falls to 15-20% (306).

7.1.2 Importance of accurate reporting

It is vitally important that the reasons for stopping a trial early are clearly articulated, so that the rest of the research community can determine if the reasons were justified. Even partial results may prevent further fruitless research or generate new hypotheses, which I would consider to be a net benefit to the communities of both researchers and trial participants.

“The registration of all interventional trials is a scientific, ethical and moral responsibility,” as stated by the WHO on its clinical trial search portal, http://www.who.int/ictrp/en/ . In 2004, the International Committee of Medical Journal Editors (ICMJE) released a statement regarding the reporting of clinical trials (317). The statement, released simultaneously across the journals they represent, stated:
The focus of their statement was trial registration, which they described as a means of achieving the goal of having full transparency in the performance and reporting of clinical trials (317). As of 2004, only clinical trials reported on a publically accessible, free-to-access registry can be published in any of the journals they represent. Any such registry must include at least the following details about every registered trial:

1. Unique registration number
2. The intervention and comparison
3. The hypothesis
4. Primary and secondary end-points
5. Registration date
6. Actual or anticipated date of commencement
7. Actual or anticipated date of final follow-up
8. Actual or anticipated date of closure for data collection
9. Actual or anticipated date of trial completion
10. Target number of participants
11. Funding source
12. Contact details for the principle investigator

It takes a long time for the results of many trials to get published – a 2013 analysis of trials completed in 2009 found a median time to publication of 21 months, with an interquartile range of 13 to 32 months. Larger trials, and those published in high impact journals, were reported sooner (median 18 and 17 months respectively, both p<0.001). Furthermore, despite

“Altruism and trust lie at the heart of research on human subjects. Altruistic individuals volunteer for research because they trust that their participation will contribute to improved health for others and that researchers will minimize risks to participants.”
compulsory registration, under-reporting of clinical trials in both adult (318) and paediatric (319) populations continues. Together, these threaten the integrity of the clinical trial evidence base (320). Twenty to thirty percent of all clinical trials remain unreported four years after completion (319,320) – a situation which has been described as evidence of the “medical research community […] failing its moral pact with research participants, patients, and the public” (320).

7.2 Study Aims

In this chapter I aimed to understand early-stopping of trials in infectious diseases. I also aimed to establish the relationship between early cessation and final publication. I hypothesized that the prevalence of under-reporting would be even higher in clinical trials stopped early, than in trials running to completion. Focusing on trials in infectious meningitis, I set out to describe the rates of early cessation, and non-publication of results.

Furthermore, I aimed to appreciate the decision-making process, reporting mechanisms, and issues faced by the DSMB which recommending stopping CryptoDex early. By doing so, I aimed to identify lessons which could be learned for future clinical trials.

7.3 Methods

7.3.1 Reporting of meningitis clinical trials

My intention was to identify a sufficient but manageable number of trials related to my primary area of focus – cryptococcal meningitis. I estimated that extracting, coding, and recording data for each record identified would take ten minutes. This would equate to approximately 8 hours of data extraction work for every 50 records identified; I decided to target 500 relevant records due to practical considerations.
I first investigated the main clinical trial databases to see which would be most comprehensive and user friendly. I ran pilot searches on the following three databases using the search term “cryptococcal meningitis”, without a time limit or any other filters:

a. ClinicalTrials.gov, “a registry and results database of publicly and privately supported clinical studies of human participants conducted around the world”, which is administered by the US National Institute of Health. 
https://clinicaltrials.gov/

b. The European Union Clinical Trials Register, which allows one “to search for protocol and results information on: interventional clinical trials that are conducted in the European Union (EU) and the European Economic Area (EEA) and clinical trials conducted outside the EU / EEA that are linked to European paediatric-medicine development”, and is administered by the European Medicines Authority. 
https://www.clinicaltrialsregister.eu/ctr-search/search

c. The International Clinical Trials Registry Platform Search Portal, which “provides access to a central database containing the trial registration data sets provided by the registries listed [below]. It also provides links to the full original records”, and is administer by the WHO.
http://apps.who.int/trialsearch/Default.aspx

Using the search platform thus identified, I proceeded to optimize my search strategy by running the following searches:

1. “infectious diseases” AND “intervention” with a five year time limit, but no other filters

2. “cryptococcc*” without a time limit or other filters

3. “AIDS” AND “meningitis” without a time limit or other filters
4. “meningitis” without a time limit or other filters

5. “meningitis” without a time limit but filtered for phase 2 and 3

Having settled on an appropriate search strategy, I extracted every trial’s start date, end date, target and actual number of recruits, and status from the registry. I excluded trials not related to infectious disease. Next, I searched for published results for all remaining trials within the trial registry, the PubMed database https://www.ncbi.nlm.nih.gov/pubmed, or via Google scholar http://scholar.google.com.vn/. I downloaded all papers, conference abstracts, and posters thus identified and reviewed the text to identify the size of the trial, whether the trial had a DSMB, whether the trial was stopped early, and if so what reasons were given for stopping early. I recorded all details in an Excel spreadsheet. I highlighted the completeness of data, according to the WHO’s list of essential data - Unique Identifier, Start Date, Target Sample, Actual Sample, and Trial Status.

To investigate what affected reporting of trials, I considered the trial unreported if results had not been published within 2 years of the trial stopping. Relevant trials were identified in three stages. First, I included all trials with an end date more than two years prior to data extraction. Next, I reviewed the trials with no stated end date. I included those with a start date more than four years prior to data extraction, and a current status ‘complete’ or ‘not recruiting’. Finally, I excluded trials for which there was no evidence they had ever started.

To address my main hypothesis, that stopping early was associated with failure to publish results, I calculated the proportion of trials reporting their results within two years overall, and amongst those stopped early. I compared proportions with Fisher’s exact test. I performed logistic regressions to see if stopping early was associated with failure to publish results. I also used the logistic regression model to ask whether the presence of a DSMB affected reporting of results. Finally, I looked at whether the presence of a DSMB or the size of
the trial affected the likelihood of the trial stopping early. I defined a trial as ‘small’ by a conventional cut-off of fewer than 100 participants (321).

### 7.3.2 CryptoDex case study

I reviewed the data provided to the DMEC at each interim analysis, and their reports. All statistical methods employed by the DMEC in producing their reports were as described in the statistical methods for the CryptoDex trial (chapter 6.5). I illustrated the decision making process of DMECs with regards to stopping trials based on our experience with the CryptoDex trial, and identified lessons learned.

### 7.4 Results

#### 7.4.1 Reporting of meningitis clinical trials

I performed the pilot searches to select the search platform on the 23rd January 2017. Searching ClinicalTrials.gov with “cryptococcal meningitis” generated 42 records. The portal reported the use of ‘*Cryptococcus neoformans* meningitis’ and ‘Meningitis due to *Cryptococcus*’ as synonyms. The search function was user-friendly, and the list of trials returned was subjectively comprehensive. However, the platform did not allow results to be exported. Retrieving the full record from their database required following one link.

The same search on the European Union Clinical Trials Register was user-friendly, and quick, and returned 40 records. This registry does not automatically search on synonyms. This registry also lacked a facility for exporting results, and retrieving the full record required a separate search.

Repeating the search on The International Clinical Trials Registry Platform (ICTRP) Search Portal returned 48 records, corresponding to 47 trials (there are more records than trials because some trials are registered on multiple registries). This portal provides details of trials registered with all of the organizations listed in Table 7-3, and is updated at least every four
weeks. The search function was user friendly. The portal automatically searches on synonyms, results can be exported, and retrieving the full record required following two links.

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**Clinical trial registries contributing to the WHO’s International Clinical Trials Registry Platform**

- Australian New Zealand Clinical Trials Registry
- Chinese Clinical Trial Registry
- ClinicalTrials.gov
- EU Clinical Trials Register (EU-CTR)
- ISRCTN
- The Netherlands National Trial Register
- Brazilian Clinical Trials Registry (ReBec)
- Clinical Trials Registry – India
- Clinical Research Information Service - Republic of Korea
- Cuban Public Registry of Clinical Trials
- German Clinical Trials Register
- Iranian Registry of Clinical Trials
- Japan Primary Registries Network
- Pan African Clinical Trial Registry
- Sri Lanka Clinical Trials Registry
- Thai Clinical Trials Register (TCTR)
- Peruvian Clinical Trials Registry (REPEC)

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Table 7-3 List of trial registries contributing data to the World Health Organisation (WHO) International Clinical Trial Registry Platform as of January 2017

A subjective review of the results returned from each platform indicated that there was a great deal of overlap in the results returned. Given the better performance of the ICTRP platform in terms of the number of records returned, and the facility to export results, I used this platform for all subsequent searches.

I searched the ICTRP platform as described in the methods, on the 25th January 2017. Searching “infectious diseases“ AND “intervention” with a five year time limit but no other filters, returned 1105 records for 1041 trials. Searching “cryptococc*” without a time limit or other filters returned 62 records for 61 trials. Searching “AIDS” AND “meningitis” without a time limit or other filters returned 49 records for 47 trials. Searching “meningitis” with a filter for phase 2 and 3, but without a time limit, returned 182 records for 145 trials. Searching “meningitis” without a time limit of any filters returned 527 records for 417 trials. This
approximated the target of 500 clinical trials, and so the data was exported. On initial review and cleaning of this database, I identified and excluded 42 entries which were unrelated to infectious meningitis, leaving 375 trials for further review.

Basic details about the trials identified are presented in Table 7-4. Of the 375 trials identified, 226 were randomized controlled trials, 148 were not randomised, and 1 could not be classified. Of the RCTs, 38 of 226 stated they had a DSMB (17%) compared with 1 of the 148 non-RCTs (<1%) (p<0.001 by Fisher’s exact test).

<table>
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<th>Terminated</th>
<th>Withdrawn prior</th>
<th>Unknown</th>
<th>Total</th>
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<td>1</td>
<td>351</td>
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<table>
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<th>Terminated</th>
<th>Withdrawn prior</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>133,635</td>
<td>357,183</td>
<td>150</td>
<td>1002</td>
<td>NA</td>
<td>200</td>
<td>492,070</td>
</tr>
<tr>
<td>Median (IQR) participants*</td>
<td>300 (106,805)</td>
<td>330 (137,773)</td>
<td>150 (150,150)</td>
<td>50 (28,198)</td>
<td>NA</td>
<td>200 (200,200)</td>
<td>---</td>
</tr>
</tbody>
</table>

Table 7-4 Number of meningitis clinical trials, and participants in those trials, as extracted from ICTRP database in January 2017. Results presented by trial status at the time of data extraction. *p-value = 0.116, by Wilcoxon rank-sum test

Data regarding completeness of data are presented in Table 7-5.
A total of 228 trials had a recorded end date prior to 25\textsuperscript{th} January 2015. Of the trials with no end-date recorded but which had commenced before 25\textsuperscript{th} January 2013, 33 had a status ‘complete’ and 31 were ‘not recruiting’, giving a new total of 291. I excluded 35 trials for which there was no evidence of ever having started recruitment (no start date, no active recruitment phase, and no result), leaving a total of 257 trials for the stopping-early analyses. Of these 29 (11.3\%) had stopped early, 167 (65\%) had run to completion, and it was not possible to tell for 61 (23.7\%).

The overall proportion of trials that had published their results after at least 2 years from completion was 177/257 (68.9\%). For trials stopped early, 22/29 (75.9\%) had reported their results vs 152/167 (91\%) of trials that ran to completion (p=0.65, by Fisher’s exact test). For trials where early-stopping could not be determined, only 3/61 (4.9\%) had reported their results.

The odds ratio for publication of results if a trial stopped early was 0.39 (95\% confidence interval (CI) 0.12 to 1.54). The odds ratios for publication by the presence of a DSMB or a small trial were 5.48 (95\%CI 1.01 to 102.67) and 0.95 (95\%CI 0.33 to 2.59) respectively (presented graphically in Figure 7-1). The odds ratio for stopping early if a DSMB was present was 3.07.
(95% CI 1.21 to 7.51), and for small trials the odds ratio was 0.57 (95% CI 0.24 to 1.34) (presented graphically in Figure 7-2).

Figure 7-1 Odds ratios for trials stopped early, trials with a DSMB, or small trials publishing their results within 2 years. Analysis by logistic regression of data extracted from ICTRP on 25th January 2017 on meningitis clinical trials completed by 25th January 2015.

Figure 7-2 Odds ratios for trials with a DSMB or small trials stopping early. Analysis by logistic regression of data extracted from ICTRP on 25th January 2017 on meningitis clinical trials completed by 25th January 2015.

7.5 CryptoDex Case Study

The CryptoDex trial was formally introduced in chapter 1. Here I will review only the parts of the trial’s published protocol (298) relevant to the early stopping of trials – ie. those sections dealing with the Data Monitoring and Ethics Committee (DMEC, referred to elsewhere as Data Safety Monitoring Board, DSMB).
An independent DMEC oversaw the trial, in accordance with a formal charter. They were responsible for formal interim analyses of results six monthly, or after every 50 deaths, whichever came first. At the outset, we expected to observe around 247 deaths during the course of the study. Thus, we expected four to five formal interim analyses. We reported all unexpected serious adverse events to the DMEC as they occurred, within a maximum of 10 days.

For interim analyses, the trial statistician sent the DMEC blinded reports of mortality, serious adverse events, grade 3 and 4 adverse events, and estimates of the rate of CSF sterilisation during the first 14 days. The trial pharmacist provided the randomization list which allowed the DMEC to unblind these reports, without risk of biasing the study team. The DMEC used these data to make recommendations on the continuation, cessation or amendment of the study. Furthermore, the DMEC charter stated that they could vary the frequency of their reviews however they saw fit.

Stopping the trial for efficacy of dexamethasone was foreseen only if the benefit of adjuvant treatment with dexamethasone was shown “beyond reasonable doubt.” The DMEC used the Haybittle-Peto boundary, requiring $P < 0.001$, as a guide to consider stopping for efficacy. The DMEC was to consider stopping for harm from dexamethasone if an unfavourable trend emerged, sufficiently large to rule out a clinically relevant benefit. We did not seek conclusive evidence of dexamethasone being harmful, as continued exposure of patients to a non-beneficial and potentially harmful treatment was considered unethical. The DMEC received conditional power curves in addition to the summaries of results, to help inform their decisions.

The first interim analysis was completed on data accrued up to the 31st of August 2013. The second was completed on data up to the 30th of January 2014, and the final on data up to
the 30th June 2014. I provide summaries of each analysis and the resulting actions in the following sections, ending with a review of the trends that emerged.

### 7.5.1.1 First interim analysis

By the time of first interim analysis, 49 deaths had occurred. The overall hazard ratio for mortality by 10 weeks for patients receiving dexamethasone was 1.04 (95% CI 0.59 to 1.82; p=0.9). Full details of the primary outcome are presented in Table 7-6.

<table>
<thead>
<tr>
<th></th>
<th>Dexamethasone (events/n)</th>
<th>Placebo (events/n)</th>
<th>HR (95%CI); p-value</th>
<th>Test for heterogeneity (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>24/56</td>
<td>25/61</td>
<td>1.04 (0.59,1.82); p=0.9</td>
<td></td>
</tr>
<tr>
<td>Continent:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Africa</td>
<td>9/23</td>
<td>10/24</td>
<td>0.99 (0.4,2.47); p=0.99</td>
<td>0.91</td>
</tr>
<tr>
<td>- Asia</td>
<td>15/33</td>
<td>15/37</td>
<td>1.06 (0.52,2.18); p=0.87</td>
<td></td>
</tr>
<tr>
<td>Glasgow coma score:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-15</td>
<td>16/44</td>
<td>12/42</td>
<td>1.3 (0.61,2.75); p=0.5</td>
<td>0.68</td>
</tr>
<tr>
<td>- &lt;15</td>
<td>8/12</td>
<td>13/19</td>
<td>0.97 (0.4,2.35); p=0.94</td>
<td></td>
</tr>
<tr>
<td>Baseline fungal count:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;5 log10 CFU/ml</td>
<td>8/24</td>
<td>10/30</td>
<td>0.92 (0.36,2.35); p=0.87</td>
<td>0.55</td>
</tr>
<tr>
<td>- 5-6 log10 CFU/ml</td>
<td>8/9</td>
<td>9/14</td>
<td>1.78 (0.65,4.86); p=0.26</td>
<td></td>
</tr>
<tr>
<td>- &gt;6 log10 CFU/ml</td>
<td>1/2</td>
<td>4/8</td>
<td>0.85 (0.08,9.44); p=0.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 7-6 Hazard ratios for the primary outcome of death by 10 weeks, in the first interim analysis of the CryptoDex trial, October 2013. Stratified by continent, baseline Glasgow coma score, and baseline fungal count. Hazard ratios estimated by the Kaplan-Meier method.

