CALCIFIED NEUROCYSTICERCOSIS:

RISK FACTORS FOR CALCIFICATION AND ASSOCIATED FACTORS FOR SEIZURE RELAPSE

By

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
January 2020

ABSTRACT

Objective: Neurocysticerosis (NCC) is the leading cause of epilepsy in endemic areas. In the final stage of NCC, the viable intraparenchymal cysts die, either resolving completely or leaving calcified scars. In a proportion of patients they are accountable for the subsequent morbidity, mainly manifesting as epilepsy. These patients experience breakthrough seizures during their antiepileptic treatment and can later present with seizure relapse after their medication is withdrawn. This doctoral dissertation is intended to evaluate the proportion of calcification and its risk factors in patients with viable cysts who receive antiparasitic drugs (study 1) and the incidence and associated factors to seizure events in patients with already calcified NCC lesions during their antiepileptic treatment (study 2), and after the antiepileptic drug treatment is withdrawn (study 3).

Methods: For the first study we evaluated the data of 220 adult patients with viable parenchymal NCC in a multilevel study from three previous clinical trials where patients received standard Albendazole (15 mg/kg/d), increased ABZ (22.5 mg/kg/d), or standard ABZ plus Praziquantel (PZQ, 50 mg/kg/d), and corticosteroids. Patients had MRI exams at baseline and at day 180 to assess cyst resolution and a CT scan at day 360 to assess calcification. For the second and third study we took advantage of a big cohort of patients with calcified NCC and neurological symptoms in Lima, Peru and developed a time-to-event study. For study two we selected patients with diagnoses of epilepsy who were under AED treatment (n=210) and for study three we included those patients from the cohort of 210 patient in whom their antiepileptic drug (AED) was withdrawn (n=62). In both studies we followed the participants for breakthrough seizure (study two) or seizure relapse (study three) from the time of enrollment until event, loss to follow-up, administrative censoring (36 months of follow-up or until November 2017) or withdrawal of AED (in case of study 2). During the follow-up in both studies, patients had a clinical evaluation every three months and were contacted by the study team by phone every two weeks. All seizure events were classified according the ILAE guideline.

Results: The first study included 147 patients with a total of 497 cysts, with an overall percentage of calcification at one year after antiparasitic treatment of 37.8% (188/497). In the multilevel model, we found in seven predictors of calcification, two at cyst level: cysts larger than 14 mm and cysts with surrounding edema, and five at patient level: patients with more than 2 years of seizures, having mild antibody responses on EITB, an increased dose of ABZ alone, receiving low dose of dexamethasone, and no early re-treatment with antiparasitic drug. Study two involved 210 patients and 103 of those (49.1%) presented breakthrough seizure during the follow-up. In the time-to-event analysis the Kaplan-Meier method showed that only 36.0% remains free of epilepsy during the study time (36 months). The risk factors in the multivariate Cox analysis were having a seizure event in the year previous to the enrollment and a history of 10 or more seizure events. In the subsequent cohort (Study 3) 62 participants who withdrew AED were included, from them 17 (27.4%) presented with seizure relapses. The Kaplan-Meier estimator showed that the probability of being free of seizures after 3 years of AED withdrawal was 58.7%. The Cox model showed that the main risk factors were having seizures in the last two years before AED withdrawal which was observed in 31 participants; these participants had 9.2 times greater chances of having a seizure relapse. Because of that we perform a sub-analysis in this group and found that having a history of 10 or more seizures before AED withdrawal and a history of partial seizures with generalization or generalized seizures had a higher risk of having a seizure relapse, but without statistical significance.

Conclusions: Residual calcified lesions after antiparasitic treatment in NCC represent less than half of cases and may be a preventable outcome. Some patients who present with remaining calcified scars developed breakthrough seizures under AED treatment and their management should be individualized. Finally, withdrawal of antiepileptic treatment should only be considered after at least two years since last seizure event.

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ACKNOWLEDGEMENTS

My greatest gratitude is with Hugo and Silvia. Hugo has been, above all, a friend and an unconditional guide from medical school through the different stages of my personal and academic life. His friendship and reliance in me have been the biggest support to successfully accomplish this phase of my life. Since I met him in medical school he has been an inspiring person in academic and personal life. His leadership of the research group, effort en each study, and knowledge of the disease are invaluable contribution not only for science but for the best care of people who suffer from this disease. My gratitude goes also to Silvia, Hugo's wife, for her sincere friendship and motivation during the first steps in my academic life. She leaved us very early and we will always miss her presence, joy and care for each patient, Silvia more that anyone taught to all of us who work with her that, at the end of the day, we are doing all this work to alleviate some of the suffering of people with this disease.

I have to thank Professor Gilman for his permanent support and advice throughout this process. His guidance has been very important during the execution of the doctoral program and the writing of the thesis, but above all and more importantly, at professional and personal level. At the end of the day, Bob, whit his example, has been the main responsible in my decision to devote my live to research. His passion for research never leaves indifferent to people who know him. And I have been lucky not only to know him, but to have his guidance as a teacher, advisor and, above all, as a friend during this time.

To all my professors, during this time I found not only really brilliant professionals but mainly wonderful persons who in several ways care our steps in this journey. I am especially grateful, beside Hugo and Bob, with professor Muñoz for his unconditional support and methodological advices, especially during the crucial final steps. Also the administrative persons have been absolutely kind and efficient. And will not be fair not to mention the special Cristina's help and motivation during all these years.

FINANCIAL SUPPORT

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LIST OF ABBREVIATIONS AND ACRONYMS

ABZ	Albendazole
AED	Antiepileptic drugs
APT	Antiparasitic treatment
BTS	Breakthrough seizure
CI	Confidence interval
CNS	Central nervous system
CSF	Central spinal fluid
CT	Computed Tomography
DXM	Dexamethasone
EEG	Electroencephalogram
EITB	Electroimmunotransfer blot
ELISA	Enzyme-linked immunosorbent assay
FLAIR	Fluid attenuation inversion recovery
F-U	Follow-up
HR	Hazard Ratio
HS	Hippocampal sclerosis
ILAE	International League Against Epilepsy
LLGP	Lentin-lectin glycoproteins
INCN	Instituto Nacional de Ciencias Neurologicas
IQR	Interquartile range
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
NCC	Neurocisticercosis
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NINDS	Neurological Disorders and Stroke
OR	Odds Ratio
PWE	People with epilepsy
PZQ	Praziquantel
RR	Risk ratio
SANCC	subarachnoidal neurocysticercosis
SCG	Solitary cysticercus granuloma
SCCG	Solitary cerebral cysticercus granuloma
SD	Standard deviation
SE	Standard error
SEL	Single enhancing lesion
US	United States
UPCH	Universidad Peruana Cayetano Heredia
WB	Western Blot
WHO	World Health Organization

CHAPTER ONE

1. INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 INTRODUCTION

Neurocysticercosis (NCC), defined as an infection of the central nervous system by the larval stage of the pork tapeworm *Taenia solium*, remains as a major challenge in public health mainly due to associated secondary epilepsy.[1-3] It has been consistently demonstrated that NCC is the single most important risk factor for acquired epilepsy in poor regions, and most likely in the world, considering that 80% of the disease burden caused by epilepsy is located in developing countries.[4] In endemic countries, cases of NCC are frequently diagnosed in hospitalized patients with epilepsy. A consistent association between NCC and seizures has also been shown in most population studies, where around 30% of all seizure cases are attributable to NCC.[5-8] NCC is also increasingly diagnosed in industrialized countries due to immigration from endemic zones, with approximately 2,000 new cases per year diagnosed in the US only.[9-11] Financially, during a 10-year period (2003-2012), NCC was responsible for more than US $908 million in US hospitals, and hospitalizations and associated charges for NCC exceeded the totals for malaria and all other neglected tropical diseases combined.[12]

T. solium larvae establish in the brain parenchyma as viable cysts. As part of their natural life cycle and as an outcome of antiparasitic treatment, these cysts degenerate, either resolving or leaving a small calcified lesion in the brain parenchyma.[13] Brain calcifications are commonly found in endemic areas for cysticercosis. In the general population, the proportion of asymptomatic individuals with calcified NCC ranges from 5% to 25%.[14-18] Cerebral calcifications can persist in the host's brain for many years, and in endemic areas these calcifications have been associated with seizures in population-based studies. In cross-sectional studies, the prevalence of NCC findings, mostly calcified

lesions, ranges from 20% to 54%.[6-8] In hospital-based studies, NCC also represents the primary cause of secondary epilepsy in endemic areas. [6, 19-22]

Although calcified NCC has a major role in seizure burden, the causal factors involved in the calcification process and the physiopathology of epileptic seizures in patients with calcified lesions are still inadequately understood.[23] The proportion of viable and degenerating cysts that result in calcified scars ranges from 10% to 60% and use of antiparasitic treatment seems to leave more calcified scars compared with only symptomatic treatment or placebo.[24-26] However, the factors or mechanisms involved in the calcification process have not been elucidated. It is still unknown why some cysts progress to a calcified lesion while others are completely removed. [27]

The incidence of seizure relapse in patients with calcified NCC has been inadequately studied. Our research group reported an incidence of 35.6 per 100 person-year, but due to a limited sample size it was not possible to explore the risk factors associated with seizure relapse. Moreover, this study involved patients with only a single event, thus the diagnosis of epilepsy was not clearly established.[28] Patients with epilepsy and calcified NCC typically remain under antiepileptic treatment for several years. After a period of two or more years without seizures, the antiepileptic drug is gradually withdrawn. A percentage of these patients will experience seizure relapse. Incidence of seizures and its risk factors have not been properly studied and very few studies report the relapse rate in this kind of patient. We have found only three publications which report a very high rate of seizure relapse of above 80%. [29-31] However, these studies present some limitations, such as few patients (30 or less) and a short follow-up period (12 months or less).

This thesis research includes three separate studies. The first study explores risk factors associated with calcification in patients with viable cysticercosis who have received antiparasitic treatment. The second study evaluates risk factors for breakthrough seizure in patients with calcified NCC under antiepileptic treatment. Lastly, the third

study evaluates risk factors for new seizures events in patients in whom the antiepileptic treatment has been withdrawn.

1.2 NEUROCYSTICERCOSIS

1.2.1 Life cycle

Taenia solium is one of many cestode species that can infect humans and belongs to the phylum Platyhelminthes, class Cestoidea, order Cyclophyllidea, family Taenidae.[32] *T. solium* has a complex two-host life cycle. Humans are the definitive host and harbor the adult intestinal tapeworm, whereas both humans and pigs act as intermediate hosts by harboring the larvae or cysticerci. Other mammals can act as a definitive or intermediate host, but have minimal relevance for clinical disease or parasite transmission. Humans are infected through incidental ingestion of *Taenia* eggs in fecal-oral contamination (Figure 1.6.1). The embryos are liberated from the eggs, penetrate the intestinal wall mucosa, access the bloodstream, and are distributed throughout the body. Once they reach a small terminal vessel, the embryos establish and evolve into metacestodes (post-oncospheral stage) and encyst to form larval vesicles or cysticerci, reaching their definitive size in two to three months.[33] Outside the nervous system, cysticercosis causes few or no symptoms and the cysts are usually identified and destroyed by the host's immune response. Clinical symptoms predominantly result from involvement of the eyes or the central nervous system (CNS). [34]. According to their location in the CNS, we can identify several types of NCC, thus, patients can have one or mixed presentations of this disease, such as intraparenchymal, intraventricular, subarachnoidal, retinal, medular, and also spinal cyst. In this study we will only evaluate intraparenchymal cysticercosis which by far is the most frequent cerebral presentation.

1.2.2 Intraparenchymal Cyst Stages

Viable cysts are typically round or oval-shaped vesicles of approximately 1 to 2 cm, with a translucent membrane through which the invaginated scolex is visible as a

small nodule. The vesicular wall has a festooned appearance and is composed of three layers; the cuticular mantle, an external eosinophilic layer, a middle cellular layer formed by a pseudo-epithelial structure, and an inner layer composed of circular muscle and reticular fibers.[35] The number of cysts is variable and they are most frequently located in the brain and muscles. The vesicular content is primarily composed of water, but it also includes calcium, glycoproteins, cholinesterase, and coproporphyrin, components that confer the fluid's antigenic properties.[3] Vesicles vary in content according to their evolutionary stage. After an undefined length of time, due to antiparasitic treatment or as consequence of their natural development, cyst degeneration begins mainly as a result of the host's immunological reaction. The vesicular fluid becomes opaque and dense, while the cyst's edges become irregular and shrink. The calcification process follows, usually starting in the cephalic portion of the parasite and progressing to the vesicular wall. A round, hard, whitish, and calcareous nodule forms as a residual calcification. In endemic areas, radiographic images of calcifications are considered highly suggestive of NCC. [13] Four distinct stages have been clearly identified for intraparenchymal cysticerci.[36] Changes in the surrounding brain tissues that are directly related to these stages have been observed. These changes have been clearly presented by Del Brutto and collaborators as shown in the following table (Table 1.7.1). [36]

1.2.3 Radiological findings

The four stages can be identified by neuroimaging; viable cysts without inflammation (vesicular stage) and with inflammation (colloidal stage), degenerating cysts ("enhancing lesions", granular stage), and calcified cysts (calcified stage). These stages will be defined for this study according to their appearance on neuroimaging, by either CT or MRI. (Table1.7.2) Summary definitions follow:[37,38,52]

Viable cysts: Viable cysts are include in the vesicular and colloidal stages. These contain a parasitic vesicle with discernible liquid content in pre-contrast imaging series and may have associated signs of inflammation (perilesional edema and contrast enhancement) or not. Cysticercal cysts are hypodense with a thin smooth wall, and an interior central or

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eccentric density. The scolex is occasionally identifiable. Although cysts unrecognized by the host do not have significant enhancement or edema, those with enhancement and/or edema that are hypodense on T1 and FLAIR MRI techniques are considered viable.

Enhancing lesion: Degenerating cyst whose liquid content is no longer visible on pre-contrast CT scan or MRI images.

Calcification: Nodular images of calcium density on CT or MRI images. Calicified cysts are more readily detectable on CT rather than MRI.

1.2.4 Diagnosis of NCC

Standard methods to diagnose NCC involve neuroimaging techniques, either computed tomography (CT) or magnetic resonance imaging (MRI).[34, 37] There are no studies to evaluate the real sensitivity and specificity of CT or MRI scan for viable intraparenchymal NCC. For viable cysts MRI has a better performance than CT scan, however CT sensitivity is good for medium or large cysts.[39-43] Also, in granulomas due to NCC, MRI has a better performance than CT scan, however in this scenario, use of a contrast substance, such as gadolinium, improves significantly the sensitivity of both techniques.[40, 44-47]. On the other hand, CT scan is the test of choice for calcified lesions given the enhancement of the calcium signal.[39-41, 43, 48-51]

In general, MRI is more accurate than CT for viable and degenerating cysts, providing the best evaluation of the degree of infestation, location, size, and evolutionary stage of the parasite. Perilesional edema and degenerative changes of the parasite are easier to detect on MRI, as are small cysts, cysts located in the ventricles, and racemose vesicles at the level of the posterior fossae and basal cisterns.[37, 52] A CT scan, however, is more sensitive for the detection of calcifications, which are the overwhelmingly predominant form in the community setting.[18, 53]

Serology using electroimmunotransfer blot (EITB Western Blot) technique which uses partially purified (lentil-lectin) glycoproteins as the antigenic parasite extracts (LLGP) provides specific confirmation of the diagnosis, with 100% specificity.[54, 55] The sensitivity of immunostained Western blot in cases with viable parasites is estimated to be over 95%, although it is somewhat lower (50% to 60%) in patients with a single cyst or a single degenerating parasite. Patients with calcified NCC may be seronegative.[56-58] Other techniques to detect serum antibodies, such as ELISA, have lower performance and cross reaction with antigens from other cestodes.[59, 60] Detection of circulating antigens using ELISA has lower sensitivity and specificity than immunostained Western blot. ELISA, however, seems promising as a tool to monitor the post-treatment evolution of patients with subarachnoid disease.[61-64] Neuroimaging and serological tests (mainly EITB and Western blot) are two key tools to for diagnosis; however other approaches may be considered to achieve diagnosis. Thus in this thesis, we will follow the diagnostic criteria proposed by del Brutto and collaborators to establish a diagnosis of NCC (Table 1.7.3).[65,66]

1.2.5 Treatment of Neurocysticercosis

Up to 1978, therapeutic options for NCC were restricted to surgery for cyst excision, colocation of ventricular-peritoneal shunts, or steroids to decrease cyst-associated inflammation. The discovery in Mexico that PZQ was effective for NCC in the mid-1980s provided the first effective specific drug.[67, 68] Later, ABZ was studied as a cheaper and more effective agent.[69, 70] The success of antiparasitic agents in neurocysticercosis was apparent: viable brain cysts disappeared soon after treatment and initial retrospective series showed that treated patients had much lower rates of seizure relapse.[71, 72] However, some claimed that because symptoms only appear at the time of the cyst death and the associated host immune response to the released parasite antigens, it is counter indicated to hasten the natural inflammation by using parasiticidal drugs.[73, 74] The discordance between these two positions led to a discussion in the literature. The main argument was whether antiparasitic treatment or natural involution of the parasite

will lead to less scarring and brain damage and thus a better prognosis in terms of the evolution of the seizure disorder and epilepsy. Unfortunately, some important aspects have been overlooked. First, there are subgroups of NCC patients that not only benefit from antiparasitic therapy but can progress and even die if not treated, like those with Subarachnoid neurocysticercosis (SANCC).[26, 75, 76] Similarly, complications in the treatment of NCC with antiparasitic drugs may be frequent, but severe complications are unusual.

Antiparasitic treatment has shown important usefulness in orbital, medullary, or intraspinal cysticercosis. A simple summary would be that antiparasitic agents effectively destroy the cysticerci in the human brain at the cost of acute inflammation. In addition, combined antiparasitic therapy is more effective in killing parasites than monotherapy.[25,77] Whether this is the most beneficial option for intraparenchymal cysticercosis in the long-term should be demonstrated, but in any case, the risks associated with this treatment are small. Additionally, it is published that increased doses and longer course of corticosteroid treatment can decrease symptoms, including seizures, in the days following treatment.[78]

In contrast to parenchymal NCC, there is wide consensus that extraparenchymal forms lead to progressive disease and should be aggressively managed.[75, 76, 79] Critically, there have been no controlled studies to establish optimal therapy. Treatment of SANCC is currently based on long (one-month) courses of ABZ and ventricular-peritoneal shunting when necessary. Efficacy of the first one-month course of ABZ therapy for SANCC is less than 40%.[80] A recent American guideline has been published, and its recommendations are the state of the art regarding the management of this pleomorphic and challenging disease.[81]

1.3 NEUROCYSTICERCOSIS AND CALCIFIED LESIONS

In some cases, the last stage in the degenerating process of brain cysts is calcification in which the parasite remnants appear as a solid calcified nodule. In 1982,

Grau and collaborators presented strong evidence favoring the hypothesis that the calcification process of cysticerci cysts is similar to that observed in other ectopic calcification, where the transformation of amorphous calcium generates hydroxyapatite and whitlockite.[82] They analyzed calcified lesions from 19 autopsied human brains, describing the appearance of calcified cysticerci as irregular shaped stones. Compared with viable cysts, calcified lesions had significantly more calcium and phosphorus, mainly amorphous calcium phosphate with small amounts of hydroxyapatite and whitlockite. It also has been demonstrated that calcareous corpuscles contained only calcium carbonate. In addition, they identified organic components in hyaline and calcifications including proteins corresponding to extracellular fibers (approximately 30 to 40% of the organic material), carbohydrates represented by glucose, and cholesterol. Thus, their study argues against the idea that the calcareous corpuscles play an important role as a nucleation center of calcification. Other findings include that initial calcium deposits occur in structures other that the scolex and that calcification is also observed in racemose cysts that do not contain a scolex.

In two reports, the histopathological findings of surgically-removed calcified nodules in patients with perilesional edema and seizures also show organic components inside the calcified nodules. The calcified nodules were shown to be composed of collagen and degenerating cyst structures including the scolex, suckers, and calcareous corpuscle. One of these reports described the presence of amorphous calcifications, most likely related to an early phase of calcification.[83, 84]

Calcified lesions are no longer considered "inert nodules". Initially, Nash and collaborators and then other authors[85-88] hypothesized that calcified lesions can undergo intermittent morphological changes due to bone remodeling or other mechanisms. These processes could theoretically expose parasitic antigenic material to the immune system causing a transitory inflammatory response and edema that could be the causal mechanism for seizures, headaches or other neurological symptoms. Additional evidence to support this theory was exposed by Zurabian et al. (2005) who reported the presence of a protein band of ~260 kDa at Western blot assay associated with the calcareous

corpuscle in calcifications. This protein may also play a role in the host immune response against calcified lesions.[89]

The morphologic change from degenerating cyst (granuloma) to calcified lesion can occur over months to years. In some patients, and for reasons not well understood, this process does not progress, resulting in reduced calcification or absence of calcification. In individuals with a single degenerating lesion, the rate of residual calcification at one year ranges between 20% and 30% and antiparasitic treatment seems to reduce the likelihood of a subsequent calcified scar in patients with degenerating lesions.[90-95]

Sharma and collaborators published a cohort study in which 78 patients with solitary granuloma and new diagnosis of epilepsy were followed for 6 months. Brain CT scans done at month six showed residual calcifications in 21 (27%) and complete resolution in 57 (63%).[96] The proportion of calcifications resulting after antiparasitic treatment of viable cysts varies from 10% to 60%, and unlike single granulomas, in viable brain cysts the use of antiparasitic drugs appears to be associated with a higher proportion of residual calcifications.[24-26] However, risk factors for subsequent calcification have not been appropriately studied.

1.4 NEUROCYSTICERCOSIS AND EPILEPSY

1.4.1 Epilepsy

Introduction. Epilepsy is one of the most common serious neurological conditions that affects more than 70 million people worldwide and is characterized by an enduring predisposition to produce spontaneous epileptic seizures[97] This disease has several causes, and recently the International League Against Epilepsy (ILAE) has divided it into six categories: genetic, structural, metabolic, infectious, immune, and unknown.[98] Among the infectious causes, parasitic infections, including cysticercosis, is one of the most common preventable factors for epilepsy around the world.[99]

Epilepsy and Seizure Classification. The first classification of seizures and epilepsy was done by Gastaut in 1969.[100] After the ILAE proposed classification of seizures in 1981,[101] where seizures were dichotomized to partial (restricted to a system of neurons and limited to part of one cerebral hemisphere) or generalized (involving both hemispheres). Then, in 1985, the ILAE proposed a revised classification of epilepsy [102] introducing a new epilepsy classification of idiopathic or symptomatic. In 1989[103] the classification of cryptogenic epilepsy was added. Fisher et al.,[104] in 2005 defined "*An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure*", it was practically applied as having two unprovoked seizure events 24 hours apart.

In 2010, the term partial seizure was replaced by focal seizure by a new revision by the ILAE,[105] and most importantly, generalized and focal were redefined for "*seizures as occurring in and rapidly engaging bilaterally distributed networks (generalized) and*

within networks limited to one hemisphere and either discretely localized or more widely distributed (focal)". After that, in 2014 the ILAE task force published a new practical clinical definition of epilepsy that defined epilepsy as a disease that fulfill any of the following conditions: (1) At least two unprovoked (or reflex) seizures occurring more than 24 hours apart; (2) one unprovoked (or reflex) seizure and a probability of at least 60% of having further seizures occurring over the next 10 years; (3) diagnosis of an epilepsy syndrome.[97] Finally, in 2017 the ILAE published an operational classification where the onset of seizure takes the main role, and classify the epileptic seizure as focal, generalized or unknown onset.[106] For practical reasons, for the analysis in our studies, we classified the epileptic seizure into two categories according the extent of brain involvement: focal seizures and generalized seizures (which includes focal onset seizures with bilateral generalization and generalized onset seizures).