The Kaplan-Meier curves for survival until 6 months are shown in Figure 7-3. As in the full trial, there is evidence of non-proportional hazards. A non-significant early trend towards benefit from dexamethasone is followed by a later non-significant trend towards harm.
The conditional power curves relating to 10 week mortality are shown in Figure 7-4. The unconditional power was based on the pre-trial estimate that 247 deaths would be observed by the end of the trial. This number of events gave rise to a power of 82% to detect a hazard ratio of 0.7 in favour of dexamethasone, as stated in the CryptoDex trial protocol. The power had dropped to approximately 65% by the first interim analysis, still well above the 15-20%
power usually taken to indicate continuing the trial would be futile.

Figure 7-4 Conditional power curves for survival until 10 weeks in the CryptoDex trial. Data are taken from first DMEC interim analysis report of October 2013. The unconditional curve is shown in blue, based on the per protocol expected number of observed deaths of 247. The conditional power curve is shown in red. This was adjusted according to accumulating data about dexamethasone’s survival impact.

To inform their decisions, the CryptoDex DMEC also considered key secondary endpoints, and the incidence of adverse events in each group. Key secondary end-points are presented in Table 7-7. Although the trends in disability outcomes, visual outcomes, relapse, and fungal clearance are all in favour of placebo, none of the differences were statistically significant.
<table>
<thead>
<tr>
<th></th>
<th>Dexamethasone (N=56)</th>
<th>Placebo (N=61)</th>
<th>Estimate (95% CI); p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disability status at week 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Good</td>
<td>6 (18.8%)</td>
<td>10 (26.3%)</td>
<td>0.65 (0.21, 2.03); p=0.45</td>
</tr>
<tr>
<td>- Intermediate</td>
<td>4 (12.5%)</td>
<td>7 (18.4%)</td>
<td></td>
</tr>
<tr>
<td>- Severe disability</td>
<td>5 (15.6%)</td>
<td>2 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>- Death</td>
<td>17 (53.1%)</td>
<td>19 (50%)</td>
<td></td>
</tr>
<tr>
<td>Visual status at week 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Normal</td>
<td>12 (80%)</td>
<td>19 (100%)</td>
<td>0 (0, Inf); p=1</td>
</tr>
<tr>
<td>- Blurred</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Finger counting</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Movement perception</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Light perception</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- No Light perception</td>
<td>1 (6.6%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Unable to assess</td>
<td>1 (6.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Relapse by week 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Relapse</td>
<td>1 (1.8%)</td>
<td>0 (0%)</td>
<td>609,308,706 (0, Inf); p=1</td>
</tr>
<tr>
<td>- Prior Death</td>
<td>28 (50%)</td>
<td>25 (41%)</td>
<td></td>
</tr>
<tr>
<td>- Censored</td>
<td>27 (48.2%)</td>
<td>36 (59%)</td>
<td></td>
</tr>
<tr>
<td>Rate of change of fungal count (log_{10} CFU/ml/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- All</td>
<td>-0.18 (-0.22, -0.15)</td>
<td>-0.21 (-0.24, -0.18)</td>
<td>0.03 (-0.02, 0.07); p=0.21</td>
</tr>
</tbody>
</table>

Table 7-7 Results for secondary outcomes from the first interim analysis of the CryptoDex trial, October 2013.

Finally, the DMEC compared the occurrence of adverse events between the arms. Those results are presented in Table 7-8, and show the total number of adverse events in the dexamethasone arm was greater (183 vs 110). However, the differences were not statistically significant when events were expressed as patients with at least one event, for any of the predefined categories of adverse event.
Table 7-8 Adverse events by treatment arm at the first interim analysis of CryptoDex trial, October 2013. Unless otherwise stated, figures refer to the number of patients with at least one adverse event of the respective type. All comparisons are based on Fisher’s exact test.

The DMEC recommended that the trial continue as planned, with the next interim analysis to be scheduled after another 50 deaths, or six months, whichever came soonest. They requested that we provide clarification around adverse events, as a majority of those occurring were categorized as ‘other adverse event’, which they thought could affect their ability to interpret the next set of results. They also requested that fungal clearance data be stratified by baseline fungal counts.

7.5.1.2 Second interim analysis

In accordance with the DMEC charter and trial protocol, we scheduled the second interim analysis after observing 100 deaths. The database was closed on the 31st January 2014, we then queried and cleaned the database before sending to the DMEC. At that time 109 deaths had been observed. The hazard ratios for 10 week mortality, stratified by continent, baseline Glasgow coma score, and baseline fungal counts, are presented in Table 7-9.
<table>
<thead>
<tr>
<th></th>
<th>Dexamethasone (events/n)</th>
<th>Placebo (events/n)</th>
<th>HR (95%CI); p-value</th>
<th>Test for heterogeneity (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>53/146</td>
<td>56/152</td>
<td>0.93(0.64,1.35); p=0.7</td>
<td></td>
</tr>
<tr>
<td>Continent:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Africa</td>
<td>27/76</td>
<td>30/76</td>
<td>0.82(0.49,1.38); p=0.46</td>
<td>0.51</td>
</tr>
<tr>
<td>- Asia</td>
<td>26/70</td>
<td>26/76</td>
<td>1.06(0.61,1.82); p=0.84</td>
<td></td>
</tr>
<tr>
<td>Glasgow coma score:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 15</td>
<td>39/120</td>
<td>35/118</td>
<td>1.06(0.67,1.68); p=0.79</td>
<td>0.33</td>
</tr>
<tr>
<td>- &lt;15</td>
<td>14/26</td>
<td>21/34</td>
<td>0.75(0.38,1.5); p=0.42</td>
<td></td>
</tr>
<tr>
<td>Baseline fungal count:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;5 log10 CFU/ml</td>
<td>24/59</td>
<td>22/57</td>
<td>0.93(0.52,1.66); p=0.81</td>
<td>0.51</td>
</tr>
<tr>
<td>- 5-6 log10 CFU/ml</td>
<td>12/17</td>
<td>13/29</td>
<td>1.54(0.67,3.55); p=0.31</td>
<td></td>
</tr>
<tr>
<td>- &gt;6 log10 CFU/ml</td>
<td>6/12</td>
<td>6/13</td>
<td>0.78(0.25,2.48); p=0.67</td>
<td></td>
</tr>
</tbody>
</table>

Table 7-9 Hazard ratios for the primary outcome of death by 10 weeks, in the second interim analysis of the CryptoDex trial, April 2014. Stratified by continent, baseline Glasgow coma score, and baseline fungal count. Hazard ratios estimated by the Kaplan-Meier method.

The Kaplan-Meier chart for survival to six months in patients given dexamethasone vs patients given placebo is shown in Figure 7-5. The pattern seen in the first interim analysis is replicated here, although the difference at six months appears less marked. Another difference is that the curves cross later in this analysis than in the first.
The conditional power curves from the second interim analysis for 10 week mortality are shown in Figure 7-6. At this analysis, the ‘unconditional’ power was updated to reflect our recognition that the total number of deaths we were likely to observe was going to exceed the pre-trial estimate of 247. Based on the overall rates of mortality we were observing, we now expected to observe 380 deaths. As can be seen in Figure 7-6, this increased the ‘unconditional’ power to detect a true hazard ratio of 0.7 from 82% to 94%. The conditional power, incorporating accumulating data on the survival impact of dexamethasone, also increased from 65% to 80% to detect a true hazard ratio of 0.7.
Figure 7-6 Conditional power curves for survival until 10 weeks in the CryptoDex trial. Data are taken from second DMEC interim analysis report of April 2014. The unconditional curve is shown in blue, based on observing a total of 380 deaths, given the mortality rate observed in the trial to that date. The conditional power curve is shown in red. This was adjusted according to accumulating data about dexamethasone’s survival impact.

The key secondary outcomes from the second interim analysis are presented in Table 7-10. At this time, the odds ratio for a good outcome in terms of disability for those treated with dexamethasone was 0.47 (95% CI 0.22 to 0.98), with a borderline significant p-value of 0.05. The DMEC also saw that the rate of clearance of fungus from the cerebrospinal fluid was slower in patients receiving dexamethasone, with the biggest effect seen in those with the highest baseline fungal burden.
<table>
<thead>
<tr>
<th>Disability status at week 10</th>
<th>Dexamethasone (N=146)</th>
<th>Placebo (N=152)</th>
<th>Estimate (95%CI); p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=91</td>
<td>n=91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Good</td>
<td>13 (14.3%)</td>
<td>24 (26.4%)</td>
<td>0.47 (0.22, 0.98); p=0.05</td>
</tr>
<tr>
<td>- Intermediate</td>
<td>20 (22%)</td>
<td>17 (18.7%)</td>
<td></td>
</tr>
<tr>
<td>- Severe disability</td>
<td>16 (17.6%)</td>
<td>11 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>- Death</td>
<td>42 (46.2%)</td>
<td>39 (42.9%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual status at week 10</th>
<th>n=49</th>
<th>n=54</th>
<th>OR of normal vision:</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=91</td>
<td>n=91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Normal</td>
<td>43 (87.8%)</td>
<td>52 (96.3%)</td>
<td>0.28 (0.05, 1.44); p=0.13</td>
</tr>
<tr>
<td>- Blurred</td>
<td>2 (4.1%)</td>
<td>1 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>- Finger counting</td>
<td>0 (0%)</td>
<td>1 (1.9%)</td>
<td></td>
</tr>
<tr>
<td>- Movement perception</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Light perception</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- No Light perception</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>- Unable to assess</td>
<td>2 (4.1%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relapse by week 10</th>
<th>n=146</th>
<th>n=152</th>
<th>HR of relapse:</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=91</td>
<td>n=91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Relapse</td>
<td>1 (0.7%)</td>
<td>2 (1.3%)</td>
<td>0.53 (0.05, 5.82); p=0.6</td>
</tr>
<tr>
<td>- Prior Death</td>
<td>61 (41.8%)</td>
<td>60 (39.5%)</td>
<td></td>
</tr>
<tr>
<td>- Censored</td>
<td>84 (57.5%)</td>
<td>90 (59.2%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rate of change of fungal count (log_{10} CFU/ml/day)</th>
<th>n=106</th>
<th>n=112</th>
<th>Difference in change:</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>-0.16 (-0.18, -0.13)</td>
<td>-0.23 (-0.26, -0.21)</td>
<td>0.08 (0.04, 0.11); p=0</td>
</tr>
<tr>
<td>Baseline fungal count:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=59</td>
<td>n=57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 log_{10} CFU/ml</td>
<td>-0.12 (-0.15, -0.09)</td>
<td>-0.19 (-0.22, -0.15)</td>
<td>0.07 (0.03, 0.11); p=0.001</td>
</tr>
<tr>
<td>n=17</td>
<td>n=29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-6 log_{10} CFU/ml</td>
<td>-0.22 (-0.28, -0.16)</td>
<td>-0.26 (-0.3, -0.21)</td>
<td>0.04 (-0.03, 0.11); p=0.28</td>
</tr>
<tr>
<td>n=12</td>
<td>n=13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6 log_{10} CFU/ml</td>
<td>-0.19 (-0.25, -0.13)</td>
<td>-0.32 (-0.38, -0.25)</td>
<td>0.12 (0.03, 0.21); p=0.007</td>
</tr>
</tbody>
</table>

Table 7-10 Results for secondary outcomes from the second interim analysis of the CryptoDex trial, April 2014. Early fungicidal activity outcomes presented for the whole population, and stratified by baseline fungal count.

Adverse events by type are presented in Table 7-11. As per the request of the DMEC, these were also broken down by subtype. The analysis revealed a statistically significant difference between two of the subtypes. ‘Hyperglycaemia’ occurred in 7 (4.8%) of patients in the dexamethasone arm, and 0 (0%) in the placebo arm (p=0.006). For ‘sepsis not otherwise specified’, the difference was 21 (14.4%) in dexamethasone arm, and 9 (5.9%) in the placebo arm (p=0.02).
<table>
<thead>
<tr>
<th>Clinical Adverse Events</th>
<th>Dexamethasone (n=146)</th>
<th>Placebo (n=152)</th>
<th>Comparison (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of adverse events</td>
<td>379</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>Number of patients with at least one event</td>
<td>105 (71.9%)</td>
<td>104 (68.4%)</td>
<td>0.53</td>
</tr>
<tr>
<td>New neurological event (NNE)</td>
<td>30 (20.6%)</td>
<td>26 (17.1%)</td>
<td>0.46</td>
</tr>
<tr>
<td>New AIDS defining illness (NADI)</td>
<td>34 (23.3%)</td>
<td>29 (19.1%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Immune reconstitution inflammatory syndrome (IRIS)</td>
<td>1 (0.7%)</td>
<td>4 (2.6%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Other Adverse Event</td>
<td>90 (61.6%)</td>
<td>88 (57.9%)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 7-11 Adverse events by treatment arm at the second interim analysis of CryptoDex trial, April 2014. Unless otherwise stated, figures refer to the number of patients with at least one adverse event of the respective type. All comparisons are based on Fisher’s exact test.

The DMEC recommended that the trial continue without any adjustments to the protocol. They requested a repeat analysis after 50 deaths or six months, whichever came first. For the next analysis, they requested that all other adverse events be categorized by body system to assist with their interpretation of the clinical relevance of emerging trends. Furthermore, they requested that grade 3-4 adverse event numbers be reported as total numbers, and number of patients experiencing any adverse events.

7.5.1.3 Third interim analysis

At the time of the third interim analysis, 172 of 411 participants had died. The overall hazard ratio for mortality by 10 weeks for patients receiving dexamethasone was 1.09 (95% CI 0.81 to 1.47); p=0.57. Full details of the primary outcome are presented in Table 7-12. There were no statistically significant differences overall, nor in any pre-defined strata.
<table>
<thead>
<tr>
<th></th>
<th>Dexamethasone (events/n)</th>
<th>Placebo (events/n)</th>
<th>HR (95%CI); p-value</th>
<th>Test for heterogeneity (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>90/204</td>
<td>82/207</td>
<td>1.09(0.81,1.47); p=0.57</td>
<td></td>
</tr>
<tr>
<td>Continent:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Africa</td>
<td>55/112</td>
<td>46/112</td>
<td>1.19(0.81,1.76); p=0.38</td>
<td>0.48</td>
</tr>
<tr>
<td>- Asia</td>
<td>35/92</td>
<td>36/95</td>
<td>0.96(0.6,1.53); p=0.86</td>
<td></td>
</tr>
<tr>
<td>Glasgow coma score:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;15</td>
<td>71/171</td>
<td>51/160</td>
<td>1.34(0.93,1.91); p=0.11</td>
<td>0.10</td>
</tr>
<tr>
<td>- &gt;15</td>
<td>19/31</td>
<td>31/47</td>
<td>0.82(0.46,1.47); p=0.51</td>
<td></td>
</tr>
<tr>
<td>Baseline fungal count:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;5 log 10 CFU/ml</td>
<td>44/97</td>
<td>35/89</td>
<td>1.08(0.69,1.69); p=0.73</td>
<td>0.98</td>
</tr>
<tr>
<td>- 5-6 log 10 CFU/ml</td>
<td>19/35</td>
<td>24/50</td>
<td>1(0.54,1.84); p=0.99</td>
<td></td>
</tr>
<tr>
<td>- &gt;6 log 10 CFU/ml</td>
<td>10/21</td>
<td>9/23</td>
<td>1.14(0.46,2.86); p=0.77</td>
<td></td>
</tr>
</tbody>
</table>

Table 7-12 Hazard ratios for the primary outcome of death by 10 weeks, in the third interim analysis of the CryptoDex trial, August 2014. Stratified by continent, baseline Glasgow coma score, and baseline fungal count. Hazard ratios estimated by the Kaplan-Meier method.

The Kaplan-Meier survival chart for dexamethasone vs placebo at the time of the third interim analysis is shown in Figure 7-7. The pattern seen in the first and second interim analyses is replicated here, with signs of non-proportional hazards, and the suggestion of early benefit and late harm from dexamethasone.
Figure 7-7 Kaplan-Meier survival curve for all patients in 2016 CryptoDex trial, dexamethasone vs placebo, at the time of the third interim analysis in August 2014

Figure 7-8 shows the conditional power curves for the third interim analysis for 10 week mortality. This time, the ‘unconditional’ power was based on an expectation of observing 396 deaths. As at the second interim analysis, the ‘unconditional’ power to detect a true hazard ratio of 0.7 was approximately 94%. The conditional power to detect a true hazard ratio of 0.7, incorporating accumulating data on the survival impact of dexamethasone, decreased from 80% to 33%. By that time, only if the true hazard ratio for treatment with dexamethasone was less than 0.6 was the power over 80%.
Figure 7-8 Conditional power curves for survival until 10 weeks in the CryptoDex trial. Data are taken from third DMEC interim analysis report of August 2014. The unconditional curve is shown in blue, based on observing a total of 396 deaths, given the mortality rate observed in the trial to that date. The conditional power curve is shown in red. This was adjusted according to accumulating data about dexamethasone’s survival impact.

The impacts of dexamethasone on key secondary outcomes are shown in Table 7-13. The DMEC would have observed that the odds ratio for a good disability outcome fell from 0.47 (95% CI 0.22 to 0.98; p=0.05) to 0.36 (95% CI 0.2 to 0.64; p<0.00049). The odds ratio for having normal vision also worsened, to 0.3 (95% CI 0.1 to 0.9; p=0.03). In terms of the microbiological outcomes, the rate of clearance was statistically significantly worse for all participants receiving dexamethasone, regardless of baseline fungal counts.
<table>
<thead>
<tr>
<th>Disability status at week 10</th>
<th>Dexamethasone (N=204)</th>
<th>Placebo (N=207)</th>
<th>Estimate (95%CI); p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>n=154</td>
<td>n=163</td>
<td>OR of status Good: 0.36(0.2,0.64); p=0.00049</td>
</tr>
<tr>
<td>Intermediate</td>
<td>20 (13%)</td>
<td>48 (29.5%)</td>
<td></td>
</tr>
<tr>
<td>Severe disability</td>
<td>35 (22.7%)</td>
<td>31 (19%)</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>23 (14.9%)</td>
<td>17 (10.4%)</td>
<td></td>
</tr>
<tr>
<td>Visual status at week 10</td>
<td>n=79</td>
<td>n=97</td>
<td>OR of normal vision: 0.3(0.1,0.9); p=0.03</td>
</tr>
<tr>
<td>Normal</td>
<td>67 (84.8%)</td>
<td>92 (94.9%)</td>
<td></td>
</tr>
<tr>
<td>Blurred</td>
<td>3 (3.8%)</td>
<td>3 (3.1%)</td>
<td></td>
</tr>
<tr>
<td>Finger counting</td>
<td>2 (2.5%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Movement perception</td>
<td>2 (2.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Light perception</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>No Light perception</td>
<td>1 (1.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Unable to assess</td>
<td>4 (5.1%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Relapse by week 10</td>
<td>n=204</td>
<td>n=207</td>
<td>HR of relapse time: 1(1,1); p=NaN</td>
</tr>
<tr>
<td>Relapse</td>
<td>102 (50%)</td>
<td>117 (56.5%)</td>
<td></td>
</tr>
<tr>
<td>Prior Death</td>
<td>1 (0.5%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
<tr>
<td>Censored</td>
<td>101 (49.5%)</td>
<td>88 (42.5%)</td>
<td></td>
</tr>
<tr>
<td>Rate of change of fungal</td>
<td>n=159</td>
<td>n=167</td>
<td>Difference in change: 0.07 (0.04,0.1); p=0</td>
</tr>
<tr>
<td>count (log_{10} CFU/ml/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.16 (-0.18,-0.14)</td>
<td>-0.23 (-0.26,-0.21)</td>
<td></td>
</tr>
<tr>
<td>Baseline fungal count:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;-5 log10 CFU/ml</td>
<td>-0.12 (-0.15,-0.1)</td>
<td>-0.19 (-0.22,-0.16)</td>
<td>0.07 (0.03,0.11); p=&lt;0.0001</td>
</tr>
<tr>
<td>5-6 log10 CFU/ml</td>
<td>-0.21 (-0.25,-0.17)</td>
<td>-0.28 (-0.32,-0.24)</td>
<td>0.07 (0.01,0.12); p=0.02</td>
</tr>
<tr>
<td>-&gt;6 log10 CFU/ml</td>
<td>-0.21 (-0.25,-0.16)</td>
<td>-0.3 (-0.34,-0.25)</td>
<td>0.09 (0.03,0.15); p=0.005</td>
</tr>
</tbody>
</table>

Table 7-13 Results for secondary outcomes from the third interim analysis of the CryptoDex trial, August 2014. Early fungicidal activity outcomes presented for the whole population, and stratified by baseline fungal count.

The occurrence of adverse events by type are shown in Table 7-14. At this analysis, we categorized all ‘other adverse events’ according to MedRA system organ class. Overall there were 511 adverse events in the dexamethasone arm, and 358 in the placebo arm. The number of patients experiencing at least one adverse event was not statistically significantly different between treatment groups. However, 41/204 (20.1%) participants in the dexamethasone arm experience a severe adverse event categorized as ‘infection or infestation’ compared with 16/207 (7.7%) in the placebo arm (p<0.001). ‘Gastrointestinal disorders’ and ‘cardiac disorders’ also occurred more often in patients receiving dexamethasone than placebo (12.2% vs 5.8%, p=0.02 and 3.9% vs 0%, p=0.003, respectively).
### Clinical Adverse Events

<table>
<thead>
<tr>
<th>Event</th>
<th>Dexamethasone (n=204)</th>
<th>Placebo (n=207)</th>
<th>Comparison (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of adverse events</td>
<td>511</td>
<td>358</td>
<td></td>
</tr>
<tr>
<td>Number of patients with at least one event</td>
<td>154 (75.5%)</td>
<td>149 (72%)</td>
<td>0.44</td>
</tr>
<tr>
<td>New neurological event (NNE)</td>
<td>39 (19.1%)</td>
<td>38 (18.4%)</td>
<td>0.9</td>
</tr>
<tr>
<td>New AIDS defining illness (NADI)</td>
<td>53 (26%)</td>
<td>47 (22.7%)</td>
<td>0.49</td>
</tr>
<tr>
<td>Immune reconstitution inflammatory syndrome (IRIS)</td>
<td>6 (2.9%)</td>
<td>6 (2.9%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

### Other adverse events

<table>
<thead>
<tr>
<th>Event</th>
<th>Dexamethasone (n=204)</th>
<th>Placebo (n=207)</th>
<th>Comparison (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections and infestations</td>
<td>41 (20.1%)</td>
<td>16 (7.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gastrointestinal Disorders</td>
<td>25 (12.2%)</td>
<td>12 (5.8%)</td>
<td>0.02</td>
</tr>
<tr>
<td>Renal and urinary disorders</td>
<td>16 (7.8%)</td>
<td>8 (3.9%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders</td>
<td>6 (2.9%)</td>
<td>11 (5.3%)</td>
<td>0.32</td>
</tr>
<tr>
<td>Hepatobiliary disorders</td>
<td>8 (3.9%)</td>
<td>3 (1.5%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Vascular disorders</td>
<td>9 (4.4%)</td>
<td>4 (1.9%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>8 (3.9%)</td>
<td>0 (0%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Endocrine disorders</td>
<td>3 (1.5%)</td>
<td>1 (0.5%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>4 (2%)</td>
<td>0 (0%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Immune system disorders</td>
<td>1 (0.5%)</td>
<td>1 (0.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>2 (1%)</td>
<td>1 (0.5%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Injury, poisoning and procedural complications</td>
<td>2 (1%)</td>
<td>1 (0.5%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Reproductive system and breast disorders</td>
<td>1 (0.5%)</td>
<td>0 (0%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Pregnancy, puerperium and perinatal conditions</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Systemic</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 7-14 Adverse events by treatment arm at the third interim analysis of CryptoDex trial, August 2014. Unless otherwise stated, figures refer to the number of patients with at least one adverse event of the respective type. All comparisons are based on Fisher’s exact test.

Following their analysis of the data, the DMEC recommended the trial be discontinued. At that time, they provided the following information to us:
7.5.1.4 Adverse trends identified by the DMEC

Increased rates of adverse events, mainly infections

By the time of the third interim analysis, with clear categorization of adverse events in place, there were more infectious adverse events in the dexamethasone arm. This trend had been present in the second interim analysis, as ‘sepsis, not specified elsewhere’ but was obscured in the first interim analysis where they were categorized simply as ‘other adverse events’. Although the total number of adverse events was consistently higher in the dexamethasone arm, the number of patients experiencing at least one adverse event was not significantly higher in the dexamethasone arm at any analysis. At the final trial analysis, this
pattern was maintained, except the increased incidence of ‘renal and urinary disorders’ had also reached statistical significance (chapter 6.7).

*Increased CSF fungal counts over the first 14 days*

The rate of decline of fungal counts (log10 CFU/ml/day) was consistently slower in the dexamethasone arm at every analysis, although it only became statically significant at the second analysis. This difference was also maintained at the final trial analysis (chapter 6.7).

*There is little chance of finding a beneficial effect of adjunctive dexamethasone therapy if the study will be continued*

I present the conditional power results from each analysis on the same chart in Figure 7-9, to highlight one of the trends guiding the decision of the DMEC. The conditional power lines, in red, were affected by both the number of events expected based on overall mortality and the accumulating data on dexamethasone’s survival impact. A higher number of expected events increases the power to detect an anticipated hazard ratio, whilst an observed hazard ratio closer than anticipated to 1, or above 1, decreases that power. At the second interim analysis, there was an artefactual increase in power, because the expected number of events rose sharply, and little data had been accumulated about the survival impact of dexamethasone. By the time of the third interim analysis, the power reduced to around 0.3. Although this didn’t reach the standard statistical level of 0.15-0.2 for futility, the DMEC charter was clear that it should act on trends if dexamethasone appeared to harmful. The combined evidence they observed was sufficient to recommend stopping the trial.
Figure 7-9 Conditional power curves for survival until 10 weeks in the CryptoDex trial. Data are taken from DMEC interim analysis reports of October 2013 (dotted lines), April 2014 (dashed lines), and August 2014 (solid lines). The unconditional curves are shown in blue. These were adjusted according to observed overall mortality at each analysis. At the first interim analysis, per the protocol, the expected number of observed deaths was 247. This was updated at the second and third interim analyses to 380 and 396 deaths, respectively. The conditional power curves are shown in red. These were adjusted according to accumulating data about dexamethasone’s survival impact.

### 7.5.1.5 Trial stopping procedures

Having received the recommendation of the DMEC on the 30th August, we immediately suspended recruitment to the trial and arranged an urgent meeting of the trial steering committee. This trial steering committee met on the 2nd of September and we reached a consensus to continue the trial suspension. For patient safety, we decided that patients currently receiving the study intervention need not be unblinded, but that they should have their treatment tapered. Since the physiological dose of dexamethasone is approximately 0.75mg, we recommended reducing the dose to 1mg orally for 1 week and then stopping, for any patient currently taking more than 1mg/day. Otherwise, for all patients (including those who have completed the study intervention), management was to continue as per the
protocol with all data collection, scheduled investigations and follow-up to the final 6 month time-point.

The trial data were then formally reviewed by the trial statistician and the independent members of the trial steering committee. They confirmed the recommendation of the DMEC, and the trial was formally discontinued on the 12th September 2014. Details of the role of the DMEC in stopping the trial were included in the paper reporting our findings (322), including the stopping boundaries and the specific justification. The DMEC charter was made available as part of the published protocol (323).

7.6 Discussion

In this chapter, I set out to examine the problem of stopping early and publication of results in meningitis clinical trials, to better understand the DSMB decision-making process, and to identify lessons learned from the CryptoDex trial stopping process. The literature on stopping trials early is predominantly focused on large industry-funded trials, especially in cardiovascular medicine. Although the pioneering approach to data safety monitoring of trials by the AIDS Clinical Trials Group has been described, and a review exists of the challenges faced monitoring trials in tropical settings (324), there remains a significant gap in analysis of smaller, academic infectious disease trials. I found no detailed case-studies specifically addressing adult trials in this area.

I reviewed the outcomes of 375 meningitis trials, in which almost half a million participants had been enrolled. Levels of data completeness in the trial registries were poor. Even ‘essential’ data points were complete for as few as 76% of trials. Furthermore, although my research identified 28 trials that had been stopped early, only 8 were recorded as ‘terminated’ in the registry. Despite the widely accepted importance of independent data monitoring, only 10% of the trials I reviewed reported having a DSMB. RCTs were more likely to state they had a DSMB than non-RCTs, but the proportion was still low at 17%. This low
estimate of the presence of DSMBs may be related to poor data completeness in the registry, as well as failure to acknowledge the role of DSMBs in publications. I found no data on the frequency of independent monitoring in infectious disease trials against which to benchmark my figures. Regardless, either underutilization of DSMBs in meningitis research or a failure to emphasize their importance would be profoundly concerning.

I hypothesized that trials stopped early would be less likely to publish their results than trials which ran to completion. Although there was a trend in this direction, it was not statistically significant (68.9% for early-terminated vs 75.9% for completed trials, odds ratio 0.39; 95% CI 0.12 to 1.54). The figure for early-terminated trials is comparable to those reported in the literature, where a review of 905 early terminated trials on ClinicalTrials.gov trials found results published for 72%. On the other hand, a 2016 investigation in the Netherlands found only 33% of early-terminated trials were published, compared with 64% of completed trials (adjusted odds ratio 0.2; 95%CI 0.1 to 0.3) (325). However, this analysis from the Netherlands also found that prospective registration enhanced rates of publication, and only such trials were included in my analysis.

I found that trials with a DSMB were more likely to stop early. However, this is likely confounded by the fact that trials which terminate early are more likely to report they had a DSMB in the registry or any subsequent publication. The most common reason cited for early termination of trials is inadequate participant accrual, and this is particularly an issue with small trials (326). Again, the literature is focused on cardiovascular trials. I found no information on this topic specific to infectious disease trials, and my own analysis showed no statistically significant relationship between small trials, and stopping early.

My analysis of the stopping procedures for the CryptoDex trial shows that the trial was stopped appropriately. The reasons were clearly stated as being due to both trends towards futility with respect to the primary end-point and harm with respect to secondary outcomes.
The DSMB charter was publically available, data was complete on the ICTRP database, and results were presented within 12 months and published within 17. This ensured that all seven ethical criteria for the proper conduct of clinical trials were adhered to. However, our experience also highlights the importance of clear and consistent categorization of adverse events. Although the early *ad-hoc* coding of ‘other adverse events’ is unlikely to have affected the timing of trial termination, systematic coding would have assisted the DSMB to identify trends of adverse events in a particular body system. In our case, we used the MedRA coding system, and the harmful trend was already visible in the second interim analysis as ‘sepsis not otherwise specified’, it emerged more clearly in the third interim analysis under ‘infections and infestations’.

**7.6.1 Limitations**

The analysis I performed on factors affecting early termination and publication of results has several limitations. I decided to focus on the neglected area of infectious disease trials, specifically meningitis trials. The target number of 500 was in line with other similar studies, but after exclusions the eventual number of trials included was relatively small. This may have prevented me from identifying more statistically significant findings. Another major issue was the volume of missing data in the registry. Since I had focused on WHO ‘essential data points’, I anticipated that near completeness of data entry, but this turned out not to be the case. I had insufficient time to contact authors directly to fill in missing data (which may in any case have been impossible for older trials), and this further reduced the power of my study.

Given more time and resources, it would be interesting to repeat this exercise. Including all infectious disease trials from an appropriate discrete time period could yield more trials to analyse. Given a set of recent trials, it would also be informative to send enquiries to principle investigators wherever data were missing. With regards to our CryptoDex experience, I would encourage investigators in smaller academic trials to systematically code adverse events using
a coding system such as MedRA. This would greatly assist their DSMBs. Furthermore, I would encourage colleagues to publish key components of the decision making process wherever trials are stopped early, and to consider the sharing of case reports.

### 7.7 Conclusions

In this chapter I have described the findings of the first study of early-termination and under-reporting in meningitis trials. Many of these are smaller and sponsored by academic institutions. Systematic analysis of such trials has been neglected, despite the acknowledged challenges for their DSMBs. I found that basic data was incompletely recorded. This unreliability in the registry data, and the time-consuming process of making the data usable, makes any ongoing review of trial outcomes very cumbersome. Although compulsory registration has undoubtedly improved the situation, more work to ensure compliance is required.

It appears that the level of reporting for prospectively registered meningitis trials is similar to clinical trials in general. Surprisingly, I did not confirm my hypothesis that early-terminated trials would be reported less frequently than completed trials.

Overall, my research into meningitis trials and my case study on the early-termination of the CryptoDex trial reinforce the vital role of DSMBs, but indicate that they are under-utilized or unacknowledged. Furthermore, smaller trials should adopt the robust adverse event reporting procedures already adopted by industry-sponsored trials. To maintain the social contract that clinical trialists rely on, these issues should be urgently addressed.
7.8 Statement of contribution

The idea for this chapter was my own. I reviewed the literature on stopping trials early and developed relevant questions. With regards to the meta-analysis of trials stopped early, I gathered and cleaned all the data, and performed all statistical analyses. For the CryptoDex interim analyses I modified and reran the R code from the CryptoDex trial, in order to produce consistent results. I collated all communications with the DSMB and trial steering committee. I am working on a manuscript for publication.
8. The impact of dexamethasone vs placebo on immune responses at the site of infection in cryptococcal meningitis: report on the CryptoDex randomised controlled trial
8.1 Summary

**Introduction** The CryptoDex placebo controlled trial (Beardsley et al, NEJM 2016, ISRCTN59144167) showed dexamethasone was associated with poorer clinical and microbiological outcomes in HIV-associated cryptococcal meningitis. Here, we describe the longitudinal immune responses of participants, to understand how that harm was mediated. We hypothesised that dexamethasone lowered pro-inflammatory cytokine concentrations, and that this was associated with worse outcomes. We hypothesized that participants with the TT LTA4H genotype, associated with hyper-inflammatory responses in tuberculous meningitis, benefitted from dexamethasone.