1.4.2 Prevalence and semiology of Epilepsy due to NCC

Seizures are the most common form of clinical presentation of parenchymal NCC and often represent the primary manifestation of this parasitic disease. The association between cysticercosis, recurrent seizures, and epilepsy has been consistently demonstrated. Firstly in case series descriptions,[19, 107] and more recently in field studies using CT in the general population.[18,53,108,109] While the percentage of NCC patients presenting with seizures varies considerably, most series have shown that epilepsy may occur in up to 80% of patients. In a systematic review, neuroimaging evidence compatible with NCC among people with epilepsy was similar in clinical versus community studies (32% versus 25%, respectively). Also, the proportion of NCC was similar when adults versus children with epilepsy were compared (28% vs 25%, respectively).[8] Another recent systematic and meta-analysis evaluated publications from 23 countries with a total of 37 studies. Association between NCC and epilepsy was significant, and odds ratios ranged from 0.2 to 25.4 reported by different studies, with a common odds ratio of 2.7 (95% CI 2.1-3.6, p <0.001). In these studies, NCC can be the presumptive attributable cause of epilepsy in 63% in the exposed population.[6]

Most cases of NCC-related epilepsy occur in rural and semi-rural areas of developing countries. In these regions, neuroimaging studies have shown that parenchymal brain calcified NCC is the most common form of the disease.[18, 109] Since symptomatic patients seeking medical care represent only a small proportion of individuals with NCC, findings from hospital-based studies cannot be extrapolated to the entire population. In a hospital setting, most patients with NCC present with seizures. Moreover, the most common findings in neuroimages in these cases are viable or degenerating cysts.[71] At the population level, most infected individuals (as demonstrated by serology or brain CT) are asymptomatic.[14, 17, 18, 108, 110] Of those with NCC-associated seizures in community settings, the vast majority have calcified disease, and half of them are already seronegative.[18, 53] However, the literature is inconsistent on whether focal or generalized seizures predominate among NCC patients. Most authors concur that there is not an exact correlation between the number and location of cysticerci with clinical characteristics of the associated seizures.[71, 111] In some series, there is predominance of focal seizures with or without secondary generalization,[111-114] and in others, generalized seizures are the most common form of presentation of the disease[71, 115, 116] Also in calcified NCC with several calcifications, only one or few lesions act as a foci of seizure event.[28, 117-119]

1.4.3 Etiopathogenesis

Clinical manifestations of NCC are related to several pathogenetic mechanisms such as immuno-regulation (which allows the eggs to reach the cystic stage and to live several years in immune equilibrium with the host), inflammation (as an immunological response to foreign antigens from the parasite), granuloma formation and ectopic calcification, disruption of the brain blood barrier, and finally epileptogenesis. When cysts begin to degenerate, they provoke a variable inflammatory response that frequently results in the occurrence of one or more reactive seizures. This most likely reflects the loss of a state of immune equilibrium between the parasite and the host.[120] Degenerating cysticerci eventually regress, but often seizures continue because of an exacerbation of the inflammation or by other mechanisms at any time during the process of degeneration

[121]. Notably, antiparasitic treatment of viable parenchymal brain cysts induces host inflammatory responses and may cause symptoms like headaches or seizures [25, 122, 123]. Involution of cysts often ends in calcification, leaving parasite remnants as a solid calcified nodule.[82-84]

Calcified lesions are also implicated in the genesis and/or maintenance of seizures and epilepsy. As previously noted, however, seizure semiology is not always concordant with the localization of the lesion.[23, 28, 124] One hypothesis of epileptogenesis in NCC patients centers on disruption of the blood-brain barrier and associated inflammation. Host inflammation directed to degenerating cysts is associated with abnormal vascular permeability and neuronal dysfunction, resulting in increased cortical excitability and acute reactive seizures. However, it is unknown how these acute effects lead to the development of a chronic, focal or distant epileptic disorder. Possible mechanisms include progressive brain inflammation, reactive gliosis, cellular damage, and increased blood-brain barrier breakdown, which may contribute to inflammation by creating a positive feedback loop.[27,125] These mechanisms, together with changes in brain excitability, might lead to a chronic epileptic state. So far, the precise epileptogenic mechanisms of NCC have not been elucidated and there is no proven clinical approach for the prevention of epilepsy in asymptomatic infected individuals.[126]

1.4.4 Calcifications, gliosis and the role of hippocampal sclerosis

As noted, residual calcified lesions are the most common cerebral finding of NCC. In patients who had viable cysts, calcified cysticercosis is the chronic condition to be faced after treatment. However, the importance of disease manifestations associated with calcified lesions has not been well characterized. In fact, this parasitic stage is still regularly referred to as "inactive NCC", suggesting an end stage and/or a state of less clinical importance. It is agreed that even after a successful anti-parasitic treatment, the remaining calcified lesion persists, which in turn acts as a foci of relapsing inflammation, edema, and seizures.[127]

There is growing evidence implicating calcified cysticerci in the genesis and/or maintenance of seizures and epilepsy in endemic populations. Calcified cerebral lesions are a common CT finding in persons with seizures in endemic areas. Indeed, in community based studies, calcified scars are much more frequent than viable cysts and more prevalent in symptomatic compared to asymptomatic participants; symptoms in individuals with only calcified disease are associated in a sizable proportion of individuals with perilesional brain edema around one or more calcified lesions.[28, 84] Also, seizure semiology is frequently concordant with the localization of the calcified lesion.[23,124] In a study performed by our group, perilesional edema was present in one third of patients with only calcified lesions who presented with seizures.[84] Another prospective cohort study in 110 patients with calcified NCC demonstrated an estimated 5-year seizure incidence of 36%, and found peri-calcification edema in 50% of the patients with a recent seizure episode, compared to 8% asymptomatic matched controls.[28] Thus, peri-calcification edema occurs frequently and is associated with episodic seizure activity in calcified NCC, suggesting a unique and possibly preventable cause of seizures in this population.

In addition, it has been consistently reported that gliosis surrounding calcified lesions are related to refractory epilepsy. In one study, seizure recurrences were significantly higher in individuals with calcifications surrounded by gliosis than in those without.[128] In another randomized controlled trial of individuals with a solitary cysticercus granuloma (SCG), it was found that, irrespective of AED therapy, that the presence of perilesional gliosis was a risk factor for seizure recurrence at month 12 of follow-up.[129]

Data from specialized epilepsy surgery centers in Latin America indicate that patients with NCC and refractory epilepsy often have seizures arising from hippocampal sclerosis and not necessarily from the calcified lesion itself. In most of these cases, a seizure-free state resulted after the surgical resection of the anterior temporal lobe, and not necessarily the brain calcification.[130-133]

In a recent cross-sectional population-based study, it has been demonstrated that calcified NCC is associated with hippocampal atrophy in older adults living in an endemic rural village.[16, 134] Theoretically, calcified NCC may lead to hippocampal sclerosis (HS) by causing recurrent seizures or status epilepticus. Brain calcifications may undergo periodic morphological changes related to remodeling mechanisms,[23, 27, 121] exposing trapped proteins of the parasite to the immune system, resulting in inflammatory changes in the brain parenchyma and recurrent seizures, which can lead to hippocampal damage in the long term. The resulting HS may, in turn, exacerbate the seizure disorder. Alternatively, irrespective of the occurrence of seizures, calcified cysticerci can lead to hippocampal damage mediated by remote inflammatory mechanisms,[135] as demonstrated in experimental studies.[136, 137] Further research is required to elucidate the relationship among epilepsy calcification due to NCC and hippocampal sclerosis.

1.4.5 Breakthrough Seizures

A breakthrough seizure is defined as a new seizure event after a crisis-free time in patients under AED treatment. Some authors consider this time as 12 months, however there is no consensus about crisis-free time to consider a new convulsive event as a breakthrough crisis.[138, 139]

1.4.5.1 Breakthrough Seizures in non-NCC patients

Breakthrough seizures (BTS) have been reported in several studies. Kim and collaborators have shown that proportion of BTS at one year can reach 36%, 46% and 50% after one, three and five years of starting AED. The significant risk factors for BTS were number of previous seizures (more than 3), presence of a neurological disorder, and an epileptic abnormal EEG.[140] Beghi et al. reported the probability of BTS of 38%, 40% and 46% at one, two, and three years respectively. Moreover, the risk factors were the number of previous seizures, having mixed types of seizures, and identified etiologic

factors.[141] The proportions of BTS are similar to the above referred studies and other potential causes or triggers has been reported by the literature such as poor adherence to AED, concurrent inflammatory condition or infection, emotional stress, concomitant medication that lower the seizure threshold, sleep deprivation, diarrhea, metabolic events, flashing lights, etc.[139]

1.4.5.2 Breakthrough Seizures in calcified NCC

The incidence of seizure recurrence (breakthrough seizures) and their predictive factors in patients with calcified NCC and epilepsy under antiepileptic treatment has been poorly studied. Sharma et al.,[96] published a report where 35 patients with newly diagnosed epilepsy and with a single calcification were followed up for 6 months. In this study, 12 (34.3%) patients presented with seizure recurrence and, in their unadjusted model, the predictors of recurrence were abnormal EEG findings, a family history of epilepsy, and serial seizures at onset. In the adjusted model none of these factors were associated with seizure relapse.[96]

Nash et al. reported a longitudinal study with 64 patients with calcified lesions. In this study we found a 5-year incidence of seizure relapses of 36%. However, this cohort is composed by 55% of people with epilepsy without AED treatment, and in this group the HR was 5 times higher compared to subjects without treatment. In the group under AED treatment the prognostic factors associated with risk of seizures were the number of frontal lobe calcifications, and the number of previous generalized tonic–clonic seizures.[28]

In a recent publication, Singh and collaborators,[142] studied a cohort of 54 PWE and single calcified lesions receiving AEDs who were followed-up for one year. This cohort presented a BTS incidence of 24% (13/54) and the associated factors were history of status epilepticus, perilesional edema, and visualization of scolex at baseline neuroimaging.

1.4.6 Antiepileptic drug withdrawal and seizure relapse

There is no consensus on a single and standardized AED withdrawal approach for patients with epilepsy and NCC. Indeed, the length of AED therapy has been mostly based on expert opinion and there are few systematic data supporting AED withdrawal in these cases. The rate of seizure recurrence as well as factors that increase the risk of relapses should be considered before AED withdrawal in these cases. These factors have been extensively studied in conditions other than NCC, and such evidence might provide some helpful clues when attempting AED withdrawal in NCC patients.

1.4.7 General principles of AED withdrawal.

In all forms of epilepsy, benefits from AEDs withdrawal are mostly related to avoiding side effects like cognitive impairment or potential teratogenic effects, improving quality of life, reduction of economic burden, reduction of stigma, and elimination of the potential for drug's interactions.[143] On the other hand, disadvantages related to AED withdrawal include increasing risk of relapses, as well as loss of driving license or loss of independence in activities with risk.[144] The main outcome of AED withdrawal is having minimal or no seizure recurrence. Many studies of discontinuance of antiepileptic treatment have been reported by the literature showing variable rates of recurrence as shown in Table 1.7.4. In persons with epilepsy from causes other than NCC, several risk factors for seizure recurrence have been recognized, such as older age at seizure onset, duration of epilepsy, abnormal neurological examination or psychiatric findings, abnormal EEG findings, increased number of seizures during AED therapy or history of epileptic status, greater number of AED used during treatment, and failure in previous withdrawal attempts.[145] Theoretically, all these factors may influence the rate of relapses after AED withdrawal in patients with epilepsy and NCC.

Those reports are mostly retrospective, observational, and uncontrolled.[145, 146] Cumulative probability of seizure recurrence, reported by literature in adult population

17

ranges from 15% to 32% at 1 year of follow-up after AED withdrawal[143, 147-149] and from 29% to 43% at two years of follow-up.[147-151]

1.4.8 Seizure recurrence in NCC patients after AED withdrawal.

Most studies specifically dealing with AED withdrawal in patients with epilepsy and NCC are retrospective, small, or non-controlled as shown in Table 1.7.5.[29, 30, 152-156] In a case series of 40 patients with NCC who had been free of seizures for two years, the observed relapse rate was 50% at one year of follow-up after AED suspension.[29] In this series, residual calcification and history of both recurrent seizures and multiple viable cysts were important risk factors for seizure recurrence.

A high rate of seizure relapse was reported by the same authors in a subsequent cohort of 30 patients with calcified NCC, 15 of whom received antiparasitic treatment (as they presented with viable cysts) and 15 who presented with spontaneously calcified cysticerci.[30] Both groups had been free of seizures for two years, and were followed for twelve months after AED withdrawal. The rate of recurrence was similar in both groups, and 83% of them presented with recurrences within six months of AED discontinuation. Results of this study argued against previous unsupported assumptions that the use of cysticidal drugs leave a more profound scar in the brain parenchyma than when the parasites calcified spontaneously.[157]

In a study conducted in India assessing risk factors associated with relapses after withdrawal in 73 patients with SCG, the presence of residual calcifications and not the length of AED therapy (six months versus two years) was associated with an increased risk of seizure recurrences.[155] In another study from India, 81 subjects with epilepsy and SCG were randomly allocated to receive six or 12 months of AEDs and were followed for one year after AED therapy.[154] Only 12% of patients showed relapses (in both groups) and all cases occurred during the first six months after AEDs withdrawal. Given that most of the relapsing cases in both groups were observed in patients with residual

calcification, the authors concluded that individuals with persistent calcified lesion might need longer AED treatment.

Three other studies in patients from India with SCG showed similar results. In one of them, 115 children were allocated to AED withdrawal after one or two years free of seizures.[153] Rates of relapses were similar in both groups, but it was noticed that patients with both a residual calcification and abnormal EEG recordings had the greatest chance of seizure recurrences after AED withdrawal. In another series, AEDs were rapidly (2-12 weeks) tapered after CT showed resolution of the SCG. Out of 185 patients, 28 had relapses; risk factors associated with relapses included the development of a residual calcification as well as history of more than one seizures or breakthrough seizures before achieving control.[90] In a third study, including 206 individuals randomly allocated to receive one or two years of AED therapy and followed up to 18 months after withdrawal, it was observed that seizure recurrences in those left with a residual calcification were significantly higher (42% versus 22%) among those treated with AED for one year.[156]

1.5 RATIONALE AND STUDY AIMS

1.5.1 Rationale

In viable parenchymal cysts, after interruption of the immunological equilibrium, we observed morphological change from granuloma to calcified lesion or complete clearance of the lesion. In single enhancing lesion the proportion of calcification has been reported in a range of 20% to 30%.[90-95] In viable parenchymal cysts, the proportion of calcification after antihelmintic treatment range from 10% to 60%,[24-26] however risk factors for calcification have not been studied.