**Materials and Methods** We included participants from Vietnam, Thailand, and Uganda. We measured cerebrospinal fluid concentrations of IFN-γ, TNF-α, GM-CSF, IL-6, IL-12p70, IL-8, MCP-1, MIP-1α, IL-4, IL-10, and IL-17 from days 1 to 7 of treatment. LTA4H genotype was determined by PCR of the promoter region SNP rs17525495. We assessed the impact of dexamethasone on cytokine dynamics and cytokine dynamics on fungal clearance with mixed effect models, powered to anticipated impacts on IFN-γ. We assessed the role of the LTA4H genotype using Cox regression.

**Results** Compared to placebo, dexamethasone was associated with faster decline of TNF-α concentration (coefficient -0.16 (95%CI -0.24 to -0.07) p-value <0.001). Faster decline of TNF-α was associated with slower fungal clearance (Pearson’s correlation -0.65 (-0.85 to -0.27)). Since IFN-γ was undetectable at baseline in the majority of patients, its association with outcome was unassessable. Dexamethasone, compared to placebo, worsened 10 week survival for participants CC and CT LTA4H genotypes (CC: HR 2.86 (95% CI 1.33 to 6.15) and CT: HR 3.32 (95% CI 1.32 to 8.35)). Survival curves suggested TT LTA4H participants benefited from dexamethasone, but this was not statistically significant (HR 0.34 (95% CI 0.05 to 2.53)).

**Conclusions** Dexamethasone’s negative effects on fungal clearance may be mediated by its impact on cytokine dynamics. Our results suggest patients with the TT LTA4H genotype may benefit from dexamethasone, and provide a biologically plausible explanation.
8.2 Background

The CryptoDex trial, described in Chapter 1, was a double blind randomised placebo controlled trial of adjuvant treatment with dexamethasone in HIV-associated cryptococcal meningitis (CM). In brief review, the study was justified by case series and animal data suggesting a benefit of corticosteroids in CM, their beneficial effect in other forms of meningitis (tuberculous meningitis, and bacterial meningitis in some settings), their widespread use in Asia, and the fact that their use forms part of current guidelines (181,186,278). Of particular note, data from animal studies suggest that corticosteroids prolong survival in cryptococcal disease in the absence of antifungals, and do not adversely affect fungal clearance when combined with fluconazole or amphotericin (181,278). Prior to CryptoDex, corticosteroids for the treatment of cryptococcal meningitis had never been subjected to a randomized controlled trial.

As covered in Chapter 1, we had to discontinue the CryptoDex trial after recruiting 451 participants because we observed harm among those receiving dexamethasone. The finding of harm was unexpected and this chapter, focusing on immune responses, aims to understand how harm may have been mediated. We performed lumbar puncture on CryptoDex participants according to the study protocol at study entry, days 3, 7, and 14, and more frequently if clinically indicated. The resulting stored samples of cerebrospinal fluid (CSF) present an important opportunity. I set out to determine the effect of dexamethasone on dynamic host immune responses at the site of infection, and to investigate whether any observed effects were associated with clinical and mycological outcomes. In addition, I planned to examine the relationship between immune response, inflammatory genotype, dexamethasone treatment, and patient outcomes.
8.2.1 Impact of corticosteroids on inflammatory profile in other forms of meningitis

In infectious diseases, disease phenotype is determined by both the pathogen and the host immune response. The rationale for the use of corticosteroids in infections is based upon the concept that the host immune response contributes to the damage that occurs as a result of infection. Corticosteroids have a profound effect on the human immune system in its normal state, exerting their effect mainly via suppressed production of IFN-γ and IL-12 and down-regulation of IL-12 receptors (180). In healthy volunteers, corticosteroids can reduce production of IFN-γ by 50-60% (327).

Individual trials have shown that adults with both acute and chronic meningitis can benefit from systemic corticosteroid therapy, in the presence of effective antimicrobial therapy (273,275). In microbiologically confirmed cases of bacterial meningitis in Vietnam, and probable or confirmed cases of bacterial meningitis in Europe, dexamethasone reduced the risks of death and neurological disability (273,274). A follow up study of the Vietnamese trial showed that the survival benefit conferred by dexamethasone was associated with greater reductions in the levels of IL-6, IL-8 and IL-10, providing a biological explanation for the observed effect (328). However, questions about the role of corticosteroids for bacterial meningitis remain, as meta-analyses have reached conflicting conclusions. The first major systematic review and meta-analysis was a 2007 Cochrane review of outcomes for 2,750 participants, and it found that corticosteroids reduced mortality overall (329). This was followed by a meta-analysis of individual patient data from five trials, heavily influenced by participants from Malawi, who were 1,063 out of a total 2,029 participants. Here, no beneficial effect for corticosteroids was identified in any pre-defined subgroup (330). A follow-up Cochrane meta-analysis in 2016, including 4,121 participants, concluded that corticosteroids are beneficial overall in high-income countries, reducing deafness and other neurological complications, and reducing case fatality in meningitis caused by Streptococcus
pneumoniae. However, they concluded that there was no role for corticosteroids in low-income countries (331).

In TB meningitis (TBM), a benefit of dexamethasone in reducing the risk of death has been shown (275). However, unlike in acute bacterial meningitis, a mechanistic explanation in terms of differences in cytokine expression between patients receiving dexamethasone and placebo was not seen (291). It is possible, however, that the effect of dexamethasone on cytokine expression occurs within the first few days and was missed in the TBM dexamethasone trial, since CSF was sampled infrequently (291). In the CryptoDex trial, there was a higher frequency of CSF sampling (days 1, 3, 7 and 14), and thus there is an excellent chance of detecting any acute effects on immune response.

8.2.2 Role of Polymorphisms at the Leukotriene-A4 Hydrolase (LTA4H) Gene

Host genetic factors have been implicated in the susceptibility to and disease phenotype of various infectious diseases from malaria, to viral hepatitis, to invasive bacterial diseases (332). In tuberculosis, polymorphisms in the leukotriene A4 hydrolase (LTA4H) gene can modulate the immune response and pathogenesis of disease (333). The role of LTA4H gene polymorphisms was originally identified in zebrafish predisposed to severe infection with Mycobacterium marinum, and was subsequently shown to play a similar role in Vietnamese adults with TBM (334). The LTA4H polymorphism results in variable inflammatory phenotypes: CC homozygotes have a hypo-inflammatory response, CT heterozygotes have a moderate inflammatory response, whilst TT homozygotes have a hyper-inflammatory response (Figure 8-1). The LTA4H polymorphism primarily affects the production of TNF-α. It is reasoned that both too much and too little is harmful, and observed that both the CC (hypo-inflammatory) and TT (hyper-inflammatory) genotypes have poorer outcomes from mycobacterial infections than CT heterozygotes (333).
Figure 8-1 Impact of LTA4H genotype on TNF-α production. Reduced levels of LTA4H in the CC genotype (blue box) leads accumulation of LXA4, and inhibition of TNF-α production; the CT genotype (yellow box) has balanced TNF-α production; and the TT genotype (red box) accumulates LTB4, with increased TNF-α production. For all metabolic components, font size denotes quantity produced.

Furthermore, Vietnamese adults with TBM and the hyper-inflammatory TT LTA4H genotype benefitted dramatically from treatment with dexamethasone. The Kaplan-Meier charts in Figure 8-2 are taken from Tobin et al’s 2012 paper, and show that by 400 days after randomization, no TT patients receiving dexamethasone died, compared with 50% of those receiving placebo. Conversely, those with the hypo-inflammatory CC LTA4H genotype may suffer harm as a result of adjunctive dexamethasone (333).
Figure 8-2 Kaplan-Meier charts of TBM patient survival up to 400 days stratified by LTA4H genotype (CC-blue, CT-green, and TT-red), Vietnam 2012. Panel A shows those receiving adjunctive placebo, panel B shows those receiving adjunctive dexamethasone. Image taken from Tobin et al Cell 148, 434–446, February 3, 2012.

In cryptococcosis, TNF-α has been shown to provide protective immunity in mice (178). However, the role of TNF-α in humans with cryptococcal disease is less clear. The prevalence and role of LTA4H polymorphisms in humans with CM are unknown but identifying whether the polymorphism influences outcome in CM as it does in TBM could have profound implications.

### 8.3 Study Aims

The aims of this chapter are depicted in Figure 8-3, and detailed below.

Figure 8-3 Inter-related aims of studying dynamic cytokine profile and inflammatory genotype of patients in the CryptoDex trial. Measured components are in dark blue, and factors that may have affected them are in light blue.
8.3.1 Primary aims

1. To assess the impact of dexamethasone on the immune response in CSF

2. To evaluate whether baseline and longitudinal CSF immune profiles are associated with patient outcomes and determine whether the association is different between dexamethasone and placebo groups.

8.3.2 Secondary aims

1. To establish the prevalence of known polymorphisms of the LTA4H gene in our study population and to determine whether the 3 LTA4H genotypes are associated with outcome or distinctive inflammatory profiles defined by concentrations of key cytokines in CSF.

2. To identify whether dexamethasone is beneficial in a subset of patients with HIV-associated cryptococcal meningitis who carry the hyper-inflammatory TT LTA4H genotype.

8.3.3 Hypotheses

1. Dexamethasone will have a significant impact on inflammatory response, and will specifically reduce the concentration of IFN-\( \gamma \) relative to the placebo group

2. Different patterns of baseline and longitudinal immune response will be associated with patient outcomes in terms of clinical outcome at 10 weeks, and rate of fungal clearance over the first 2 weeks. The association will be different between dexamethasone and placebo groups.

3. The TT LTA4H genotype is associated with a hyper-inflammatory CSF response as defined by higher levels of IFN-\( \gamma \) and TNF-\( \alpha \).
4. Beneficial effects of corticosteroids on clinical outcome, if any, will be restricted to patients with the hyper-inflammatory TT LTA4H genotype.

8.4 Methods

8.4.1 Study design and participants

All CryptoDex participants from Vietnam, Uganda, and Thailand who gave consent for genetic testing were included in the genotype component of this study. Participants from Thailand were not included in cytokine analyses, because we did not have ethical approval to use their cerebrospinal fluid (CSF) samples for that purpose. We measured cytokine concentrations in the stored CSF of CryptoDex participants from Uganda and Vietnam. All samples had been stored at -80°C since collection. The study involved analyses of both baseline CSF cytokine profiles, and longitudinal CSF cytokine profiles. For the baseline analyses I used all available samples collected before the administration of study drug. For the longitudinal analyses I used CSF samples from any participants with samples from at least two of the following three time windows: baseline, day 0-2, and day 4-7. In addition, the inclusion and exclusion criteria from the CryptoDex trial applied. Full inclusion and exclusion criteria are shown in Table 8-1.
Study Specific Inclusion Criteria

- CryptoDex participants from Uganda and Vietnam
- Baseline LP performed and stored sample available (for baseline analyses)
- LP performed and stored samples available for two of the following three time-points: prior to study drug administration, study day 0-2, and study day 4-7 (for longitudinal analyses)
- Consent to genetic testing (for the LTA4H analyses)

CryptoDex Inclusion Criteria

- Age ≥18 years
- HIV antibody positive
- Cryptococcal meningitis defined as a syndrome consistent with CM and one or more of:
  - Positive CSF India ink (budding encapsulated yeasts)
  - *C. neoformans* cultured from CSF or blood
  - Positive cryptococcal antigen Lateral Flow Antigen Test (LFA) from CSF
- Informed consent to participate given by patient or acceptable representative

CryptoDex Exclusion Criteria

- Pregnancy
- Active gastrointestinal bleeding, vomiting blood, or melaena stool in the previous week
- Currently receiving treatment for CM and having received ≥1 week of anti-CM therapy
- Known allergy to dexamethasone
- Current corticosteroid use defined as:
  - Currently receiving the equivalent of prednisolone 40 mg/day or more
  - Currently receiving corticosteroid therapy (any dose) for more than 3 weeks (except topical corticosteroids, which are permitted)
- Concurrent condition for which corticosteroids are indicated because of proven benefit (such as severe *Pneumocystis* pneumonia (pO₂ < 70 mm Hg) or tuberculous meningitis)
- Renal failure (defined as creatinine >3 × ULN, despite adequate hydration)

Table 8-1 Inclusion and exclusion criteria for CSF cytokine response in cryptococcal meningitis study

8.4.2 Primary endpoint assessment

All primary and secondary endpoints were assessed as in the CryptoDex trial in chapter 1.

The primary outcome was overall survival until 10 weeks.
8.4.3 Secondary endpoint assessment

Secondary endpoints included: overall survival until 6 months, disability at 10 weeks, and 6 months after randomization, and the rate of fungal clearance up to 2 weeks.

I extracted survival and disability data from the CryptoDex database. Yeast quantitative counts were already determined for CryptoDex samples, and the results of those tests were extracted from the CryptoDex database. The early fungicidal activity (EFA) was defined as the rate of change in yeast quantitative counts over the first two weeks of treatment, calculated using linear regression, as described in chapter 1.

8.4.4 Explanatory variables

The cytokines measured for this chapter are shown in Table 8-2. I selected these cytokines based on their importance in previous studies of cytokines in the CSF of meningitis patients.

<table>
<thead>
<tr>
<th>Pro-inflammatory</th>
<th>Immuno-regulatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 associated cytokines</td>
<td>Th17 associated cytokines</td>
</tr>
<tr>
<td>IFNγ</td>
<td>IL-6</td>
</tr>
<tr>
<td>TNFα</td>
<td>IL-12p70</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>IL-8</td>
</tr>
</tbody>
</table>

Table 8-2 Cytokines measured in the CryptoDex immune response sub-study, 2017, and their cellular associations

The co-primary explanatory variables were the following 6 key cytokine measures in CSF: IFN-γ, TNF-α, IL-4, IL-10, IFN-γ/IL-4 ratio, and TNF-α/IL-10 ratio. All other cytokine measurements were considered as additional explanatory variables. I measured all cytokine concentrations using R&D’s multiplex human cytokine kits (R&D Systems, Inc., Minneapolis, USA), in accordance with the manufacturer’s instructions. R&D cytokine kits are magnetic micro-bead antigen capture sandwich assays, which are analysed on the Luminex platform (Luminex corporation, Austin, USA) (335). IFNγ, TNFα, GM-CSF, IL-6, IL-8, IL-12p70, MCP1,
MIP1α, IL-4, IL-10, and IL-17 concentrations were measured, and their limits of detection are presented in Table 8-3.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Lower Range (pg/ml)</th>
<th>Upper Range (pg/ml)</th>
<th>Lower Range (log2 pg/ml)</th>
<th>Upper Range (log2 pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ</td>
<td>4.1</td>
<td>16800</td>
<td>2.04</td>
<td>14.04</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.73</td>
<td>3000</td>
<td>-0.45</td>
<td>11.55</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.37</td>
<td>1500</td>
<td>-1.43</td>
<td>10.55</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.88</td>
<td>3600</td>
<td>-0.18</td>
<td>11.81</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.71</td>
<td>2900</td>
<td>-0.49</td>
<td>11.50</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>5.86</td>
<td>24000</td>
<td>2.55</td>
<td>14.55</td>
</tr>
<tr>
<td>MCP1</td>
<td>3.09</td>
<td>2400</td>
<td>1.63</td>
<td>11.23</td>
</tr>
<tr>
<td>MIP1α</td>
<td>16.32</td>
<td>13600</td>
<td>4.03</td>
<td>13.73</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.71</td>
<td>7000</td>
<td>0.77</td>
<td>12.77</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.61</td>
<td>2500</td>
<td>-0.71</td>
<td>11.29</td>
</tr>
<tr>
<td>IL-17</td>
<td>2.88</td>
<td>2600</td>
<td>1.53</td>
<td>11.34</td>
</tr>
</tbody>
</table>

Table 8-3 Limits of detection for cytokine concentration as measure with the R&D multiplex human cytokine kits in the CryptoDex immune response sub-study, 2017. Concentrations are presented in absolute and log2 transformed pg/ml.

LTA4H genotype – classified as CC, CT or TT – was another explanatory variable.

Genotyping was achieved using an established and validated in-house TaqMan RT-PCR of the LTA4H promoter region SNP (rs17525495), as described in Dunstan et al’s 2015 paper (336).