The incidence of seizure relapse in patients with calcified NCC and epilepsy has been inadequately studied. Our research group reported an incidence of 35.6 per 100

person-year, but due to a limited sample size and the heterogeneity of the participants, it was not possible to explore the risk factors associated with seizure relapse adequately.[28] Patients with epilepsy and calcified NCC typically remain under antiepileptic treatment for several years and beside treatment, show a presence of breakthrough seizures. Largely based on expert opinions, after a period of two to three years without seizures, the antiepileptic drug is gradually withdrawn. However, some of these patients will experience seizure relapse. The relationships between the incidence of seizures, its risk factors of breakthrough seizure, and seizure relapse have not been properly studied.

This dissertation reports on the findings of three studies that explore the pathological outcomes of NCC. The first study will explore risk factors associated with calcification in patients with viable cysticercosis who have received antiparasitic treatment. The second study will evaluate risk factors for breakthrough seizure in patients with calcified NCC who are under antiepileptic treatment. The third study will evaluate risk factors for new seizures in patients in whom the antiepileptic treatment has been withdrawn.

1.5.2 Study Aims

Study 1: To estimate the incidence of calcification in patients with viable parenchymal cysticercosis after one year of antiparasitic treatment and to evaluate associated risk factors.

Study 2: To evaluate the incidence rate, cumulative incidence and risk factors of seizure breakthrough in patients with epilepsy due to calcified NCC who are under antiepileptic drug therapy.

Study 3: To evaluate the incidence rate, cumulative incidence and risk factors of seizure relapse in patients with epilepsy due to calcified NCC who had stopped their AED treatment.

1.6 Figures

1.6.1 - Life cycle of *Taenia solium*.

Human
(Definitive host)

Ingestion of infected pork, poorly cooked: **Taeniasis**

Ingestion of *T. solium* eggs by fecal contamination: **Human cysticercosis**

Ingestion of *T. solium* eggs or proglottids: **Porcine cysticercosis**

Pig
(Intermediate host)
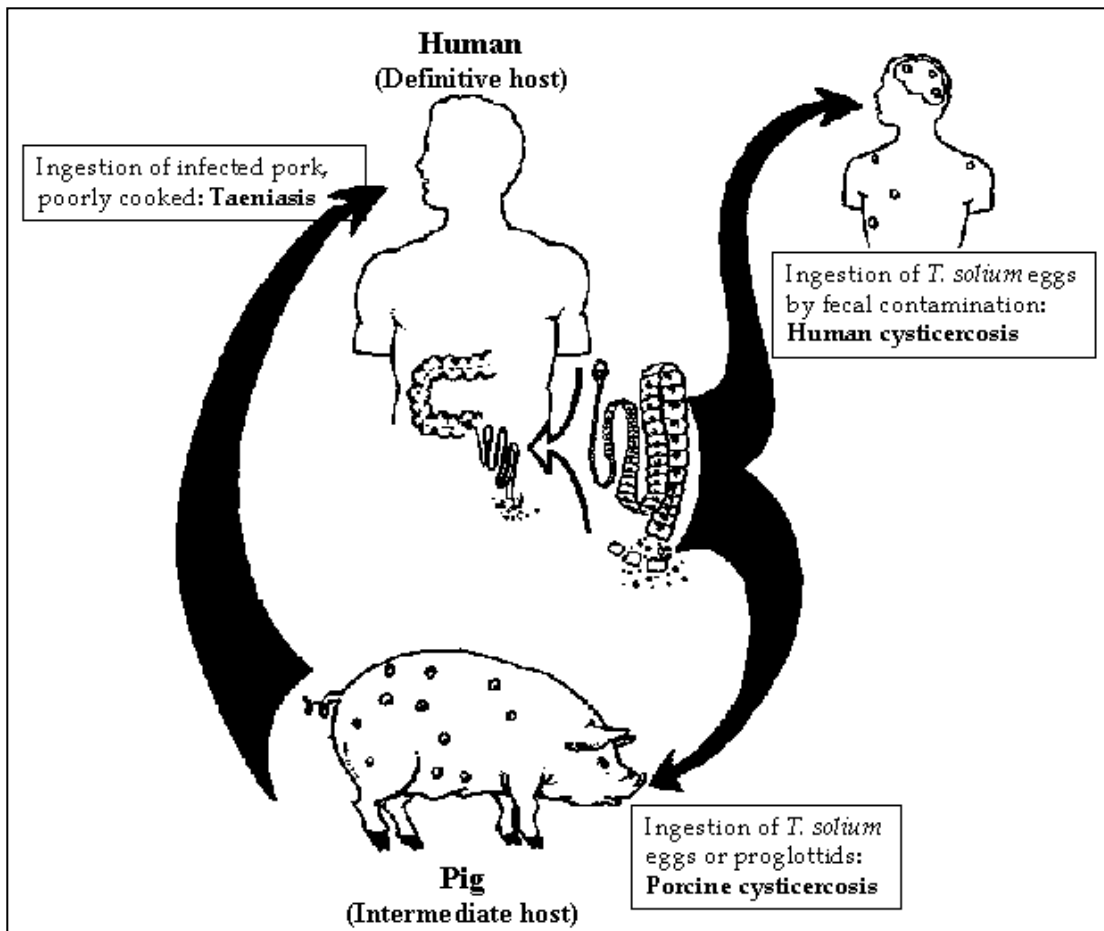
1.7 TABLES

Table 1.7.1: Pathological characterizes identified in intraparenchymal cysticerci[36]

Stage of involution	Appearance of the parasite	Pathological changes in the brain parenchyma
Vesicular stage	Translucent vesicular wall; transparent vesicular fluid; viable invaginated scolex	Scarce inflammation reaction; formation of a collagen capsule around the parasite
Colloidal stage	Thick vesicular wall; turbid vesicular fluid; scolex showing signs of hyaline degeneration	Intense inflammatory reaction that includes the parasite; thick collagen capsule around the parasite
Granular stage	Thick vesicular wall; degenerated scolex	Astrocytic gliosis around the cyst; microglial proliferation
Calcified stage	Transformation of the parasite in coarse calcified nodules	Intense gliosis; multinucleated giant cells

Table 1.7.2. Differences of evolutive stages on CT and MRI (MRI FLAIR and T2 protocols are taken as a reference)

Stage	CT (Pre-Contrast)	MRI -FLAIR	MRI-T2	Enhancement	Edema
Cyst without inflammation	Hypodense	Hypointense	Hyperintense	Not	Not
Cyst with inflammation	Hypodense	Hypointense	Hyperintense	Yes (perilesional)	Usually
Enhancing lesion	Not defined	Mixed signal	Mixed signal	Yes	Yes
Calcification	Hyperdense	Signal substraction	Signal substraction	Rare	Rare

Table 1.7.3. Diagnostic criteria for Neurocysticercosis [66]

Diagnostic criteria

Absolute
- Histological demonstration of the parasite from biopsy of a brain or spinal cord lesion
- Evidence of cystic lesions showing the scolex on neuroimaging studies
- Direct visualization of subretinal parasites by fundoscopic examination

Major
- Evidence of lesions highly suggestive of neurocysticercosis on neuroimaging studies
- Positive serum immunoblot for the detection of anticysticercal antibodies
- Resolution of intracranial cystic lesions after therapy with albendazole or praziquantel
- Spontaneous resolution of small single enhancing lesions

Minor
- Evidence of lesions compatible with neurocysticercosis on neuroimaging studies
- Presence of clinical manifestations suggestive of neurocysticercosis
- Positive CSF ELISA for detection of anticysticercal antibodies or cysticercal antigens
- Evidence of cysticercosis outside the central nervous system

Epidemiological
- Individuals coming from or living in an area where cysticercosis is endemic
- History of travel to disease-endemic areas
- Evidence of a household contact with *T. solium* infection

Degrees of diagnostic certainty

Definitive
- Presence of one absolute criterion
- Presence of two major plus one minor and one epidemiological criteria

Probable
- Presence of one major plus two minor criteria
- Presence of one major plus one minor and one epidemiological criteria
- Presence of three minor plus one epidemiological criteria

Table 1.7.4. Seizure relapse rate in patients after AED withdrawal in non-cisticercotic patients

Author (Year)	Population	Follow-up	Relapse rate* (CI 95%)	Seizure-free period (years)
Lossius (2008) [143]	Adults (n=79)	12 mo.	15%	>= 2y
MRCADW (1991) [147]	Adults(n= 883)	12 mo.	32%	>= 2y
MRCADW (1991) [147]	Adults(n= 883)	24 mo.	41%	>= 2y
Berg (1994) [148]	Meta-analysis	12 mo.	25%	NS
Berg (1994) [148]	Meta-analysis	24 mo.	29%	NS
Specchio (2002) [149]	A&Ch (n=225)	6 mo.	12%	>= 2y
Specchio (2002) [149]	A&Ch (n=225)	12 mo.	26%	>= 2y
Specchio (2002) [149]	A&Ch (n=225)	24 mo.	43%	>= 2y
Specchio (2002) [149]	A&Ch (n=225)	36 mo.	49%	>= 2y
Specchio (2002) [149]	A&Ch (n=225)	60 mo.	52%	>= 2y
Ricci (1999) [158]	Adults (n=125)	6m	15%	>1y
Avoni (1995) [159]	Adults (n=286)	>3y	53%	2y – 6y
Mastropaolo (1992) [160]	A&Ch (n=191)	NS	NS	NS
Alvarez (1989) [150]	Adults (n=50)	2y	30 %	> 2y
Alvarez (1989) [150]	Adults (n=50)	3y	42 %	> 2y
Callaghan (1988) [161]	A&Ch (n=92)	2.1y (0.5-5y)	34%	> 2y
Overweg (1987) [162]	Adults (n=62)	> 2y	66%	> 3y
Pestre (1987) [163]	Adults (n=272)	NS	49%	NS
Juul-Jensen (1964) [151]	Adults (n=200)	2y	35%	2y

(*) Relapse after withdrawal or AED reduction, **A&Ch**: Adults and children, **NS**: Not specified

Table 1.7.5 Studies on seizure recurrence in NCC patients after AED withdrawal.

Author (Year)	Participants	F-U Time	Type of lesion	Relapse rate	Seizure -free (years)	Risk factors: Associated / Non-associated	Time of Withdrawal
Del Brutto (1994) [29]	Adults (n=40)	12 mo.	Viable	50%	2y	**- Residual calcification** **- Recurrent seizures** **- Multiple cysts**	6-8 weeks
Garg (1998) [152]	A&Ch (n= 25)	< 3 mo.	SEL	16%	NS	NS	Abruptly
Singhi (2003) [153]	Children (n=55)	>12mo.	SEL	4.5%	1y	**- Residual calcification** **- Abnormal EEG**	8-12 weeks
Singhi (2003) [153]	Children (n=51)	>12mo.	SEL	5.8%	2y	**- Residual calcification** **- Abnormal EEG**	8-12 weeks
Del Brutto (1996) [30]	Adults (n=30)	12 mo.	Calcified	83.3%	2y	_- Sex, age._ _- Type and number of seizures_ _- Number of calcifications_ _- AED therapy_	NS
Rajshekhar (2004)[39]	(n=185)	>2y	SCCG	15.1%		**- Breakthrough seizures** **- Residual calcification** **- More than one seizure**	2-12 weeks
Del Brutto (1997) [41]	Children (n=13)	NS	Mixed	69%	2y	**- Residual calcification**	NS
Gupta (2002) [154]	NS (n=41)	12 mo.	SEL	12.2%	0.5 y	**- Residual calcification**	NS
Gupta (2002) [154]	NS (n=40)	12 mo.	SEL	12.8%	1 y	**- Residual calcification**	NS
Thussu (2002) [155]	A&Ch (n=47)	12 mo.	SEL	17%	0.5 y	**- Residual calcification**	3 months
Thussu (2002) [155]	A&Ch (n=26)	12 mo.	SEL	11.5%	2 y	**- Residual calcification**	3 months
Verma (2006) [156]	A&Ch (n=65)	18 mo.	SCCG	16.3%	0.5 y	**- Residual calcification**	6 Weeks
Verma (2006) [156]	A&Ch (n=62)	18 mo.	SCCG	12.0%	2 y	**- Residual calcification**	6 Weeks

F-U Time: Follow-up time, SEL: Single enhancing lesion, SCCG: Solitary cerebral cysticercus granuloma, A&Ch: Adults and children, NS: Not specified

1.8 NOTES

This introduction has been partially used to publish a review paper.

- **Bustos JA**, García HH, Del Brutto OH. Antiepileptic drug therapy and recommendations for withdrawal in patients with seizures and epilepsy due to neurocysticercosis. Expert Rev Neurother. 2016 Sep;16(9):1079-85. doi: 10.1080/14737175.2016.1194757. Epub 2016 Jun 8. Review. PubMed PMID: 27228190.