### 8.5 Statistical Methods

#### 8.5.1 Power calculation

Unpublished data from a previous trial (3) suggested that the mean fall in CSF levels of IFN-γ between day 1 and day 3 would be 0.47 log10 pg/ml with a standard deviation of 0.54 Log10 pg/ml, in the control arm. Assuming the variance of change would be the same in the control and dexamethasone groups, with at least 100 participants in each group, we would have 90% power to detect a 0.25 log10 pg/ml difference of change in levels of IFN-γ between groups with 0.05 type I error, using the two sample t-test.
8.5.2 Preliminary descriptive analyses

We summarized baseline characteristics of the selected population as median (IQR) for continuous data and n (%) for categorical data. We displayed the amount of missing data for each baseline characteristic.

We visually summarized the baseline CSF cytokine concentrations of the selected population with box plots, stratified by site. We also made box plots of baseline CSF cytokine concentrations by clinical outcome at 10 weeks and 6 months. Clinical outcome was categorized as being a good, intermediate, or poor disability outcome, as described in chapter 1, or death. We used these box plots to make preliminary visual comparisons, and undertook statistical testing where indicated.

8.5.3 Assessing the impact of dexamethasone on the immune response in CSF

We compared the co-primary cytokines described above (IFN-\(\gamma\), TNF-\(\alpha\), IL-4, IL-10, IFN-\(\gamma\)/IL-4 ratio, and TNF-\(\alpha\)/IL-10 ratio) by treatment arm using a univariate mixed model with left censoring for longitudinal cytokine data. In this model, log2 cytokine values were the outcome, time since randomization was the main covariate with an interaction between time since randomization and treatment arm. We adjusted the analysis for baseline cytokine concentrations, based on their non-linear pattern with respect to time since illness onset. These baseline concentrations were modelled by a natural spline with 5 degree of freedom.

8.5.4 Assessing whether longitudinal CSF immune profiles are associated with patient outcomes and whether the association is different between dexamethasone and placebo groups

We assessed the association between longitudinal CSF cytokine concentrations and death at 10 weeks and 6 months using logistic regression with treatment arm and patient’s estimated change in log2-cytokine concentration as the main covariates, and an interaction
term between patient’s estimated change in log2-cytokine value and treatment arm. The analyses were adjusted for the patient’s baseline CSF fungal burden, and Glasgow Coma Score.

We also assessed the association between longitudinal CSF immune profiles and EFA using a bivariate linear mixed model with longitudinal fungal counts vs. left-censored longitudinal cytokine data. With this model, we evaluated the dynamics of co-primary cytokine measurements and fungal count over the first week since randomization. The interaction between longitudinal cytokine and fungal count measurements were studied by measuring the Pearson correlation coefficients of the decline of cytokine concentration and fungal counts.

In both of the above analyses, we planned to correct for multiple testing only if there were statistically significant (p<0.05) interaction effects.

8.5.5 Assesing the prevalence of polymorphisms of the LTA4H gene and whether the 3 LTA4H genotypes are associated with unique inflammatory profiles

We summarized the proportion of patients with each of the three LTA4H genotypes in our population of patients with HIV associated cryptococcal meningitis. We compared the genotype frequency between our Vietnamese study population and the general Vietnamese population (as previously published (336)). In addition, we tested for statistically significant differences in distribution between African and Asian sites using the Chi-squared test.

We produced box plots comparing the baseline CSF white cell counts, fungal counts, and cytokine concentrations between CC, CT and TT LTA4H genotypes for visual comparison. Where indicated, we calculated statistical significance using the Wilcoxon rank-sum test for continuous data and Chi-square test for categorical data.
We assessed the difference in subjects’ estimated change in log2-cytokine concentration between the three LTA4H genotypes with a univariate mixed model of longitudinal samples vs. the LTA4H genotype. We used the same model as used for assessing the impact of dexamethasone on cytokine concentrations to address primary aim one, but with the addition of LTA4H genotype.

8.5.6 Assessing whether dexamethasone reduces case fatality in patients with the hyper-inflammatory (TT) genotype.

We produced Kaplan-Meier curves by treatment arm and stratified by LTA4H genotype as a graphical description of the impact of dexamethasone and LTA4H genotype on time to death.

We assessed the effect of dexamethasone on the association between LTA4H genotype and time to death with a Cox regression mode. Because it was established in the CryptoDex trial that hazards of mortality between placebo and dexamethasone arms were non-proportional, we added a time dependent variable. Because they were shown to be associated with outcome in Chapter 1, we adjusted these models for several baseline features, namely: CSF fungal count, Glasgow Coma Score (GCS), country of enrollment, CSF opening pressure, and CSF white cell count. We further tested the impact of genotype on mortality and 10 weeks and 6 months using similarly adjusted logistic regression models.

8.5.7 Additional planned auxiliary analyses

In order to explore which cytokines were most strongly associated with overall survival until 10 weeks and 6 months, we used a logistic regression model including the patient’s log2-baseline cytokine concentration as the covariates, adjusted for treatment arm, baseline GCS (GCS<15, GCS≥15) and baseline fungal count. We adjusted for multiple testing in regression coefficients using the R package multcomp ([https://CRAN.R-project.org/package=multcomp](https://CRAN.R-project.org/package=multcomp)). Next, in order to identify the best subset of
predictors for overall survival, we used the Lasso method for selecting variables in Cox regression models. This analysis was conducted using the R package glmnet. Finally, we performed a conventional stepwise variable selection for Cox models (R package MASS::stepAIC) to assess the robustness of the results.

### 8.6 Ethics

Study samples were obtained from patients enrolled in the CryptoDex trial, which had full ethical approval as detailed in chapter 1. All patients gave written informed consent to enter the trial, which included donation of samples to elucidate mechanisms of disease. Consent for genetic testing was obtained through an opt-in clause at the time of study enrolment; only samples from patients who consented for genetic testing were enrolled into the genotyping component of this study. The protocol for this chapter had further ethical approval from Oxford Tropical Ethics Committee (Oxford, UK), the UVRI research ethics committee (Entebbe, Uganda), the Hospital for Tropical Diseases (HCMC, Vietnam), and Cho Ray Hospital (HCMC, Vietnam).

### 8.7 Results

Baseline lumbar punctures were performed on 274 patients from Uganda and Vietnam. Two hundred and sixty nine patients had sufficient longitudinal lumbar punctures (LP) to be included in the longitudinal modeling analysis. We had consent from 343 patients for the genotype component of the study. See the study flow chart in Figure 8-4.
8.7.1 Baseline clinical characteristics

The distribution of key baseline clinical characteristics between the sub-population included in the immune response study, and the wider population of Vietnamese and Ugandan participants in CryptoDex is provided in Table 8-4. We found no important differences between the two groups.
Baseline characteristics of cytokine study sub-population and residual CryptoDex population, displayed as median (IQR) for continuous data and n (%) for categorical data.

8.7.2 Baseline cytokine concentrations

The log2 concentrations of all measured cytokines by continent are displayed as box plots for visual comparison in Figure 8-5, and in tabular form in Table 8-5.
<table>
<thead>
<tr>
<th>Cytokine conc. log2pg/ml</th>
<th>n</th>
<th>Africa (N=195)</th>
<th>n</th>
<th>Asia (N=61)</th>
<th>Comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=195</td>
<td></td>
<td>N=61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNγ</td>
<td>191</td>
<td>142/191 (74%)</td>
<td>61</td>
<td>44/61 (72%)</td>
<td>0.74</td>
</tr>
<tr>
<td>- &lt;=30pg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &gt;30pg/ml</td>
<td>192</td>
<td>5.62(4.23,7.04)</td>
<td>61</td>
<td>5.52(4.59,6.65)</td>
<td>0.875</td>
</tr>
<tr>
<td>TNFα</td>
<td>194</td>
<td>10.42(9.40,11.66)</td>
<td>61</td>
<td>11.18(9.91,12.20)</td>
<td>0.005</td>
</tr>
<tr>
<td>MCP-1</td>
<td>194</td>
<td>9.44(8.66,10.05)</td>
<td>61</td>
<td>9.37(9.00,10.23)</td>
<td>0.52</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>192</td>
<td>2.19(1.00,3.73)</td>
<td>61</td>
<td>1.06(-0.58,2.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>192</td>
<td>7.33(4.99,9.53)</td>
<td>61</td>
<td>7.11(5.19,8.63)</td>
<td>0.424</td>
</tr>
<tr>
<td>IL-6</td>
<td>192</td>
<td>10.17(8.60,11.90)</td>
<td>61</td>
<td>10.42(8.99,11.46)</td>
<td>0.899</td>
</tr>
<tr>
<td>IL-8</td>
<td>192</td>
<td>3.06(1.55,3.57)</td>
<td>61</td>
<td>1.55(1.55,2.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-12</td>
<td>192</td>
<td>4.58(4.05,4.92)</td>
<td>61</td>
<td>4.76(4.16,5.10)</td>
<td>0.271</td>
</tr>
<tr>
<td>IL-4</td>
<td>191</td>
<td>3.59(2.06,5.13)</td>
<td>61</td>
<td>2.07(0.64,3.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-10</td>
<td>194</td>
<td>2.82(1.66,3.93)</td>
<td>61</td>
<td>2.59(2.18,3.13)</td>
<td>0.238</td>
</tr>
</tbody>
</table>

| Table 8-5 Comparison of baseline log2 cytokine concentrations by site, shown as median (IQR) for continuous data and n (%) for categorical data. Statistical testing with the Wilcoxon rank-sum test for continuous data and Chi-square test for categorical data. |

Box plots for cytokine concentrations by disability and death, at 10 weeks and 6 months, are displayed in Figure 8-6 and Figure 8-7 respectively. The corresponding tables are Table 8-6 for 10 week outcomes, and Table 8-7 for 6 month outcomes.
Figure 8-6 Box plots of baseline cytokine CSF concentration versus disability endpoints by 10 weeks (box = median and IQR; whisker = range; point = outliers)

<table>
<thead>
<tr>
<th>Cytokine conc.</th>
<th>Good (N=33)</th>
<th>Intermediate (N=69)</th>
<th>Severe disability (N=38)</th>
<th>Death (N=113)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>log2pg/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNγ &lt;=30pg/ml</td>
<td>21/32 (66%)</td>
<td>43/69 (62%)</td>
<td>33/38 (87%)</td>
<td>86/110 (78%)</td>
<td>0.016</td>
</tr>
<tr>
<td>IFNγ &gt;30pg/ml</td>
<td>11/32 (34%)</td>
<td>26/69 (38%)</td>
<td>5/38 (13%)</td>
<td>24/110 (22%)</td>
<td></td>
</tr>
<tr>
<td>TNFα</td>
<td>5.44 (4.36,6.48)</td>
<td>5.90 (4.81,7.48)</td>
<td>5.44 (4.15,6.77)</td>
<td>5.70 (4.15,7.11)</td>
<td>0.544</td>
</tr>
<tr>
<td>MCP-1</td>
<td>9.99 (9.30,11.3)</td>
<td>10.25 (9.49,11.00)</td>
<td>11.06 (9.54,12.02)</td>
<td>10.82 (9.76,12.00)</td>
<td>0.028</td>
</tr>
<tr>
<td>MIP-1a</td>
<td>9.26 (8.60,9.77)</td>
<td>9.44 (8.71,10.15)</td>
<td>9.61 (8.87,10.20)</td>
<td>9.41 (8.76,10.05)</td>
<td>0.802</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1.77 (0.83,3.17)</td>
<td>2.19 (1.29,3.71)</td>
<td>1.68 (0.56,3.05)</td>
<td>1.71 (0.15,3.58)</td>
<td>0.436</td>
</tr>
<tr>
<td>IL-6</td>
<td>6.63 (5.41,9.85)</td>
<td>8.47 (6.15,10.06)</td>
<td>6.64 (4.76,9.20)</td>
<td>6.98 (5.05,8.75)</td>
<td>0.109</td>
</tr>
<tr>
<td>IL-8</td>
<td>9.98 (8.51,11.8)</td>
<td>10.45 (9.21,11.94)</td>
<td>9.99 (8.41,11.80)</td>
<td>10.22 (8.77,11.45)</td>
<td>0.721</td>
</tr>
<tr>
<td>IL-12</td>
<td>2.70 (1.55,3.30)</td>
<td>2.92 (1.55,3.49)</td>
<td>2.80 (1.55,3.57)</td>
<td>2.84 (1.55,3.40)</td>
<td>0.8</td>
</tr>
<tr>
<td>IL-4</td>
<td>4.55 (4.02,4.97)</td>
<td>4.67 (4.02,5.05)</td>
<td>4.54 (4.04,4.80)</td>
<td>4.63 (4.07,4.97)</td>
<td>0.786</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.94 (1.27,3.71)</td>
<td>3.78 (2.22,5.30)</td>
<td>3.57 (1.76,5.14)</td>
<td>3.23 (1.56,4.55)</td>
<td>0.129</td>
</tr>
<tr>
<td>IL-17</td>
<td>2.79 (2.00,3.91)</td>
<td>2.85 (1.99,4.79)</td>
<td>3.30 (2.05,3.94)</td>
<td>2.68 (1.66,3.20)</td>
<td>0.073</td>
</tr>
</tbody>
</table>

Table 8-6 Comparison of baseline log2 cytokine concentrations by clinical outcome at 10 weeks, shown as median (IQR) for continuous data and n (%) for categorical data. Statistical testing with the Wilcoxon rank-sum test for continuous data and Chi-square test for categorical data.
Figure 8-7 Box plots of baseline cytokine CSF concentration versus disability endpoints by 6 months (box = median and IQR; whisker = range; point = outliers)

<table>
<thead>
<tr>
<th>Cytokine conc.</th>
<th>Clinical Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>log2pg/ml</strong></td>
<td>Good (N=57)</td>
</tr>
<tr>
<td>IFNγ &lt;=30pg/ml</td>
<td>33/56 (59%)</td>
</tr>
<tr>
<td>&gt;30pg/ml</td>
<td>23/56 (41%)</td>
</tr>
<tr>
<td>TNFα</td>
<td>5.87 (4.95,6.80)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>10.37 (9.6,11.69)</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>9.43 (8.85,10.10)</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>2.19 (1.00,3.71)</td>
</tr>
<tr>
<td>IL-6</td>
<td>8.20 (5.51,9.84)</td>
</tr>
<tr>
<td>IL-8</td>
<td>10.38 (9.14,11.9)</td>
</tr>
<tr>
<td>IL-12</td>
<td>2.69 (1.55,3.58)</td>
</tr>
<tr>
<td>IL-4</td>
<td>4.66 (4.03,4.96)</td>
</tr>
<tr>
<td>IL-10</td>
<td>3.49 (1.98,4.72)</td>
</tr>
<tr>
<td>IL-17</td>
<td>3.17 (2.30,4.09)</td>
</tr>
</tbody>
</table>

Table 8-7 Comparison of baseline log2 cytokine concentrations by clinical outcome at 6 months, shown as median (IQR) for continuous data and n (%) for categorical data. Statistical testing with the Wilcoxon rank-sum test for continuous data and Chi-square test for categorical data.
8.7.3 Impact of dexamethasone on longitudinal immune responses in CSF

The results of the univariate mixed model of cytokine data by treatment arm are displayed graphically in Figure 8-8.

Figure 8-8 Concentrations of IL-10, IL-4, TNFα, and TNFα:IL-10 over time. All data from patients receiving placebo are shown in blue, from those receiving dexamethasone in red. Bold lines in blue and red are the linear regressions from the univariate model. The dashed line is the lower limit of detection for each cytokine.

Full results of the regression are displayed in Table 8-8, and show that TNF-α concentrations declined faster in the dexamethasone than the placebo arm over the first seven days of treatment (coefficient of regression lines -0.16 (95%CI -0.24 to -0.07) adjusted p-value <0.001). Dexamethasone was also associated with more rapid declines in the TNF-α: IL-10 ratio over the first seven days (-0.14 (-0.21 to -0.06) adjusted p-value <0.001), indicative of a shift to a Th2 type immune response. Because the majority of patients (55%) already had
IFN-γ concentrations below the lower limit of detection at baseline, we dichotomised the variable at above or below 30pg/ml, and included it in the longitudinal model as an odds ratio.

The level of 30pg/ml was selected based on the approximate mid-point of our IFNγ log2 concentration distribution curve. However, this dichotomization meant the planned analyses based on IFNγ : IL4 ratios could not be performed.