CHAPTER TWO

– RESEARCH PAPER 01 –

FREQUENCY AND DETERMINANT FACTORS FOR CALCIFICATION IN NEUROCYSTICERCOSIS

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Keywords: Cysticercosis, *Taenia solium*, calcification, risk factors, Perú.

2.1 ABSTRACT

2.1.1 Introduction

Neurocysticercosis (NCC) is a major cause of acquired epilepsy in endemic areas. Along its natural course of evolution or as an effect of antiparasitic treatment, the host´s inflammatory response leads to cyst degeneration, which in some cases ends as a calcified scar. This study assessed the proportion of residual calcification at one year in parenchymal brain cysts that resolved after antiparasitic treatment, and the risk factors associated with their calcification.

2.1.2 Methods

We evaluated data from 220 adult patients with viable parenchymal NCC from three previous randomized clinical trials where patients received standard albendazole (ABZ) (15mg/kg/d), increased ABZ (22.5mg/kg/d), standard ABZ plus praziquantel (PZQ 50mg/kg/d) or placebo (PCB), and concomitant anti-inflammatory therapy with dexamethasone (DXM). Patients had MRI exams at baseline and at day 180 to assess cyst resolution and a CT scan at day 360 to assess residual calcifications. We selected cases with complete follow up (MRI at day 180 and CT at day 360). Multilevel Poisson regression model with random intercepts adjusted by covariates at the patient and cyst levels was used to estimate risk ratios of calcification.

2.1.3 Results

A total of 497 cysts in 147 patients were evaluated, with an overall 37.8% of calcification at one year after treatment of (188/497). Positive predictors for residual calcification at the cyst level were cysts larger than 14 mm (RR 1.34, p=0.035), and cysts with surrounding edema at baseline (RR 1.39 p=0.023). At the patient level, a history of more than 24 months with seizures (RR: 1.25; p=0.003), mild antibody response (less than four antibody bands on western blot, RR: 1.14; p=0.020), increased dose of ABZ (RR: 1.26; p<0.001), lower dose of DXM (RR: 1.36; p=0.037), no early re-treatment in patients with incomplete resolution of cysts (RR: 1.45 p=0.012) or complete cure (RR: 1.48 p<0.001]).

2.1.4 Conclusions

Approximately 40% of parenchymal NCC cyst resulted in a residual calcification after antiparasitic treatment. Some risk factors are modifiable (steroid dose, antiparasitic regime, early re-treatment) and may contribute to decrease or avoid calcification, potentially decreasing the risk for seizure relapses.

2.2 INTRODUCTION

Neurocysticercosis (NCC), a parasitic infection of the human central nervous system (CNS) by larval cysticerci of the cestode *Taenia solium*, is as a global public health problem and important source of neurologic disease. The presence of the parasite in the brain, and the associated host inflammatory response, causes seizures, intracranial hypertension, and other neurological symptoms.[19, 164] The parasite initially establishes as a viable cyst that is in immunologic equilibrium and induces minimal or no inflammation. Over time, the cyst loses its integrity, either because of the natural progression of the infection or in response to antiparasitic chemotherapy. Parasite antigens become exposed to the immune system resulting in a host inflammatory response that frequently causes neurological symptoms.[13] The cyst degenerates into a granuloma as the infection is cleared, and in some instances, the degeneration process results in a calcified lesion that persists in the brain.[82]

Calcified lesions are not inert or inactive nodules as previously believed.[27, 84] It has been consistently demonstrated that calcified cysticerci can be permanent foci of seizures and other neurological symptoms, although the pathogenic mechanisms are not well understood.[23, 51, 146, 165, 166] Nash et al.[85] and subsequently others[85-89, 167] suggest the hypothesis that calcified lesions undergo morphological changes similar to bone remodeling intermittently exposing parasitic antigenic material to the immune system, and the antigen exposure triggers an inflammatory response that leads to seizures, headaches, or other neurological symptoms. It has also been proposed that direct contact of calcium with cerebral tissues can have a toxic effect resulting in seizure activity.[168] Finally, the process of perilesional gliosis, starting even when cysts are viable, could result in chronic brain tissue damage and persistent foci for seizure activity.[88, 129, 169, 170]

The morphologic change from granuloma into a calcified lesion can take months to years. In some patients, and for reasons still not well elucidated, this process is altered or even halted, resulting in little or no residual calcification.[24-26] In individuals with a single degenerating cyst, the rate of residual calcification at 1 year ranges between 20% and 30%,[90-95] where antiparasitic treatment seems to reduce the likelihood of developing

a subsequent calcified lesion.[95, 171] In individuals with viable cysts, the proportion of calcifications resulting after antiparasitic treatment has not been systematically assessed, although estimates range from 10% to 60%. Risk factors for subsequent calcification remain unknown.[24, 25] This study estimated the incidence of calcification in patients with viable parenchymal cysticercosis one year after antiparasitic treatment and identified factors associated with a higher risk for calcification.

2.3 MATERIAL AND METHODS

2.3.1 Study design. This is a retrospective cohort study using data previously collected in three clinical trials on antiparasitic treatment for viable parenchymal NCC performed by our study group in Lima, Peru, between 2006 - 2014.[77, 78, 172]

2.3.2 Study outcomes. The main outcome was evaluated at the level of the cyst rather than the participant. For each cyst that resolved after antiparasitic treatment, the presence or absence of a calcified lesion in the location of the original cyst 12 months after initiation of antiparasitic treatment was defined by topographic identification of each lesion. It was assessed using MRI at baseline and at month 6 and by CT scan at month 12 (Figure 2.7.1).

2.3.3 Study population and selection criteria. We identified eligible individuals from a total population of 220 participants in three parent clinical trials. The first trial compared two regimes of dexamethasone (either 6 mg/d for 14 days [n=32], or 8 mg/d for 28 days with a 2-week taper [n=32]) in patients receiving standard doses of albendazole (ABZ) (15 mg/kg/d for 14 days).[78] The other two parent trials assessed the pharmacokinetics, safety, and efficacy of combined ABZ plus praziquantel (PZQ) treatment. In the second trial, patients treated with ABZ at 15 mg/kg/d for 10 days were randomized to also receive either PZQ (n=16) or placebo (n=16).[172, 173] In the third trial, patients were randomized to receive 10 days of either standard ABZ treatment plus PZQ (n=41), standard ABZ treatment without PZQ (n=43), or increased (22.5 mg/kg/d) doses of ABZ without PZQ (n=40).[77]

All participants in the trials were between 16-65 years-old, had epilepsy, and had NCC with 20 or fewer viable parenchymal cysts at baseline based on brain CT and MRI scans. Detailed eligibility criteria for each trial are described in the original publications. [77, 78, 172] Trial participants underwent an MRI scan 6-months after treatment initiation to

assess cyst resolution, and a CT scan at 12-months to identify residual calcifications (typically seen as well-defined hyperdense rounded, nodular or punctuate solid lesions). Trial participants were eligible for this cohort study if they completed both the MRI and CT follow up evaluations, and if at least one of the baseline viable cysts resolved with antiparasitic treatment (Study flowchart, Figure 2.7.2).

2.3.4 Data source. Anonymized demographic, clinical and radiological information collected in the original trials was collected for this study. Data included participant age (years), sex, time-period between first seizure and enrollment (months), number of anti-*T. solium* antibody bands reactive on enzyme-linked immunoelectrotransfer blot (EITB western blot, 1 to 7 bands), previous antiparasitic treatment, previous use of antiepileptic drugs, antiparasitic treatment regimen received in the trial (standard ABZ, increased ABZ, or combined ABZ plus PZQ), length of antiparasitic treatment (10 versus 14 days), initial dose of dexamethasone (mg/day), length of dexamethasone treatment (10-12 days versus 28 days). MRIs were performed on 1.5T or 3T MRI machines using T1, T2 and Flair protocols (before and after contrast agent injection). Non-contrasted CT scan were performed at baseline and 12 months after in majority of the cases in a Siemens Somatom IV helical scanner.

Radiologic data from MRI and CT scans included the number and topographic location of the cysts and calcified lesions as well as cyst size (using the greatest diameter in millimeters), cyst content (clear or turbid), and the presence of pericystic edema (defined as hyper-intense signal on fluid-attenuation inverse recovery (FLAIR) or T2 MRI sequences). Cyst resolution at month 6 was defined as absence of discernible hyperintense content on T2 MRI sequences. In addition, study charts were revised to find out whether patients had received anti-parasitic re-treatment between their 6-month MRI scan follow up and their 12-month CT scan assessment. For the current study, two NCC experts re-evaluated all MRI and CT scans to verify the accuracy of recorded outcome data. Any potential discrepancies were resolved by consensus.

2.3.5 Statistical analysis. Main covariates were described using summary statistics (mean ± standard deviation or median with ranges for numerical variables, and percentages for categorical variables), and compared initially between included and excluded participants to rule out selection biases. The overall percentage of residual calcification in cysts that resolved after antiparasitic treatment was calculated.

Due to the multi two-level nature of the data, we used a multilevel Poisson regression with random effects for individual intercepts to estimate risk ratios (RR) of calcification with 95% confidence intervals using covariates at the cyst and at the individual level. Bivariate models were initially performed for each covariate of interest. Subsequently, we performed a multivariate model including all covariates at the cyst level using a stepwise approach with backward selection algorithm retaining covariates with p<0.05 derived from Wald test. A second model was then performed with covariates selected in the first model, and including all covariates at the patient level using a similar stepwise approach. All models were estimated using mean-variance adaptive Gauss-Hermite quadrature with seven integration points for each level of random effects. Standard errors were estimated using cluster-robust variance estimates to account for correlation within each clinical trial from which the data came. All the analyses were performed in the statistical software Stata 15.1 (StataCorp, College Station, TX).

3.3.6 Human subjects rights protection. This study analyzed clinical and radiological data already obtained in previous trials. These clinical studies were reviewed and approved by their corresponding IRBs. Additionally, this secondary analysis was also approved as a separate study by the main IRB of the Universidad Peruana Cayetano Heredia (FWA 0000525). We analyzed all data without personal identifiers to ensure patient confidentiality.

2.4 RESULTS

From 220 participants in the parent trials, 73 were excluded: in 47 of them, no cysts resolved after antiparasitic treatment, 18 did not have the 12-month CT scan performed, 6 did not have MRI at 6 months, and another 2 were determined to have brain lesions that were not NCC (study flowchart, Figure 2.7.2). The 147 participants that were retained, therefore, all had at least one cyst that resolved six months after antiparasitic treatment, and had complete MRI and CT follow up.

Baseline characteristics of study participants are shown in Table 2.7.1. Included participants had a mean age of 32 years (SD: 12.2), 95 (64.6%) were male, and the median time of pre-existing seizures was 24 months (range: 0.9-294). Eighty patients (54.4%) had three or more viable cysts at baseline, and 95 (64.6%) had four or more EITB antibody bands. Previous antiparasitic treatment and previous use of antiepileptic drugs were reported by 14 (9.5%) and 71 (48.3%) patients respectively. Seventy-six patients (51.7%) received standard-dose ABZ, 29 (19.7%) received increased-dose ABZ, and 42 (28.6%) received standard-dose ABZ plus PZQ. The median daily dose of dexamethasone was 6.5 mg/day (range: 4.5-10). The duration of dexamethasone therapy was 10-12 days in 123 participants (83.7%) and 28 days in 24 participants (16.3%), with all of the latter receiving >6.5 mg per day. Characteristics of included and non-included patients at baseline were not statistically different with the exception that more participants that were excluded had a history of prior antiparasitic therapy than those who were included (20.6% [15/73] versus 9.5% [14/147], p=0.023.

Of the 147 participants, 92 (62%) had complete cyst cure, 38 (25.8%) had incomplete cyst resolution and were not re-treated with antiparasitic treatment before CT at month 12, and 17 (11.6%) had incomplete cyst resolution and were re-treated before CT exam.

Among the 147 included participants, 497 cysts resolved after antiparasitic treatment. From these, 188 calcified lesions were detected by CT in the topographic area corresponding to the original viable cyst 12 months after treatment, representing a proportion of cyst calcification of 37.8% (95% C.I.: 33.68-42.17). The proportions of cysts resulting in calcification according to study covariates are shown in Table 2.7.2.

We evaluated 18 self-selected patients with complete resolution of viable cysts who had 6-month MRI and 12-months CT scan evaluation. Subsequently, these patients had an additional long-term CT scan evaluation (mean time 3.6 years [1.0y - 6.6y]. These participants had 79 viable cysts at time zero that resolved when seen at the 6-month MRI, and 21 cysts (26.6% 21/79) that had calcified at the 12-month CT scan. In the long-term follow-up CT scan we found 3 (3.4% 3/79) new calcified lesions (in three different participants) among the resolved cysts evaluated at month six that did not show calcification at month 12.