<table>
<thead>
<tr>
<th>Impact of dexamethasone on slope (log pg/ml/day)</th>
<th>Lower 95%CI</th>
<th>Upper 95%CI</th>
<th>Unadjusted p-value</th>
<th>Adjusted p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ (log OR)</td>
<td>-0.02</td>
<td>-1.27</td>
<td>1.24</td>
<td>0.98</td>
</tr>
<tr>
<td>TNF-α (log2 pg/ml)</td>
<td>-0.16</td>
<td>-0.24</td>
<td>-0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml)</td>
<td>-0.01</td>
<td>-0.05</td>
<td>0.04</td>
<td>0.73</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml)</td>
<td>0.00</td>
<td>-0.09</td>
<td>0.09</td>
<td>1.00</td>
</tr>
<tr>
<td>log2 TNF-α : log2 IL-10</td>
<td>-0.14</td>
<td>-0.21</td>
<td>-0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log2 IFN-γ : log2 IL-4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 8-8 Results of univariate mixed model of longitudinal cytokine concentrations by treatment arm. IFN-γ is presented as an odds ratio of being above 30pg/ml. P-values were adjusted with the Hochberg method.

**8.7.4 Impact of longitudinal CSF cytokine concentrations on mortality and EFA**

**8.7.4.1 Mortality**

The results of analyses of variance on the logistic regression model of cytokine concentration slope vs mortality at 10 weeks and 6 months are presented in Table 8-9. In this analysis, we found no evidence that the rate of change in cytokine concentrations explained the variance in mortality at 10 weeks or 6 months.

<table>
<thead>
<tr>
<th>Cytokine slope</th>
<th>ANOVA for 10 weeks mortality model (p-value)</th>
<th>ANOVA for 6 months mortality model (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ (log OR/day)</td>
<td>0.22</td>
<td>0.10</td>
</tr>
<tr>
<td>TNFα (log2 pg/ml/day)</td>
<td>0.85</td>
<td>0.79</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml/day)</td>
<td>0.69</td>
<td>0.57</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml/day)</td>
<td>0.23</td>
<td>0.36</td>
</tr>
<tr>
<td>TNFα : IL-10 (/day)</td>
<td>0.74</td>
<td>0.77</td>
</tr>
<tr>
<td>IFNγ : IL-4 (/day)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 8-9 Results of ANOVA on logistic regression model for 10 week and 6 month mortality (p<0.05 is significant). IFN is presented as an odds ratio of being above 30pg/ml.
The full results of the logistic regression are shown in Table 8-10, confirming the findings from the ANOVA. Furthermore, no significant effect was noted when an interaction term for dexamethasone was added.

<table>
<thead>
<tr>
<th>Cytokine slope</th>
<th>Mortality at 10 weeks</th>
<th>Mortality at 6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>IFN-γ (log OR/day)</td>
<td>1.15 (0.85, 1.58)</td>
<td>0.37</td>
</tr>
<tr>
<td>TNF-α (log2 pg/ml/day)</td>
<td>3.02 (0.05, 201.27)</td>
<td>0.61</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml/day)</td>
<td>1.14 (0.01, 134.58)</td>
<td>0.96</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml/day)</td>
<td>2.62 (0.03, 196.14)</td>
<td>0.66</td>
</tr>
<tr>
<td>TNFα : IL-10 (/day)</td>
<td>2.29 (0.25, 21.25)</td>
<td>0.46</td>
</tr>
<tr>
<td>IFNγ : IL-4 (/day)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 8-10 Results of logistic regression on cytokine slope for 10 weeks and 6 month mortality

8.7.4.2 Early fungicidal activity

The linear regression on EFA by treatment arm is shown graphically in Figure 8-9, showing that dexamethasone was associated with slower rates of decline of fungal counts in this study population (as already shown for the overall CryptoDex study population in Chapter 1).
Figure 8-9 Fungal counts (CFU/ml) over time (days) by treatment arm. Dashed line is the lower limit of detection for fungal counts.

The results of the correlation analyses on the bivariate mixed model of longitudinal cytokine concentrations and EFA are presented in Table 8-11. They show a strong negative correlation between the rate of decline of IL-10 and EFA, and a moderate negative correlation between TNFα and EFA (ie. faster rates of decline in these cytokine concentrations were associated with slower rates of fungal clearance). The interaction term for dexamethasone in this model showed that dexamethasone had a statistically significant association with the slope of TNFα (coefficient -0.26, p<0.001) and IL-4 (-0.13, p=0.01), but not IL-10 (-0.14, p=0.3). In all cases, dexamethasone was associated with faster rates of decline in the individual cytokine’s concentration.
### Table 8-11 Correlation coefficient (95% confidence interval) between cytokine slope and early fungicidal activity in the seven days following randomization.

<table>
<thead>
<tr>
<th>Cytokine slope</th>
<th>Correlation coefficient with early fungicidal activity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFα (log2 pg/ml/day)</td>
<td>-0.65 (-0.85 to -0.27)</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml/day)</td>
<td>-0.42 (-0.76 to 0.1)</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml/day)</td>
<td>-0.76 (-0.93 to -0.29)</td>
</tr>
</tbody>
</table>

8.7.5 **Prevalence of polymorphisms of the LTA4H gene in study population**

The proportion of patients from Vietnamese and Thai vs Ugandan sites with the three LTA4H genotypes are presented in Figure 8-10. The Hardy-Weinberg equilibrium was confirmed for all groups, indicating that the distribution of genotypes conforms to a hypothetical stable distribution. Furthermore, I note that the genotype distribution of Vietnamese participants in this study did not differ from the broader Vietnamese population (Chi-Squared test $p=0.35$). Across the whole study population, we identified 20 patients with the TT genotype, 122 with the TC genotype, and 201 with the CC genotype (see Table 8-12). The pro-inflammatory TT genotype was more prevalent in Asian that African participants (10% vs 1%, $p <0.001$).
Figure 8-10 LTA4H Genotype distribution by site (n=343). Testing for Hardy-Weinberg equilibrium gave p-values >0.05 for all groups, indicating the condition was met.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Vietnam Number (%) [n=105]</th>
<th>Thailand Number (%) [n=66]</th>
<th>Asian Sites Combined Number (%) [n=171]</th>
<th>Uganda Number (%) [n=172]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>9 (9%)</td>
<td>9 (14%)</td>
<td>18 (11%)</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>TC</td>
<td>53 (50%)</td>
<td>34 (52%)</td>
<td>87 (51%)</td>
<td>35 (20%)</td>
</tr>
<tr>
<td>CC</td>
<td>43 (41%)</td>
<td>23 (35%)</td>
<td>66 (39%)</td>
<td>135 (78%)</td>
</tr>
</tbody>
</table>

Table 8-12 Genotype by site. Chi-Squared testing of difference between proportions in Asian vs African sites gives p <0.001.

8.7.6 Impact of LTA4H polymorphisms on inflammatory profiles

Baseline white cell counts and fungal burden by genotype are compared in box plots in figure 8-11, and baseline concentrations of all cytokines by genotype are presented in Figure 8-12.
Figure 8-11 Baseline white cell counts (log10 cell/ml of CSF) and baseline fungal counts (log10 colony forming unit (CFU) /ml CSF, by genotype (box = median and IQR; whisker = range; point = outliers)

Figure 8-12 Concentrations of baseline cytokine (box = median and IQR; whisker = range; point = outliers) by LTA4H genotype
I found that baseline fungal counts were lower in patients with the TT genotype, than in patients with the CT or CC groups (TT 3.44 (95% CI 2.60 to 4.87) vs CT 4.92 (3.05 to 5.80) vs CC 4.04 (1.90 to 5.43) log10 CFU per ml of CSF (p= 0.004)). White cell count did not vary by genotype. Although baseline concentrations of IFNγ and TNFα appeared higher in the TT group, this difference failed to reach statistical significance. Full statistical comparisons are presented in Table 8-13.

<table>
<thead>
<tr>
<th></th>
<th>TT Genotype</th>
<th>TC Genotype</th>
<th>CC Genotype</th>
<th>Comparison (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ (log OR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=7</td>
<td>N=56</td>
<td>N=156</td>
<td></td>
<td>0.521</td>
</tr>
<tr>
<td>- &lt;=30[pg/ml]</td>
<td>4/7 (57%)</td>
<td>39/56 (70%)</td>
<td>112/152 (74%)</td>
<td></td>
</tr>
<tr>
<td>- &gt;30[pg/ml]</td>
<td>3/7 (43%)</td>
<td>17/56 (30%)</td>
<td>40/152 (26%)</td>
<td></td>
</tr>
<tr>
<td>TNFα (log2 pg/ml)</td>
<td>7.17(5.50,7.65)</td>
<td>5.61(4.79,6.67)</td>
<td>5.61(4.29,7.04)</td>
<td>0.547</td>
</tr>
<tr>
<td>MCP-1 (log2 pg/ml)</td>
<td>10.85(10.05,11.98)</td>
<td>10.64(9.59,12.07)</td>
<td>10.55(9.60,11.88)</td>
<td>0.519</td>
</tr>
<tr>
<td>MIP-1a (log2 pg/ml)</td>
<td>9.88(8.62,10.16)</td>
<td>9.55(9.08,10.16)</td>
<td>9.43(8.68,10.05)</td>
<td>0.644</td>
</tr>
<tr>
<td>GM-CSF (log2 pg/ml)</td>
<td>1.60(-0.42,4.56)</td>
<td>1.80(-0.24,3.53)</td>
<td>2.05(0.57,3.52)</td>
<td>0.771</td>
</tr>
<tr>
<td>IL-6 (log2 pg/ml)</td>
<td>7.14(6.01,9.78)</td>
<td>7.08(4.93,8.81)</td>
<td>7.42(5.16,9.48)</td>
<td>0.768</td>
</tr>
<tr>
<td>IL-8 (log2 pg/ml)</td>
<td>10.67(9.74,11.96)</td>
<td>10.22(8.88,11.91)</td>
<td>10.18(8.77,11.66)</td>
<td>0.815</td>
</tr>
<tr>
<td>IL-12 (log2 pg/ml)</td>
<td>2.66(2.09,3.08)</td>
<td>2.75(1.55,3.28)</td>
<td>2.84(1.55,3.48)</td>
<td>0.388</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml)</td>
<td>5.10(3.77,5.13)</td>
<td>4.83(4.22,5.08)</td>
<td>4.59(4.02,4.94)</td>
<td>0.308</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml)</td>
<td>3.05(0.39,5.61)</td>
<td>2.66(0.90,4.15)</td>
<td>3.44(1.79,4.91)</td>
<td>0.065</td>
</tr>
<tr>
<td>IL-17 (log2 pg/ml)</td>
<td>2.21(2.07,2.99)</td>
<td>2.80(2.13,3.28)</td>
<td>2.73(1.66,3.54)</td>
<td>0.845</td>
</tr>
<tr>
<td>Fungal count (log10 CFU/ml CSF) N=19</td>
<td>N=112</td>
<td>N=192</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>White cell count (log10 cells/ml CSF) N=20</td>
<td>N=117</td>
<td>N=194</td>
<td>0.464</td>
<td></td>
</tr>
</tbody>
</table>

Table 8-13 Comparison of baseline log10 cytokine concentrations, white cell counts, and fungal counts by genotype, shown as median (IQR) for continuous data and n (%) for categorical data. Statistical testing with the Wilcoxon rank-sum test for continuous data and Chi-square test for categorical data.

The results of the ANOVA on the univariate mixed model for longitudinal samples vs. the LTA4H genotype are summarized in Table 8-14, showing no overall impact of genotype on the dynamics of individual cytokines.
ANOVA for impact of genotype on cytokine concentration slope (p-value)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ (log OR)</td>
<td>0.78</td>
</tr>
<tr>
<td>TNFα (log2 pg/ml)</td>
<td>0.23</td>
</tr>
<tr>
<td>MCP-1 (log2 pg/ml)</td>
<td>0.65</td>
</tr>
<tr>
<td>MIP-1a (log2 pg/ml)</td>
<td>0.52</td>
</tr>
<tr>
<td>GM-CSF (log2 pg/ml)</td>
<td>0.33</td>
</tr>
<tr>
<td>IL-6 (log2 pg/ml)</td>
<td>0.98</td>
</tr>
<tr>
<td>IL-8 (log2 pg/ml)</td>
<td>0.53</td>
</tr>
<tr>
<td>IL-12 (log2 pg/ml)</td>
<td>0.02</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml)</td>
<td>0.74</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml)</td>
<td>0.24</td>
</tr>
<tr>
<td>IL-17 (log2 pg/ml)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Table 8-14 Results of ANOVA on univariate mixed model for longitudinal cytokine concentrations vs LTA4H genotype (<0.05 is significant). IFN is presented as an odds ratio of being above 30pg/ml.

However, full results of the model are presented in Table 8-15. They show that the rate of decline of TNFα was generally slower in the TT than CT (slope coefficient -0.96 95%CI (-2.28 to 0.37), p=0.16) and CC groups (-1.29 (-2.55 to -0.02), p=0.05). The different genotypes responded differently to dexamethasone; TT patients receiving dexamethasone had a more rapid decline of TNFα than their counterparts in CT (0.41 (0.02 to 0.81), p=0.04), or CC groups (0.44 (0.05 to 0.82), p=0.03).
<table>
<thead>
<tr>
<th></th>
<th>TT vs CT</th>
<th>TT vs CC</th>
<th>Impact of dex on TT vs CT coefficient</th>
<th>Impact of dex on TT vs CC coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>p-value</td>
<td>Coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>IFNγ (log OR)</td>
<td>1.39 (-3.49 to 6.28)</td>
<td>0.58</td>
<td>1.77 (-2.86 to 6.41)</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td><strong>0.05 (-1.09 to 1.20)</strong></td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (log2 pg/ml)</td>
<td>-0.96 (-2.28 to 0.37)</td>
<td>0.16</td>
<td>-1.29 (-2.55 to -0.02)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.41 (0.02 to 0.81)</td>
<td>0.04</td>
<td>0.44 (0.05 to 0.82)</td>
<td>0.03</td>
</tr>
<tr>
<td>MCP-1 (log2 pg/ml)</td>
<td>0.57 (-0.46 to 1.6)</td>
<td>0.28</td>
<td>0.37 (-0.62 to 1.36)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>-0.15 (-0.5 to 0.2)</td>
<td>0.41</td>
<td>-0.19 (-0.53 to 0.15)</td>
<td>0.28</td>
</tr>
<tr>
<td>MIP-1a (log2 pg/ml)</td>
<td>-0.23 (-0.98 to 0.51)</td>
<td>0.54</td>
<td>-0.32 (-1.04 to 0.4)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>0.04 (-0.2 to 0.29)</td>
<td>0.72</td>
<td>-0.01 (-0.25 to 0.22)</td>
<td>0.92</td>
</tr>
<tr>
<td>GM-CSF (log2 pg/ml)</td>
<td>-0.4 (-2.04 to 1.24)</td>
<td>0.63</td>
<td>-0.21 (-1.79 to 1.37)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>1.34 (-0.29 to 2.97)</td>
<td>0.11</td>
<td>1.4 (-0.23 to 3.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>IL-6 (log2 pg/ml)</td>
<td>-0.49 (-2.79 to 1.81)</td>
<td>0.68</td>
<td>-0.46 (-2.69 to 1.76)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>0.47 (-0.59 to 1.53)</td>
<td>0.38</td>
<td>0.42 (-0.62 to 1.47)</td>
<td>0.43</td>
</tr>
<tr>
<td>IL-8 (log2 pg/ml)</td>
<td>-0.45 (-1.64 to 0.75)</td>
<td>0.46</td>
<td>-0.69 (-1.84 to 0.45)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>0.36 (-0.11 to 0.82)</td>
<td>0.14</td>
<td>0.4 (-0.05 to 0.85)</td>
<td>0.08</td>
</tr>
<tr>
<td>IL-12 (log2 pg/ml)</td>
<td>0.15 (-0.58 to 0.88)</td>
<td>0.69</td>
<td>0.35 (-0.35 to 1.05)</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>0.27 (-0.37 to 0.92)</td>
<td>0.41</td>
<td>0.35 (-0.29 to 0.99)</td>
<td>0.28</td>
</tr>
<tr>
<td>IL-4 (log2 pg/ml)</td>
<td>0.32 (-0.53 to 1.17)</td>
<td>0.46</td>
<td>0.36 (-0.46 to 1.17)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>-0.01 (-0.25 to 0.24)</td>
<td>0.94</td>
<td>0.04 (-0.2 to 0.28)</td>
<td>0.75</td>
</tr>
<tr>
<td>IL-10 (log2 pg/ml)</td>
<td>-0.66 (-1.97 to 0.66)</td>
<td>0.33</td>
<td>-0.07 (-1.33 to 1.19)</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>0.25 (-0.2 to 0.7)</td>
<td>0.28</td>
<td>0.26 (-0.18 to 0.69)</td>
<td>0.25</td>
</tr>
<tr>
<td>IL-17 (log2 pg/ml)</td>
<td>0.48 (-0.53 to 1.48)</td>
<td>0.35</td>
<td>0.35 (-0.62 to 1.32)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>-0.2 (-0.56 to 0.17)</td>
<td>0.29</td>
<td>-0.2 (-0.55 to 0.15)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 8-15 Results of univariate mixed model on longitudinal cytokine concentration, genotype and treatment arm. 'dex' = dexamethasone. **Impact of dexamethasone in TT vs CT and CC.
8.7.7 Effect of dexamethasone on mortality by LTA4H genotype

Kaplan-Meier curves by treatment arm, stratified by LTA4H genotype, are displayed up to 10 weeks in Figure 8-13, and up to 6 months in Figure 8-14. In these charts, survival appears better for TT patients receiving dexamethasone than TT patients receiving placebo. This is in contrast to CC and CT patients who appear to have worse outcomes with dexamethasone (a pattern similar to the overall survival outcomes in the CryptoDex trial). This effect is further highlighted in the final set of Kaplan-Meier charts, Figure 8-15, with outcomes by genotype split into two charts – one for placebo and one for dexamethasone.

The hazard ratios for mortality from the Cox regression model are presented alongside the relevant Kaplan-Meier charts, in Table 8-16, Table 8-17, and Table 8-18. These show, for example, that dexamethasone was associated with increased mortality from day 21 to day 70 for CC (HR 2.86 (95% CI 1.33 to 6.15)) and CT patients (HR 3.32 (95% CI 1.32 to 8.35)), but not TT patients (HR 0.34 (95% CI 0.05 to 2.53)). CC and CT patients had lower hazards of mortality than TT patients in the placebo arm, but higher hazards of mortality in the dexamethasone arm.
Figure 8-13 Kaplan-Meier curves of survival up to 10 weeks in placebo (blue) and dexamethasone (red) arms. Displayed by all participants and those with each of the three LTA4H genotypes: CC, CT, and TT (highlighted).

**Table 8-16 Hazard ratios from Cox regression on 10 week mortality related to dexamethasone therapy, by genotype, with time-dependent variable to account for non-proportional hazards.**

<table>
<thead>
<tr>
<th></th>
<th>Up to day 21</th>
<th>Day 21 - day 70</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (CI)</td>
<td>HR (CI)</td>
</tr>
<tr>
<td>All</td>
<td>0.82 (0.54 to 1.26)</td>
<td>2.34 (1.80 to 2.88)</td>
</tr>
<tr>
<td>CC</td>
<td>1.03 (0.61 to 1.74)</td>
<td>2.86 (1.33 to 6.15)</td>
</tr>
<tr>
<td>CT</td>
<td>0.65 (0.32 to 1.31)</td>
<td>3.32 (1.32 to 8.35)</td>
</tr>
<tr>
<td>TT</td>
<td>0.31 (0.06 to 1.59)</td>
<td>0.34 (0.05 to 2.53)</td>
</tr>
</tbody>
</table>

Analysis corrected for baseline fungal count, Glasgow coma score, opening pressure of CSF, CSF white cell count, and participant’s country of origin.
Figure 8-14 Kaplan-Meier curves of survival up to 6 months in placebo (blue) and dexamethasone (red) arms. Displayed by all participants and those with each of the three LTA4H genotypes: CC, CT, and TT (highlighted).

### Time-dependent hazard ratio for mortality related to dexamethasone therapy, within each genotype

<table>
<thead>
<tr>
<th></th>
<th>Up to day 21</th>
<th>Day 22 - day 43</th>
<th>Day 43 - 180</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (CI)</td>
<td>HR (CI)</td>
<td>HR (CI)</td>
</tr>
<tr>
<td>All</td>
<td>0.81 (0.53 to 1.23)</td>
<td>2.29 (1.07 to 4.87)</td>
<td>6.85 (2.45 to 19.15)</td>
</tr>
<tr>
<td>CC</td>
<td>0.56 (0.22 to 1.42)</td>
<td>1.59 (0.51 to 5)</td>
<td>4.09 (1 to 16.74)</td>
</tr>
<tr>
<td>CT</td>
<td>0.44 (0.16 to 1.19)</td>
<td>1.32 (0.09 to 18.67)</td>
<td>5.83 (1.24 to 27.42)</td>
</tr>
<tr>
<td>TT</td>
<td>0.33 (0.07 to 1.68)</td>
<td>0.76 (0.08 to 6.91)</td>
<td>0.8 (0.03 to 21.88)</td>
</tr>
</tbody>
</table>

Table 8-17 Hazard ratios from Cox regression on 6 month mortality related to dexamethasone therapy, by genotype, with time-dependent variable to account for non-proportional hazards. Analysis corrected for baseline fungal count, Glasgow coma score, opening pressure of CSF, CSF white cell count, and participant’s country of origin.
Figure 8-15 Kaplan-Meier curves up to 10 weeks, with survival of patients with the CC (blue), CT (yellow) and TT (red) genotypes shown by placebo (left chart) and dexamethasone (right chart).

### Time-dependent hazard ratio for mortality related to genotype, within each treatment arm

<table>
<thead>
<tr>
<th></th>
<th>Up to day 70</th>
<th>Day 21 - day 70</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT vs TT</td>
<td>0.65 (0.26 to 1.59)</td>
<td></td>
</tr>
<tr>
<td>CC vs TT</td>
<td>0.54 (0.22 to 1.33)</td>
<td></td>
</tr>
<tr>
<td><strong>Dexamethasone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT vs TT</td>
<td>1.34 (0.29 to 6.19)</td>
<td>4.06 (0.53 to 31.19)</td>
</tr>
<tr>
<td>CC vs TT</td>
<td>1.79 (0.41 to 7.78)</td>
<td>4.66 (0.61 to 35.49)</td>
</tr>
</tbody>
</table>

Table 8-18 Hazard ratios from Cox regression on 10 week mortality related to dexamethasone therapy, by genotype, with time-dependent variable to account for non-proportional hazards. Analysis corrected for baseline fungal count, Glasgow coma score, opening pressure of CSF, CSF white cell count, and participant’s country of origin.

I performed an ANOVA comparing Cox models with and without a genotype:dexamethasone interaction term to assess whether this interaction was able to explain the variance in mortality - the p-value for the 10 week model was 0.19 and for the 6 month model it was 0.37.
The logistic regression model of overall mortality by genotype gave odds ratios for mortality at ten weeks (95% CI) of 0.63 (0.09 to 4.29) for CT vs TT, and 0.66 (0.1 to 4.22) for CC vs TT. By six months, these results were 0.99 (0.14 to 6.79) for CT vs TT, and 0.75 (0.11 to 4.86) for CC vs TT.

8.7.8 Additional planned auxiliary analyses

Our estimates on the ability of all baseline variables (including cytokine concentrations) to predict mortality at 10 weeks using Lasso and stepwise AIC methods on all variables are presented in Table 8-19.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of appearances per variable</th>
<th>10 week mortality</th>
<th>6 month mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>log₂ IL-4</td>
<td></td>
<td>Lasso 0 Stepwise AIC 13</td>
<td>Lasso 6 Stepwise AIC 10</td>
</tr>
<tr>
<td>log₂ IL-6</td>
<td></td>
<td>1 20</td>
<td>19 20</td>
</tr>
<tr>
<td>log₂ IL-8</td>
<td></td>
<td>1 0</td>
<td>0 0</td>
</tr>
<tr>
<td>log₂ IL-10</td>
<td></td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>log₂ IL-12</td>
<td></td>
<td>0 4</td>
<td>3 1</td>
</tr>
<tr>
<td>log₂ GM-CSF</td>
<td></td>
<td>1 6</td>
<td>6 13</td>
</tr>
<tr>
<td>log₂ IFNγ</td>
<td></td>
<td>5 8</td>
<td>10 9</td>
</tr>
<tr>
<td>log₂ TNFα</td>
<td></td>
<td>0 0</td>
<td>1 0</td>
</tr>
<tr>
<td>log₂ MCP-1</td>
<td></td>
<td>20 2</td>
<td>20 0</td>
</tr>
<tr>
<td>log₂ MIP-1a</td>
<td></td>
<td>0 20</td>
<td>6 20</td>
</tr>
<tr>
<td>log₂ IL-17</td>
<td></td>
<td>20 0</td>
<td>20 0</td>
</tr>
<tr>
<td>log₁₀ CSF fungal count</td>
<td></td>
<td>13 11</td>
<td>7 20</td>
</tr>
<tr>
<td>Genotype TT</td>
<td></td>
<td>7 -</td>
<td>6 -</td>
</tr>
<tr>
<td>Genotype TC</td>
<td></td>
<td>0 20</td>
<td>0 20</td>
</tr>
<tr>
<td>Genotype CC</td>
<td></td>
<td>0 9</td>
<td>10 4</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td>19 9</td>
<td>19 1</td>
</tr>
<tr>
<td>GCS</td>
<td></td>
<td>20 0</td>
<td>20 0</td>
</tr>
<tr>
<td>Africa vs Asia</td>
<td></td>
<td>13 20</td>
<td>18 20</td>
</tr>
</tbody>
</table>

Table 8-19 Results of Lasso and Stepwise AIC variable selection analyses, to identify best predictors of mortality at 10 weeks and 6 months

8.8 Discussion

In this chapter I used baseline and longitudinal CSF cytokine concentrations to describe the immune response in HIV-associated cryptococcal meningitis in relation to dexamethasone
therapy and the patient’s LTA4H genotype. I then looked for how these factors were associated with clinical and microbiological outcomes.

I found that dexamethasone has a measurable impact on the cytokine profile in the CSF of patients with HIV associated cryptococcal meningitis. It appears to accelerate the shift in the immune response from a Th1- to a Th2-type response, as indicated by accelerated rates of decline of TNF-α relative to IL-10. The rate of decline of TNF-α was negatively correlated with EFA, which may explain the observed impact of dexamethasone on microbiological outcomes – it was established in the CryptoDex trial, and confirmed in this subset of patients, that dexamethasone slows the rate of fungal clearance. However, although EFA is often used as a surrogate marker for treatment success, in this study I found no statistically significant evidence for an impact of cytokine dynamics on mortality or disability.

Interestingly, the general harmful effect of dexamethasone was not universal. When I investigated the role of polymorphisms of the LTA4H gene I found that patients with the hyperinflammatory TT genotype may actually benefit from dexamethasone.

I will discuss my findings with regard to the hypotheses stated in section 8.3.

8.8.1.1 Does dexamethasone have a significant impact on inflammatory response? Specifically, does dexamethasone reduce the concentration of IFN-γ relative to the placebo group?

I did not find IFN-γ to be a useful longitudinal marker in this study. At baseline, 55% of patients had CSF IFN-γ concentrations below the lower limit of detection, and the proportion rapidly increased over the seven days from randomization. We attempted to include it in analyses as a dichotomized variable (above or below 30pg/ml), but this meant we could not do the planned analyses of IFN-γ : IL-4 ratios, and we lost richness in the data. As a result of this, I was unable to reject the null hypothesis that dexamethasone has no effect on IFN-γ concentrations.
However, other cytokine parameters provided evidence of dexamethasone having a significant effect on the immune response over the first seven days. TNF-α CSF concentrations fell significantly more quickly in patients receiving dexamethasone, than those receiving placebo. Furthermore, the ratio of TNF-α : IL-10 also fell more quickly – this ratio is used as an indicator of the underlying balance of Th1 : Th2 immune responses. Its quicker decline in patients receiving dexamethasone indicates that such therapy may be accelerating the shift from a Th1 to a Th2 immune response. These impacts of corticosteroid therapy are consistent with the literature described in my introduction; however, this is the first time they have been described in HIV-associated cryptococcal meningitis. Indeed, apart from Mai et al’s 2009 paper on corticosteroids in bacterial meningitis (328), no other study has described a clear impact of corticosteroids on CSF cytokine concentrations for any infectious meningitis.

8.8.1.2 Are different patterns of baseline and longitudinal immune response associated with patient outcomes? And is the association different between dexamethasone and placebo groups?

First, addressing the association of baseline cytokine concentrations and clinical outcome, these data lend additional support to the understanding that higher baseline concentrations of IFN-γ are associated with reduced mortality in HIV-associated cryptococcal meningitis. At both 10 weeks and 6 months, a higher percentage of participants with good or intermediate outcomes had IFN-γ >30pg/ml CSF, when compared to those with a poor outcomes and those who died. This difference was statistically significant at 10 weeks, and just failed to reach significance at 6 months. No other individual baseline cytokine concentrations had a clear relationship with outcome.

Given the role of T-helper type 1 cells and M1 activated macrophages in clearing cryptococcal infection described in my introduction, I would have expected to see higher concentrations of TNF-α in the CSF of patients with good outcomes. However, recent work by Scriven et al (337) and Jarvis et al (338) showed that it is the capacity of stimulated cellular
effectors to secrete TNF-α, rather than the concentration of circulating cytokine, that is associated with outcome. I did not assess cell phenotypes in this study, so would have missed this role of TNF-α in outcome. No previous human studies have shown a relationship between higher Th2 type cytokines and clinical outcome, and none was seen here.

A primary focus of this study was to look at the impact of longitudinal cytokine profiles on outcome. We estimated the slope of cytokine concentration over the first week of treatment using linear regression, and assessed the impact of this parameter on mortality. The biggest effect size was seen in the slope of TNF-α – an increase in the rate of decline of 1 log2 pg/ml/day was associated with odds ratios for mortality (95% CI) of 3.02 (0.05 to 201.27) at 10 weeks and 2.28 (0.04 to 148.85) at 6 months. However, for this and all other parameters the confidence intervals were extremely wide, and p-values non-significant. This likely reflects the large inter-patient variability and resultant imprecision in estimates of the slope of cytokine concentration decline over time.

With no statistically significant effect demonstrated for any cytokine slope’s impact on mortality, we were unable to reject the null hypothesis. Models including a dexamethasone interaction term didn’t differ significantly from models without this interaction term, so we were also unable to show a dexamethasone mediated effect of cytokine profile on mortality.

However, we did identify an interesting interaction between cytokine concentration slope and early fungicidal activity (EFA), which is frequently used as a surrogate marker of clinical outcome. The model used for this part of the study is complex, and it was necessary to limit the cytokine variables to TNF-α, IFN-γ, IL-4, and IL-10. However, because of the high proportion of IFN-γ concentrations under the lower limit of detection, imputation of missing values was not appropriate, and the final model contained just TNF-α, IL-4, and IL-10. We found significant negative correlations between the slopes of TNF-α and IL-10 and the rate of decline of CSF fungal counts. This means that faster declines in the concentrations of these
cytokines were associated with slower fungal clearance. As we identified above, dexamethasone was associated with faster declines of TNF-α, and in the current model the interaction term for dexamethasone was statistically significant.

So, although we were unable to demonstrate an effect of longitudinal cytokine slopes on mortality, we have demonstrated that cytokine concentration slopes are associated with fungal clearance, and that this effect is mediated by dexamethasone. It is well established that the use of TNF-α antagonists predispose humans to invasive fungal infections, including cryptococcal infections (339). A 2016 article by Xu et al used a mouse model to demonstrate the central role of TNF-α in fighting cryptococcal infections - mice deficient in TNF-α failed to mount an appropriate Th1 response, had more disseminated disease, and failed to clear infection (340). Furthermore, it has previously been shown that even transient inhibition of TNF-α in mice can lead to an abnormal initial immune response to Cryptococcus, and predispose to chronic infection (341). Our data are the first to demonstrate the association between corticosteroid-mediated TNF-α depletion, and reduced capacity to clear cryptococcal infection, in real-world human participants.

8.8.1.3 Is the TT LTA4H genotype associated with hyper-inflammatory CSF response as defined by higher levels of IFN-γ and TNF-α?

More participants with the TT LTA4H genotype had IFN-γ >30pg/ml than those with the CT or CC genotypes (43% vs 30% and 26% respectively). However, the difference didn’t reach statistical significance. Similarly, TT participants had higher concentrations of TNF-α than their counterparts in the CT and CC groups (7.17 vs. 5.61 and 5.61 respectively) although again the difference was not statistically significant. I was unable to reject the null hypothesis in either case. There were only 20 participants in the TT genotype group, so we lacked power. There was no difference in baseline CSF white cell counts. However, we did identify that baseline fungal counts were lower in the TT group than CT or CC groups (3.44 log10 CFU/ml CSF (95% CI
2.60 to 4.87) for TT vs. 4.92 (3.05 to 5.80) for CT and 4.04 (1.90 to 5.43) for CC; p=0.004), and lower baseline fungal counts have been linked to lower baseline concentrations of IFN-γ and TNF-α (184).

Interestingly, in TB meningitis, Thuong et al (342) saw some similarities in baseline CSF cytokine concentrations to our observations. Their results are shown in Figure 8-16. Concentrations of IFN-γ and TNF-α also appear higher in TT patients, though failing to reach statistical significance. Thuong et al detected a statistically significant difference in IL-6 concentrations between the genotypes, but we see no evidence of this in our participants. Another difference is that their plot for HIV-positive patients shows no variability in median cytokine concentrations, in contrast to ours (which only includes HIV positive patients).
Are there beneficial effects of corticosteroids on clinical outcome for patients with the TT LTA4H genotype?