In univariate analysis, variables at the cyst level significantly associated with risk of calcification included: cyst size greater than 14 mm, the location of cyst in the parietal lobe, and the presence of surrounding edema. At the patient level, the risk of calcification was associated with having seizures for more than 24 months, having 3 or fewer reactive antibody bands on EITB, receiving an increased dose of ABZ compared to combined treatment (ABZ plus PZQ), receiving a daily low dose of dexamethasone (less than 6.5 mg/patient), and not having received re-treatment in the presence of incomplete cyst resolution at month 6 (Table 2.7.2).

Multivariate analyses, model 1, included cyst covariates only, and demonstrated a significantly higher risk of residual calcification for cysts with a diameter greater than 14 mm (RR: 1.30 [95% CI: 1.07-1.57, $p=0.007$]) and for cysts with surrounding edema (RR: 1.36 [95% CI: 1.16-1.59, $p<0.001$]). In model 2, which also included patient level covariates, the risk of calcification remained higher for cysts larger than 14 mm (RR: 1.34 [95% CI: 1.02-1.75, $p=0.035$]) and for cysts with surrounding edema (RR: 1.39 [95% CI: 1.05-1.85, $p=0.023$]). Additional patient-level covariates associated with a

greater risk of calcification, model 2, included having a history of more than 24 months with seizures (RR: 1.25 [95% CI: 1.08-1.46, p=0.003]), having fewer than four reactive antibodies bands on EITB (RR: 1.14 [95% CI: 1.02-1.27, p=0.020]), receiving an increased dose of ABZ compared to combined treatment with ABZ plus PZQ (RR: 1.26 [95% CI: 1.14-1.39, p<0.001]), and receiving lower doses of dexamethasone (RR: 1.36 [95% CI: 1.02-1.81, p=0.037). Receiving standard-dose ABZ was not associated with a different risk of calcification compared to combined treatment.

Interestingly, the proportion of calcification in cysts that resolved after antiparasitic treatment was lower in individuals who had incomplete cyst cure and receive re-treatment, compared to both participants with incomplete cure who did not receive re-treatment (RR: 1.45 [95% CI: 1.08-1.93, p=0.012]) and to participants with complete cure at month 6 (RR: 1.48 [95% CI: 1.29-1.71, p<0.001]) (Table 2.7.2)

2.5 DISCUSSION

Despite the important role of post-treatment calcifications in the pathogenesis of seizures and resulting morbidity in NCC, there is still very little knowledge about how common this outcome is and how it can be avoided. This study shows that approximately two of each five (37.8%) viable brain parenchymal cysts result in residual calcification 12 months after antiparasitic treatment. In addition, the design of our study allowed a systematic assessment of factors associated with residual calcification. Large cysts, and cysts with perilesional edema prior to treatment, were more likely to result in calcification. Other factors associated with increased risk of calcification include a longer duration of disease (epilepsy), a mild antibody response, increased doses of ABZ versus combined antiparasitic treatment (ABZ-PZQ), lower doses of corticosteroids, and lack of antiparasitic re-treatment among those with incomplete cyst cure.

While there are multiple reports of the frequency of residual calcification in Indian patients with a single degenerating brain cysticercus, that is a milder presentation of NCC.[91, 95, 152, 166, 171, 174-179] Few studies have reported the proportion of viable parenchymal cysts resulting in calcification after antiparasitic treatment compared with placebo. In a prior study by our group, the proportions of residual calcifications were higher than what we report here, at 62% and 38.4% for the ABZ and placebo groups respectively.[25] In that study, however, the occurrence of calcifications was measured at 2 years (versus 12 months here), a difference that might explain the higher proportions of observed calcification. Carpio et al. [26] found 81.8% and 76.7% after a year of follow up in the albendazole versus placebo group, respectively, and showed similar proportion of calcification at the one and six month evaluations. On the other hand Das et al.[24] followed patients for 5 years after antiparasitic treatment and showed that the likelihood of calcification increased over time from 13.5% to 33.8% at 1 and 5 years, respectively, after antiparasitic treatment versus 9.3% to 22.7% at 1 and 5 years of follow up in the

placebo group. We only found an increment of 3.4% of calcification in a span of 2 to 6 years after treatment in a subgroup of patients.

Overall, the factors associated with the development of a calcified lesion suggest a significant role of the host inflammatory reaction in the pathogenesis of calcification, including baseline perilesional edema (reflecting significant ongoing inflammation), the use of lower corticosteroid doses (poorer modulation of inflammation), larger cysts (greater host exposure to parasite antigen), use of a higher dose of ABZ (causing important cyst damage but not lethal effects such as is seen with ABZ-PZQ and thus enhancing and extending inflammation), or lack of antihelmintic retreatment (failing to eliminate cyst remnants).

Our findings are consistent with those reported for solitary cysticercus granuloma (SCG), in which moderate to severe baseline edema is strongly associated with subsequent calcification (OR 3.3 95%CI 1.50–7.36).[165] However, patients with SCG who received anti-inflammatory treatment compared to a conservative approach (symptomatic treatment), both in the absence of antiparasitic treatment, have similar rates of calcification.[176-180] However, in some of these studies the CT scans were done only 2 to 6 months after intervention, likely underestimating the rates of calcification and missing any differential effect.

An increased-dose ABZ was associated with a higher risk of subsequent calcification, compared to combined ABZ plus PZQ, or standard dose ABZ. Since this regime was more effective than standard dose ABZ but less effective than combined ABZ plus PZQ, we are tempted to hypothesize that increased-dose ABZ causes greater damage and exposition of antigens than standard-dose ABZ (thereby requiring a prolonged, enhanced inflammatory reaction and thus more calcification), but does not reach the high efficacy levels of combined ABZ plus PZQ. In the combined ABZ plus PZQ group, the simultaneous use of two antiparasitic drugs may promote more aggressive damage to the cyst, and faster parasite death, reducing the overall time with inflammation.

In individuals who did not cure all their cysts (assessed at month 6 after treatment), a new course of treatment is indicated. Some, but not all of these individuals received early courses of retreatment (6 to 10 months after the first course of treatment). Interestingly, early retreatment was strongly associated with lower proportions of subsequent calcification for the subgroup of cysts that had already resolved by month 6. Resolved cysts that were not exposed to a second course of treatment were 1.5 times more likely to result in calcification than those that were exposed to a second treatment. This phenomenon would be equivalent to what occurs in NCC patients with a single enhancing lesion, in which the cyst has already started the degenerating process. The use of ABZ in these individuals results in less residual calcification.[95, 171] There is no clear mechanistic explanation for why the use of antiparasitic treatment in an already dying parasite can help to decrease the proportion of calcified lesions. One possible explanation is that antiparasitic treatment helps eliminate active or living parasite remnants that continue causing an inflammatory reaction.

This study has limitations. While MRI is the best neuroimaging modality to assess the viability of cysts, and CT scan allows more prominent visualization of calcifications, the sensitivity and specificity of these neuroimaging techniques are not perfect. Participants in the original trials may have been evaluated using different neuroimaging machines and this could have introduced a measurement bias. However, this was likely a non-differential bias that did not significantly alter the results observed in this study. Although there was some loss to follow-up in the original trials, this was also likely non-differential in nature.

2.6 CONCLUSION

This study adds important evidence to understand the factors involved in the calcification process, and suggests that inflammation plays a key role. Because residual calcification clearly increases the risk of epilepsy, clinical interventions to reduce calcification may reduce the risk of subsequent seizures and associated morbidity. The differential risk seen with varying regimens of both antiparasitic drugs and corticosteroids strongly suggests that that effective antiparasitic treatment aid by an optimized control of the resulting inflammatory reaction may mitigate the calcification process, reducing future seizure recurrence. The application of combined ABZ-PZQ treatment, higher doses of corticosteroids, and early re-treatment in patients with partial or even complete cure may result in lower rates of calcification, and should be tested in adequately powered trials.

2.7 ANNEXES

FIGURES

Figure 2.7.1. Consecutive T2 MRI slides showing two viable cysts at baseline in T2 MRI (left), both lesions resolve by month 6 after antiparasitic treatment (middle), and only the bigger cyst calcify at month 12 (right). Lesions are marked by red arrows.
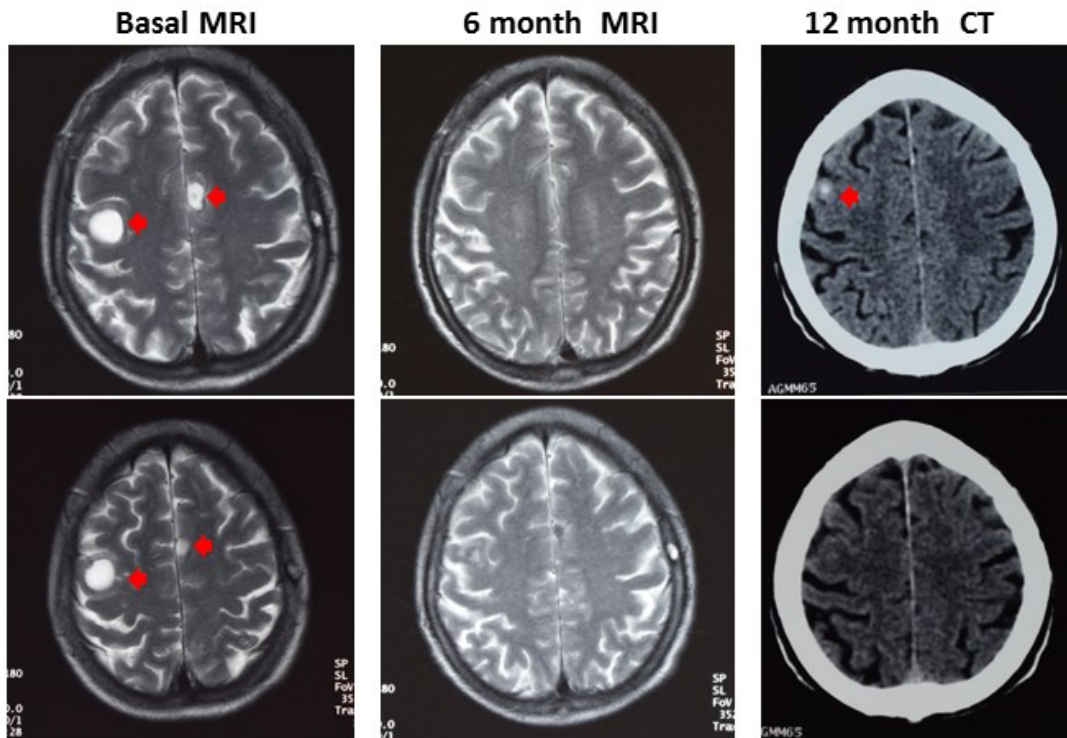
Figure 2.7.2. Flowchart of selection of study participants
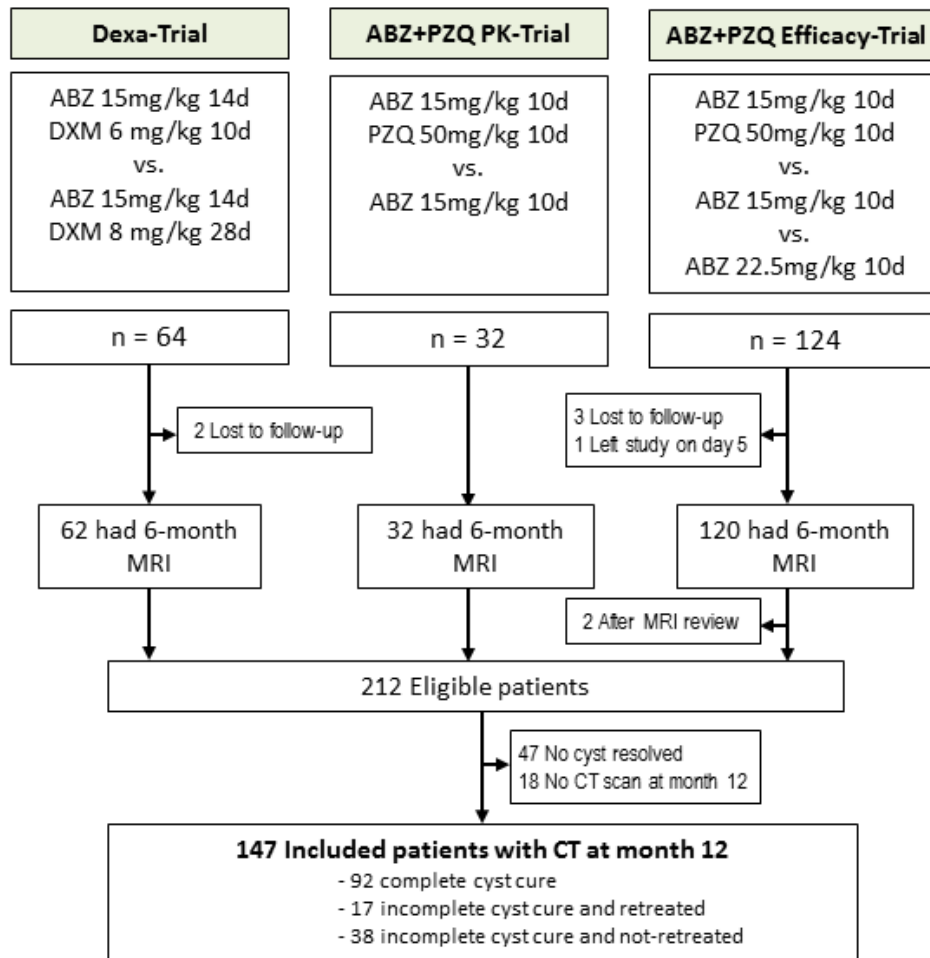
Table 2.7.1 Demographic, clinical and radiological characteristics of included and excluded participants at baseline (n=220)

Characteristics		Included (n=147)	Excluded (n=73)	*p* value
Age (years) [†]		32.0 ± 12.2	33.6 ± 12.8	0.367
Sex	Female	52 (35.4%)	28 (38.4%)	0.665
	Male	95 (64.6%)	45 (61.6%)	
Disease time (months) [‡]		24 (0.9-294)	24 [0.1-181]	0.329
Basal cysts	1-2 cysts	67 (45.6%)	36 (50.7%)	0.477
	≥3 cysts	80 (54.4%)	35 (49.3%)	
Basal calcifications	None	42 (28.6%)	17 (26.2%)	0.717
	1 or more	105 (71.4%)	48 (73.8%)	
EITB bands[b]	≤3 bands	52 (35.4%)	20 (27.4%)	0.265
	>3 bands	95 (64.6%)	53 (72.6%)	
Previous APT	No	133 (90.5%)	58 (79.5%)	0.023
	Yes	14 (9.5%)	15 (20.5%)	
Previous AED	No	76 (51.7%)	33 (45.2%)	0.364
	Yes	71 (48.3%)	40 (54.8%)	
APT scheme	ABZ+PZQ	42 (28.6%)	15 (20.6%)	
	Standard ABZ	76 (51.7%)	47 (64.4%)	0.202
	Increased ABZ	29 (19.3%)	11 (15.1%)	
Days with APT	10 days	102 (69.4%)	54 (74.0%)	0.481
	14 days	45 (30.6%)	19 (26.0%)	
Dose DEXA (mg/d) [‡]		6.5 (4.5-10%)	6.5 (4-10%)	0.403
Days with DEXA	10-12days	123 (83.7%)	65 (89.0%)	0.288
	28 days	24 (16.3%)	8 (11.0%)	

Abbreviations: APT (antiparasitic treatment), AED (antiepileptic drug), ABZ(albendazole), PZQ (praziquantel), DEXA (dexamethasone). EITB (electro immunotransfer blot), CNS (central nervous system)

[†]Mean ± standard deviation

[‡]Median (min-max)

Table 2.7.2 Risk ratios (RR) for the development of residual calcifications after antiparasitic treatment in patients with viable brain parenchymal NCC

Study variables	Calcified / cysts resolved (%)	Univariate models		Multivariate model 1[b]		Multivariate model 2[b]	
		RR (95% CI)	p value[c]	RR (95% CI)	p value[c]	RR (95% CI)	p value[c]
CYST LEVEL							
Cyst size							
≤14 mm	166/453 (36.6)	Ref.		Ref.		Ref.	
>14 mm	22/44 (50.0)	1.35 (1.14-1.60)	<0.001	1.30 (1.07-1.57)	0.007	1.34 (1.02-1.75)	0.035
Cyst content							
Clear	158/435 (36.3)	Ref.					
Turbid	30/62 (48.4)	1.30 (0.18-2.15)	0.313				
Cyst location							
Frontal lobe	67/172 (39.0)	1.02 (0.78-1.35)	0.866				
Parietal lobe	60/182 (33.0)	0.82 (0.67-1.00)	0.047				
Occipital lobe	27/63 (42.9)	1.13 (0.85-1.52)	0.402				
Temporal lobe	36/98 (36.7)	0.98 (0.70-1.38)	0.902				
Cyst edema							
No	125/365 (34.3)	Ref.		Ref.		Ref.	
Yes	63/132 (47.7)	1.37 (1.17-1.62)	<0.001	1.36 (1.16-1.59)	<0.001	1.39 (1.05-1.85)	0.023
PATIENT LEVEL							
Age (tertiles)							
16 to 25 years	67/177 (37.9)	Ref.					
26 to 35 years	56/156 (35.9)	0.98 (0.69-1.38)	0.899				
>35 years	65/164 (39.6)	1.03 (0.81-1.32)	0.796				
Sex							
Female	63/172 (36.6)	Ref.					
Male	125/325 (38.5)	1.04 (0.83-1.30)	0.733				
Disease time							
≤24 months	79/229 (34.5)	Ref.				Ref.	
>24 months	109/268 (40.7)	1.17 (1.13-1.21)	<0.001			1.25 (1.08-1.46)	0.003
N° of Cysts							
1 to 2	38/78 (48.7)	Ref.	Ref.				
3 or more	150/419 (35.8)	0.74 (0.44-1.25)	0.261				
N° of Calcifications at baseline							
None	39/98 (39.8)	Ref.					
1 or more	149/399 (37.3)	0.95 (0.55-1.69)	0.891				
EITB bands							
≥4 bands	140/388 (36.1)	Ref.	Ref.			Ref.	
≤3 bands	48/109 (44.0)	1.21 (1.14-1.28)	<0.001			1.14 (1.02-1.27)	0.020
Previous APT							
No	173/440 (39.3)	Ref.					
Yes	15/57 (26.3)	0.69 (0.36-1.30)	0.249				
Previous AED							
No	92/254 (36.2)	Ref.					
Yes	96/243 (39.5)	1.11 (0.99-1.23)	0.064				
APT scheme							
ABZ+PZQ	78/224 (34.8)	Ref.				Ref.	
Standard ABZ	73/190 (38.4)	1.09 (0.84-1.41)	0.525			1.04 (0.71-1.51)	0.861

Study variables	Calcified / cysts resolved (%)	Univariate models RR (95% CI)	p value[c]	Multivariate model 1[b] RR (95% CI)	p value[c]	Multivariate model 2[b] RR (95% CI)	p value[c]
Increased ABZ	37/83 (44.6)	1.27 (1.18-1.36)	<0.001			1.26 (1.14-1.39)	<0.001
Days with APT							
10 days	136/361 (37.7)	Ref.	Ref.				
14 days	52/136 (38.2)	0.99 (0.88-1.13)	0.958				
Dose of DXM							
>6.5 mg /day	71/217 (32.7)	Ref.	Ref.			Ref.	Ref.
≤6.5 mg /day	117/280 (41.8)	1.26 (1.04-1.52)	0.018			1.36 (1.02-1.81)	0.037
Days with DXM							
10-12 days	163/429 (38.0)	Ref.	Ref.				
28 days	25/68 (36.8)	0.95 (0.86-1.06)	0.366				
Cyst cure and re-treatment							
Incomplete cure and re-treated	19/66 (28.8)	Ref.	Ref.			Ref.	Ref.
Complete cure and not re-treated	111/280 (39.6)	1.41 (1.21-1.63)	<0.001			1.48 (1.29-1.71)	<0.001
Incomplete cure and not re-treated	58/151 (38.4)	1.33 (1.16-1.53)	<0.001			1.45 (1.08-1.93)	0.012

Abbreviation:
Risk ratio (RR), Confidence interval (CI), Electro immunotransfer blot (EITB), Antiparasitic treatment (APT), Antiepileptic drugs (AED), Albendazole (ABZ), Praziquantel (PZQ), Dexamethasone (DXM).

[†]Risk ratios from univariate and multivariate models were estimated using multilevel Poisson regression with random-effects for individual intercepts.

[‡]Multivariate multilevel Poisson regression model 1 initially included all covariates at the cyst level using a stepwise approach with backward selection algorithm to retain covariates with p value <0.05 for Wald Test.

[#]Multivariate multilevel Poisson regression model 2 included covariates retained in model 1 and all covariates at the patient level using a similar stepwise approach for selection of covariates.

All regression models were estimated using mean-variance adaptive Gauss-Hermite quadrature with seven integration points for each level of random effects. Standard errors were estimated with cluster-robust variance estimates to allow extra correlation due to the original trial from which the data came.

CHAPTER THREE

– RESEARCH PAPER 02 –

RISK FACTORS FOR BREAKTHROUGH SEIZURES IN PATIENTS WITH ACTIVE EPILEPSY DUE TO CALCIFIED NCC

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Competing interests: No competing interests are reported.

3.1 ABSTRACT

3.1.1 Introduction: Neurocysticercosis (NCC), an infection of the central nervous system with the larval vesicles of the parasite *Taenia solium*, is a leading cause of acquired epilepsy worldwide. Viable cysts in the brain parenchyma often follow a degenerative progression and eventually resolve completely or leave a calcified scar. Some patients with calcified NCC lesions develop epilepsy, and a proportion of them present breakthrough seizures despite appropriate treatment with antiepileptic drugs (AED). This study assessed the incidence and risk factors of breakthrough seizures (BTS) in patients with epilepsy due to NCC.

3.1.2 Methods: In this prospective cohort study, we assessed the incidence of and risk factors for BTS in adults with epilepsy and calcified NCC. We assembled a cohort of patients with neurological symptoms and calcified NCC as determined by computed tomography (CT) scan. Participants in this cohort received clinical examination, electroencephalogram (EEG), and enzyme-linked electroimmunotransfer blot (EITB) for detection of anticysticercal antibodies in serum. They were contacted by telephone every two weeks and had clinical evaluations every three months. From this main cohort, we selected the sub-group of individuals with epilepsy who were receiving AED, as indicated by their attending neurologist. These participants were followed until a BTS (event), withdrawal of AED, loss to follow-up, or administrative censoring. All seizures were classified according to the ILAE guidelines.

3.1.3 Results: The study cohort was composed by 210 patients that fulfilled the inclusion criteria, of which 103 (49.1%) presented BTS during the follow-up. The predefined maximum follow-up time was 36 months with a median of 10.8 months (total of observed months at risk were 2,915). The mean age was 36.3 ± 13.1 years old and 102 (48.6%) were male. In time-to-event analysis the Kaplan-Meier plot showed that only 36% (95% CI: 27.2%-44.9%) remained free of seizure during the study time and most of the events (91.2%, 94/103) occurred within the first 2 years of follow-up. In the initial

univariate Cox analysis we found that eight participants had an abnormal interictal EGG and they had a HR of BTS 9.2 times higher than the rest, and since they represented s different group we decide to exclude these eight participants for further characterization of other risk factors. In the remaining 202 participants univariate Cox analysis showed that risk factors for BTS included: being female (HR: 1.50 p=0.049), having a history of calcification without antiparasitic treatment prior to enrollment (HR 1.44 p=0.067), history of at least one seizure event in the past year prior to enrollment (HR: 2.91 p<0.001), and history of 10 or more seizures (HR: 1.77, p=0.006). The multivariate analysis showed similar trends, but only having a seizure event in the past year (HR: 2.69 p<0.001) and a history of 10 or more seizure events (HR 1.55 p= 0.038) remained statistically significant. Being female and having calcified lesions in the temporal lobe were associated with an increased risk for BTS relapse, but with marginal significance only.

3.1.4 Conclusions: Control of seizures with AED drugs in patients with calcified NCC is suboptimal. Survival time analysis showed that only 36% remain free of seizure after a follow-up of three years. Main risk factors for BTS were having a seizure within one year before enrollment, history of 10 or more seizures, and having an abnormal inter-ictal EEG. Our findings should orientate an individualized clinical management of patients with calcified NCC in a higher risk of BTS, which represent enduring epileptogenic foci.

3.2 INTRODUCTION

When cysticerci, the larvae of the pork tapeworm *Taenia solium*, infect the central nervous system, the disease is known as neurocysticercosis (NCC). NCC is the single most important factor associated with acquired epilepsy in developing countries, and probably in the world, and is thus an important public health problem.[164] Cases of symptomatic NCC are frequently seen in hospitals in endemic countries, and a consistent association with seizures has been found in most population studies.[5, 6, 8]

Larvae establish infection in the brain parenchyma by forming cystic structures, known as viable cysts when the parasite is alive. Over time, the cysts may degenerate, either resolving completely or leaving small, calcified scars in the brain. Brain calcifications are known to cause seizures, although the mechanisms are not well understood. Because calcifications can remain in the brain long after the parasite has died, perhaps even for the entire lifespan of the host, they accumulate in the population in endemic regions and are thus a major contributor of seizure burden.