We showed that corticosteroids did not reduce mortality in any of the predefined subgroups in the CryptoDex trial, described in chapter 1. However, given the beneficial effects of corticosteroids in patients with the TT LTA4H genotype in tuberculous meningitis (TBM) (342), we were eager to see if this new subgroup also benefited from dexamethasone in cryptococcal meningitis.

The Kaplan-Meier charts presented in my results section bear a striking resemblance to those published by Tobin et al in 2012 for TB meningitis (333) (see Figure 8-2).
In both cases, TT patients go from having the worst survival to having the best, when given dexamethasone. In contrast, the Kaplan-Maier charts suggest a small survival benefit for TBM patients with the CT genotype receiving dexamethasone; in our cryptococcal meningitis patients, both CT and CC groups appear to have worse survival with dexamethasone than placebo.

Because hazards were non-proportional in the CryptoDex trial, we used a time dependent variable in our Cox regressions. For the 10 week survival model, in the first 21 days hazard ratios for mortality with dexamethasone compared to placebo were: CC 1.03 (85% CI 0.61 to 1.74), CT 0.65 (0.32 to 1.31) and TT 0.31 (0.06 to 1.59), with the most pronounced effect seen in the TT group. Unfortunately, perhaps due to the low number of TT patients, confidence intervals crossed 1, in all cases. However, from day 21 to day 70, hazard ratios by genotype had diverged considerably: CC 2.86 (1.33 to 6.15), CT 3.32 (1.32 to 8.35), and TT 0.34 (0.05 to 2.53), with clear evidence of harm for CC and CT, and an indication of benefit for TT (the 95% CI still crosses 1 for TT). A similar pattern was seen in the 6 month Cox survival model. When comparing genotype survival within treatment arms, the appearances of the Kaplan-Meier charts were confirmed; TT patients have worse survival in the placebo arm, and better survival in the dexamethasone arm, compared to CC and CT patients.

Perhaps due to the low number of TT patients, I am unable to reject the null hypothesis that dexamethasone offers no survival benefit to TT patients with HIV-associated cryptococcal meningitis. Also, the result of the analysis of the interaction between LTA4H genotype and dexamethasone with regards to longitudinal TNF-α concentration strikes a note of concern: TT patients receiving corticosteroids had a faster rate of decline in TNF-α, which correlated with slower rates of fungal clearance. However, TT patients started with a higher concentration of TNF-α, and it is not possible to draw a strong conclusion on this. Overall, I find there are sufficient indicators here that corticosteroids benefit the TT LTA4H group to justify future
genotype specific studies, and these could monitor any impact of reduced fungal clearance on survival.

### 8.8.2 Limitations

This study was powered to the primary end-point, which was the effect of dexamethasone on cytokine concentrations, specifically IFN-\( \gamma \). Unfortunately, concentrations of IFN-\( \gamma \) were too often below the lower limit of detection to allow the planned analyses to be performed. I used different cytokine kits to those used in the study on which I based the power calculation, and it may have had lower sensitivity for IFN-\( \gamma \). In retrospect, it may have been better to have used the high sensitivity IFN-\( \gamma \) assay, instead of the standard version. This may have given me additional power to identify IFN-\( \gamma \) related effects, but it is hard to see how it would have materially changed my conclusions.

A major limitation of this study is inherent in all retrospective immune response studies. I only measured cytokine responses, and have no data on circulating cell types, nor their activation status. A comprehensive study would require real-time cell phenotyping. It would have been fascinating to describe the underlying cellular immune response in a prospective cohort, and to be able to conclude whether the longitudinal Th1 / Th2 balance is important in cryptococcal meningitis, and to confirm that this was shifted by dexamethasone therapy, and indicated by the cytokine data presented here.

### 8.9 Conclusions

Here I have shown that dexamethasone has a measurable impact on cytokine profiles in HIV associated cryptococcal meningitis. Although I am unable to conclude that these effects have an impact on mortality, I have shown that they do have an effect on the clearance of fungi from the CSF.
The fascinating results with respect to LTA4H genotype may help to explain the worse outcomes for African vs Asian patients receiving dexamethasone. A previously undetected sub-group of patients, those with the TT genotype, may have been getting benefit from dexamethasone – whilst this genotype was common in Vietnam and Thailand (11%), it was uncommon in Uganda (1%).

My data also draw parallels with genotype specific survival in TBM. CM and TBM are the commonest causes of sub-acute meningitis in Vietnam, and outcomes from both can be devastating. The data presented here suggest that targeted dexamethasone therapy may be of benefit in both conditions. A rapid genotyping test would allow targeted therapy, and may improve outcomes for TT patients whilst preventing harmful exposures to corticosteroids for those CC and CT patients unlikely to benefit. This hypothesis should be tested in a randomized controlled trial.
8.10 Statement of contribution

I developed the protocol for this study, as a sub-study of the CryptoDex trial. The study questions arose from the unexpected early cessation of the CryptoDex trial, and I developed appropriate statistical methods to address them with a statistics colleague. We co-authored the statistical analysis plan.

I performed all lab work. The actual statistical analyses were run by myself and a statistics colleague, through an iterative process over several months.

I was first author of, and presented, a poster containing these results at the ICCC in Brazil. I am first author on a draft manuscript relating to this work, which will soon be submitted for publication.
9. Future directions

My overarching research aim in writing this thesis was to better define the problem of cryptococcal meningitis in Vietnam, and to contribute to improving its management. Ultimately, I divided this into four sub-aims:

1. Describe the incidence and prevalence, in Vietnam, of the most serious invasive fungal infections (including cryptococcal meningitis)

2. Determine the effect of adjunctive dexamethasone therapy on clinical and microbiological outcomes in adult patients with HIV-associated cryptococcal meningitis

3. Describe the relationships between early termination of trials and publication of results, and perform a case study on the early termination of the CryptoDex trial

4. Assess the impact of dexamethasone on the immune response in CSF, and determine whether dynamic immune responses are associated with patient outcomes

My findings, which are discussed in detail at the end of each chapter, have addressed some pressing needs. Until recently, research into fungal infections had been neglected. Last year, the Royal Society noted that fungal infections cause more deaths than malaria, tuberculosis, or breast cancer, and held a summer science exhibition to promote research [https://royalsociety.org/news/2016/07/new-treatments-needed-for-fungal-infections/](https://royalsociety.org/news/2016/07/new-treatments-needed-for-fungal-infections/). Ongoing advocacy from groups such as the Global Action Fund for Fungal Infections (GAFFI) is improving the profile of fungal infections, leading to a 2017 Lancet series [http://www.thelancet.com/series/fungal-infections](http://www.thelancet.com/series/fungal-infections). I was keen to contribute to this research effort. First, I contributed directly to GAFFI’s work in estimating the global burden of fungal disease by producing the first estimate of incidence and prevalence from South East Asia. Second, our RCT in cryptococcal meningitis will help to inform future international guidelines.
on the use of adjunctive corticosteroids. Third, I have highlighted some issues particular to the proper conduct of infectious disease clinical trials, with regards to Data Safety Monitoring Boards, reporting adverse events, stopping trials early, and publishing results. Finally, my work on dexamethasone’s impact on immune responses has helped to explain the adverse outcomes seen with adjunctive dexamethasone therapy. My immune response research also indicated a new avenue of enquiry with regards to host inflammatory genotype, which may be important for future research in Asian settings, where the pro-inflammatory LTA4H is prevalent and the burden of fungal infections is likely to be high.

In chapter 1, I estimated there were 140 cases of HIV-associated cryptococcal meningitis in 2012 in Vietnam. Given the previously discussed high mortality and morbidity of cryptococcal meningitis, this conservative estimate still represents a large number. Unfortunately, the evidence from our RCT, presented in chapter 1 was that dexamethasone did not reduce mortality. In fact, it was associated with worse disability outcomes, poorer microbiological responses to therapy, and more adverse events. As discussed in chapter 7 the data monitoring and trial stopping procedures for our trial were appropriate and ethically applied, balancing the need for scientific evidence with the obligation to prevent avoidable harm to trial participants. Although the trial stopped after 451 of a proposed 880 patients had been recruited, we were still able to answer important clinical questions. Furthermore, we were able to use the stored CSF samples from participants to examine the effect of dexamethasone on the immune response. The results of my research into immune responses, presented in chapter 8, provided a plausible mechanistic explanation for why dexamethasone was harmful. This data may aid other researchers working in the area of immune modulation in cryptococcal meningitis to better predict the outcome of their proposed intervention.
9.1 **Unmet research needs**

However, there are still many unanswered questions arising from my research, and I will now discuss potential future directions for research in this area.

9.2 **Fungal disease surveillance in Vietnam**

Two issues arise from my research into the burden of disease. First, as discussed in chapter 5.6, my estimates were based on actuarial methods, and are likely to be conservative. The methods I used have not been validated in a tropical setting such as Vietnam. A formal surveillance programme, incorporating health economic analyses, would greatly advance our knowledge around the burden of fungal diseases in Vietnam, and allow better planning for healthcare resource allocation. It would also provide an opportunity to attempt to validate the actuarial approach I used. Once validated, the actuarial approach could deliver massive cost savings for other developing countries wishing to understand their burden of fungal diseases.

9.3 **Chronic pulmonary aspergillosis in Vietnam**

A second finding of my burden of disease estimates is that *Aspergillus* sp. are likely to be the major fungal pathogens in Vietnam, manifesting as chronic pulmonary aspergillosis (CPA). CPA is a spectrum of diseases causing progressive lung destruction, with an annual mortality rate of 25% (343). It generally affects structurally damaged lungs: most CPA worldwide occurs in survivors of tuberculosis (9). Twelve percent of tuberculosis survivors with residual cavities will develop CPA in the first year after tuberculosis treatment, rising to over 25% after 3-4 years (9,344–346). Vietnam has one of the highest incidences of tuberculosis in the world (239), and cavitation occurs in over 40% of patients (235,264); it is for these reasons I believe CPA is likely to be common in Vietnam.

I estimated around 1,500 patients suffer from CPA each year in Ho Chi Minh City (HCMC) alone (39). In reality, fewer than 100 cases of CPA are actually diagnosed. Based on the
discrepancy between my estimates and the observed cases, I hypothesize that CPA is being substantially under-diagnosed in Vietnam. The likely reasons are:

1. Low clinical suspicion
2. Poor availability of fungal diagnostics
3. Misdiagnosis as relapsed or drug resistant pulmonary tuberculosis

Failure to diagnose CPA is a major concern, since anti-fungal therapy improves clinical outcomes (347). Over 50% of patients with CPA respond favourably to treatment with oral azoles (itraconazole or voriconazole), as recommended by international guidelines (237,343,348–352). Misdiagnosing CPA as tuberculosis denies patients the benefits of anti-fungal therapy but also exposes them to unnecessary anti-tuberculosis therapy. Furthermore, it confounds tuberculosis surveillance programmes. Better understanding the incidence and prevalence of CPA will improve access to beneficial treatments for CPA patients and will enhance the accuracy of tuberculosis surveillance.

The majority of post-tuberculosis CPA research has been undertaken in high-income, temperate settings with low tuberculosis prevalence. Vietnam is a densely-populated low middle income country with a tropical climate and a high burden of tuberculosis. It is representative of many countries in Asia, and is the ideal setting to investigate the interplay between CPA and tuberculosis.

I propose a programme of research to investigate the burden, clinical and microbiological characteristics, and treatment of CPA in a tropical setting with a high prevalence of tuberculosis, specifically addressing the following questions:

1. What are the prevalence, incidence, and health economic impact of CPA in patients previously treated for pulmonary tuberculosis?
2. What are the clinical characteristics of post-tuberculosis CPA, which Aspergillus species are responsible for the disease, and are they susceptible to azoles?

3. What are the predictors of a good outcome from CPA treatment?

I would answer these questions by establishing a cohort of 500 survivors of tuberculosis with cavities. Using a sentinel surveillance approach, I would define the incidence and prevalence of CPA in HCMC. I would quantify its health economic burden, and calculate the incremental cost-effectiveness ratio for its diagnosis and treatment. Such a cohort would allow me to study clinical characteristics, treatment responses and prognostic features of CPA.

I could use latent class analyses to investigate the performance of available diagnostics. Furthermore, I would be able to identify which Aspergillus species cause disease, measure susceptibility to anti-fungal agents, and see how susceptibility correlates to outcome. This work would further our understanding of CPA, guide healthcare policy, and contribute to improving patient outcomes.

To justify this ambitious research programme, I would need preliminary data. I would propose the following approach.

1. Identify if there is evidence of CPA being highly prevalent amongst patients previously treated for tuberculosis

To address this aim, I would measure the titre of Aspergillus IgG in stored serum samples from patients who have completed treatment for pulmonary tuberculosis. These would include 100 patients who had cavities, and 100 patients who did not have cavities. These would be compared to 100 age- and gender-matched ‘controls’ from the serum biobank at OUCRU. For ethical reasons, it would best for all tests to be performed on anonymized historical samples.
2. Investigate the presence of antifungal resistance environmental *Aspergillus* isolates from HCMC

For all isolates of *Aspergillus fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*, collected in the above project, I would perform CLSI anti-fungal susceptibility testing for itraconazole, voriconazole, isuvaconazole, amphotericin B, caspofungin, and micafungin. All other environmental *Aspergillus* isolates could be screened for azole resistance by subculture on four-well plates containing itraconazole, voriconazole, posaconazole, and growth control as described by van der Linden *et al* in 2013 (353).

3. Establish which *Aspergillus* species are present in the environment around HCMC

This could be achieved by sampling the soil and air of HCMC at approximately 200 geographical locations. Locations would be selected purposively to cover urban, peri-urban, and rural areas. It would be interesting to select sites in / around agricultural settings where environmental azoles are used.

In light of my burden estimates, I think a focus on CPA is appropriate and important. The above programme would further our understanding of CPA, guide healthcare policy, and contribute to improving patient outcomes.

9.4 **Targeted dexamethasone therapy in cryptococcal meningitis**

Although our trial of dexamethasone for CM showed a universal approach to dexamethasone prescription was harmful, a role for corticosteroids may still exist. Guidelines recommend their use where patients have cryptococcomas with mass effect, acute respiratory distress syndrome, or IRIS. Since we saw few such events in our trial, we lacked power to test these indications. Furthermore, the evidence of non-proportional hazards may suggest a benefit of short course corticosteroids, and could justify a further clinical trial.
In chapter 8, I demonstrated that dexamethasone had a measurable impact on the immune profile in cryptococcal meningitis. The results with respect to LTA4H genotype were particularly interesting. As mentioned, the differential effects of dexamethasone with regards to LTA4H genotype may help to explain the worse outcomes for African vs Asian patients receiving dexamethasone. Patients with the TT genotype may have benefitted from dexamethasone therapy. This genotype was common in Vietnam and Thailand (11%), but not in Uganda (1%). At least in Asian patients, in whom the TT LTA4H genotype is prevalent (333), targeted dexamethasone therapy may be indicated.

To investigate this further, I would propose a clinical trial. It would be necessary to develop a rapid diagnostic PCR for the LTA4H promoter region SNP (rs17525495). Patients with the TT LTA4H genotype would then be invited to consent to being randomized to either dexamethasone or placebo. However, it would be challenging to recruit sufficient numbers of patients. Such a study would need to be multi-centred, and could utilize the Asian network from the CryptoDex study. We recruited 204 patients from Asian sites during the CryptoDex study, which would only yield 18-29 patients with TT LTA4H genotype (based on a TT LTA4H prevalence of 9-14%). However, to look at an effect on survival, even assuming a generous hazard ratio of 0.3 for dexamethasone vs placebo (with 80% power at the two-sided 5% significance level), 22 events would have to be observed. Assuming a mortality rate of 35%, this would require 63 participants. A more conservative hazard ratio of 0.7 would mean 247 events had to be observed, and would require 705 participants. The trial would likely have to rely on other outcomes, such as disability and adverse events.

Although there would be difficulties addressing these three areas of future research, results would build on the work presented here. They would be very useful for further understanding fungal disease burden and improving management for these serious, but neglected conditions.
10. Publications arising from this thesis

Introduction


Estimating the Burden of Fungal Disease in Vietnam


Multiple papers in Lancet as co-author on Global Burden of Disease estimates via University of Washington. I was a co-author as GBD expert on Vietnam, TB, and HIV.

Adjunctive Corticosteroids in HIV-associated Cryptococcal Meningitis: A Randomized Controlled Trial in African and Southeast Asian Countries


Stopping Trials Early: a Review of the Literature and a Case Report on the CryptoDex Trial

Manuscript in preparation.
The impact of dexamethasone vs placebo on immune responses at the site of infection in cryptococcal meningitis: report on the CryptoDex randomised controlled trial

Manuscript in preparation.
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12. Appendix – pdfs of publications arising from PhD research