Breakthrough seizures (BTS) are described as new seizure events that occur suddenly and unexpectedly after a seizure-free period, despite regular use of AED.[139, 181] BTS occur in about 30% of persons with epilepsy (PWE) from causes other than NCC.[182] Risk factors associated with BTS include experiencing multiple seizures before achieving control, presence of a concomitant neurological disorder, family history of epilepsy, and presence of a paroxystic EEG. Other potential causes include poor adherence to AED, concomitant inflammatory conditions or infections, emotional stress, use of medications that lower the seizure threshold, sleep deprivation, menstruation, diarrhea, metabolic events, flashing lights, etc.[139-141] Although BTS are common among PWE due to calcified NCC, these events and their risk factors have not been systematically studied among this group.[28, 96, 142] The incidence of BTS in calcified NCC has been reported to be 24% to 36% in a few small cohort studies, but associated risk factors have been poorly evaluated.[28, 96, 142]

In this study, we evaluated the incidence, cumulative incidence, and predictive factors of BTS in a cohort of patients with cerebral NCC calcifications and a well-established diagnosis of recurrent non-provoked seizures (epilepsy). Our results may help to identify the subpopulation of individuals with calcified NCC at increased risk of BTS, in order to implement individualized therapeutic approaches.

3.3 MATERIAL AND METHODS

3.3.1 Study design and objective. The objective of this prospective cohort study is to assess the incidence rate and cumulative incidence of BTS, as well as associated factors, in patients with epilepsy and calcified NCC.

3.3.2 Study population and selection criteria. This hospital-based study comprised adult patients who are part of a larger parent cohort of participants with calcified parenchymal NCC. This parent cohort started in 2012 and consecutively enrolled patients with calcified NCC and neurological symptoms (Figure 3.7. 1). From the parent cohort we selected a sub-group to evaluate incidence of and risk factors for breakthrough seizures BTS. Both men and women greater than 16 years of age were eligible for the BTS cohort. Other inclusion criteria were having been diagnosed with calcified parenchymal NCC using a non-enhanced brain CT, having acquired epilepsy as defined by ILAE (with a history two or more seizures),[105] and being currently under AED therapy. Exclusion criteria were having recurrent non-provoked seizures (not related to NCC), presence of viable and/or degenerating NCC lesions in the brain parenchyma, subarachnoid spaces, or ventricles, having a positive antigen ELISA result suggesting the presence of viable parasites,[183] intracranial hypertension or hydrocephalus, focal neurological deficits, dual neurological pathology (NCC + Stroke, or NCC + cortical dysplasia, etc.) and pregnancy. Participants with viable and/or degenerating cysts received their diagnostic test results and images and were referred for appropriate medical treatment.

3.3.3 Study activities. Participants received medical consultation by a team of study physicians, who collected clinical information regarding seizure history (months since first seizure, seizure-free months before enrollment, number of previous seizures, description of seizures, and family history of seizures) and prior use of cysticidal drugs. Brain CT scans were read first by the study physician, and subsequently by a

neuroradiologist who confirmed the number, location, and size of calcified lesions, and ruled out the presence of viable and/or degenerating cysts. Any discrepancies between reviewers were resolved by consensus. Patients also had an EEG, serum enzyme-linked electroimmunotransfer blot (EITB) assay for antibody detection,[55] and serum ELISA for antigen detection.[183]

The follow-up period was 36 months per patient, with censoring occurring for any of the following endpoints: AED withdrawal, loss to follow-up, or administrative censoring (36 months of follow-up or study end date of November 30, 2017), and the event was the occurrence of a BTS. Participants in the parent cohort were evaluated at the study site every three months, and the study team also followed them by phone contact every two weeks. All patients and close relatives were instructed to recognize and immediately report to the study team any event suggesting a seizure, and were given a diary that was reviewed by the study team at each clinical visit. A diagnosis of seizure was made initially by a study physician who interviewed the patient during the visit, with subsequent verification of the diagnosis by a study neurologist. Seizure events were classified according to the 2010 ILAE classification.[105] Further classification of epilepsy according new ILAE in 2017[106] did not change the seizure classification used in this study.

3.3.4 Outcome assessment. The primary outcome was the presence and timing of BTS. Cumulative incidence, incidence rate, and the survival curve were estimated in a time-to-event analysis during the time period between enrollment until event or censoring.

3.3.5 Covariates. We included the following covariates in our analysis: age in years (continuous variable and also as categories using tertiles), gender, prior antiparasitic treatment for viable NCC (yes/no), months since first seizure (continuous variable and also as dichotomous using >36 months), seizure-free months before enrollment (continuous variable and also as dichotomous variable using >12 months), number of prior seizures (discrete variable and also dichotomous variable using >10 seizures), family (first-degree relatives) with history of seizures (yes/no), personal history of

seizures (focal seizures/generalized seizures or focal seizures with generalization), EEG results (normal/abnormal), EITB result (≤3 reactive bands/≥4 reactive bands), number of calcifications (discrete variable and also as categories 1/ 2-10 / >10), size of calcifications (dichotomous; at least one large calcification [having a diameter >10 mm in at least one axis axial, coronal and/or sagittal planes]), and presence of edema around the calcification (dichotomous; at least one calcification had basal perilesional edema) and location of calcifications: frontal/other, parietal/other, occipital/other, and temporal/other. (See table 3.7.1)

3.3.6 Statistical analyses. Data was entered in Stata/IC 15.0 (Stata Corp, College Station, TX, US) for statistical analyses. Patient characteristics were described using summary statistics (categorical variables were expressed as percentages, whereas continuous and discrete variables were expressed using mean ± standard deviation or median and ranges respectively). The overall incidence of BTS in the cohort was reported with 95% confidence intervals, and compared between patient covariates using two-sample parametric or non-parametric tests as appropriate. The following definitions were applied for survival analysis: time origin – date of enrollment; time metric – duration of follow-up in months; and event – seizure reported by the patient and confirmed by the study neurologist. Cumulative event-free survival curves with 95% confidence intervals were calculated using the Kaplan – Meier method, and the log-rank test was used to compare survival curves by covariate. We also fitted Cox proportional hazard regression models to estimate hazard ratios (HR) of having BTS. First, bivariate models were performed, and then, a multivariate model was performed using a backward-stepwise approach for selection of covariates considering a p-value<0.05 from the Wald test. The proportional hazard assumption from the final model was assessed using the Schoenfeld residuals test and complementary log-log plots (Figure3.8.2). Predicted survival functions after Cox regression were also plotted.

3.3.7 Human subject rights protection. The parent cohort study was reviewed and approved by the IRBs of the Universidad Peruana Cayetano Heredia and of National Institute of Neurological Sciences, both in Lima, Peru. The participants were enrolled

after an informed consent process which included signing a written consent form. All study personnel who had contact with subjects or data completed a course on human subject protection. All exams that were part of the study were provided free of cost to the participants.

3.4 RESULTS

The BTS cohort included individuals followed from January 2012 to November 2017. As shown in the study flowchart (Figure 3.7.1), the original parent cohort was composed of 368 patients with NCC and neurological symptoms. The inclusion criteria for the BTS cohort included patients with calcified NCC, diagnosis of epilepsy and AED treatment; 177 individuals met the inclusion criteria and were selected in 2012. Subsequently, 33 of the 191 remaining individuals in the parent cohort became eligible during the follow-up period and were admitted into the BTS cohort. Of the 33, 26 began AED treatment for their existing diagnosis of epilepsy during the follow-up period, and 7 with a history of a single or no seizure subsequently developed a second seizure (5) or two or more seizures (2), thereby fulfilling the criteria of recurrent non-provoked seizures and starting their AED treatment. The final number of participants in the BTS cohort was 210 (Figure 3.7. 1).

Demographic, clinical, and neuroimaging characteristics of the BTS cohort are shown in Table 3.7.1. Participants had a mean age of 36.3 ± 13.1 years and 102 (48.6%) were men. The median time-period of having seizures was 79 months before enrollment (Interquartile range IQR: 36-181), and the median seizure-free time-period before enrollment was 6 months (IQR: 1-22). Participants had a median of 10 seizures prior to enrollment (IQR range: 3-33), and the majority of cases (81.9% [172/210]) reported having experienced at least one generalized seizure, or a focal seizure with secondary generalization. Twenty-six participants (12.4%) had a family history of seizures, and only eight (3.9%) had an abnormal EEG (focal abnormalities 5/8 and diffuse abnormalities 3/8). Fifty (23.8%) had a serum EITB result of ≥ 4 bands. Participants had a median of 3 (IQR: 1-10) calcifications detected by brain CT scan, and 38 (18.2%) had at least one lesion with perilesional edema. Calcifications were more often located in frontal lobes (164/210; 78.1%). Thirteen patients (6.2%) had at least one large lesion.

Of the 210 participants in the cohort, 103 presented new seizures during the follow-up period, with almost all events (94/103) occurring during the first 2 years of follow-up. The cumulative incidence of BTS was 49.1% (95% CI: 42.3%-55.8%). In the bivariate analysis using chi square test, individuals with BTS were likely to be female (p=0.027), to have had a shorter time-period ($\leq$ 12 months) without seizures before enrollment (p<0.001), to have a history of more than 10 events (p=0.007), to have reported at least one episode of generalized seizures or focal seizures with generalization (p=0.017), and to present edema around at least one calcified lesion (p=0.024). Other characteristics were not significantly different between patients with and without BTS during the follow-up period (Table 3.7.1).

Survival analysis showed a total time at risk in the cohort of 2,915 months, with a median follow-up time per subject of 10.8 months (IQR: 3.9-20.5 months). 107 patients were censored in this study: only 9 out of 210 (4.3%) were lost to follow-up, 51 underwent administrative censoring (22 due to date to study end and 29 fulfill 3 years of follow-up), and 47 stop their AED. The median seizure-free time in the cohort was 20.2 months (95% CI: 13.5-30.7). The Kaplan-Meier survival estimator showed that only 36.0% (95% CI: 27.2%-44.9%) remained free of seizures during the follow-up period (Figure 3.7.2). Given that 6 out of 8 participants with abnormal interictal EEG at baseline presented with BTS during the follow-up and five of these seizure events were observed within the first 6 months (Figure 3.7.3), we decide to exclude these eight participants for further characterization of risk factors for BTS.

Survival curves in the remaining 202 participants were statistically different between men and women, between patients with >12 and $\leq$12 months free of seizures before enrollment, and between those with >10 and $\leq$10 previous seizure episodes (Figure 3.7.4).

Analysis of risk factors for BTS is shown in Table 3.7.2. In univariate Cox regression models, significant risk factors for BTS included being female (HR: 1.50 [95% CI: 1.00-2.25], p=0.049), having 12 or less months free of seizures before enrollment

(HR: 2.91 [95% CI: 1.82-4.66], $p<0.001$), and having had more than 10 prior seizure episodes (HR: 1.77 [95% C I: 1.18-2.66], $p=0.006$). We also observed a trend of having calcification as result of natural evolution (without antiparasitic treatment) of the cyst but without statistical significance (HR: 1.31 [95% CI: 0.86-1.97], $p=0.194$).

After performing a multivariate model using a stepwise approach with backward selection, starting with a model that included all covariates and retaining variables with a $p<0.05$ from the Wald test, only two variables remain as significant risk factors for BTS; having a seizure-free time period ≤12 months (HR: 2.69 [95% CI: 1.67-4.34], $p<0.001$) and having had >10 previous seizures (HR: 1.55 [95% CI: 1.02-2.33], $p=0.038$), see Table 3.7.2. Schoenfeld's residuals tests and the plots of observed-versus-predicted survival curves corroborated the proportional-hazards assumption for covariates included in the multivariate analysis (Supplementary Information 3.8).

3.5 DISCUSSION

This prospective cohort study demonstrates a high frequency of BTS in PWE due to calcified NCC, with a total proportion around 50%. Survival analysis showed that only 36% remain free of seizures during 3-year follow-up period. Significant risk factors for BTS in the multivariate Cox regression model included having more prior and more recent seizures events (having a history of ten or more seizures, and having at least a seizure event within the previous year). An abnormal interictal EEG was strongly associated with BTS and most of these participants (6/8) presented BTS in the first year of follow up. Due to this particular characteristic, these participants with abnormal EEGs, were excluded from further univariate and multivariate time-to event analysis.

In PWE and with calcified NCC, the existing literature reports rates of BTS that are difficult to directly compare with our study results mainly because of differences in participant characteristics (number of calcifications) and follow-up time. Sharma et al.[8] reported a cohort of 35 patients with newly diagnosed epilepsy and single calcification, finding a cumulative incidence of BTS 34.3% in a follow-up time of 6 months. Singh et al.[142] studied a cohort of 54 PWE and single calcified lesions receiving AEDs who were followed-up for one year, and showing a BTS cumulative incidence of 24.04% (13/54). Nash and collaborators reported a 5-year longitudinal study with 110 patients with multiple calcified lesions. They reported seizure incidence of 36%; however, this study included a mix of patients with (60) and without (50) AED treatment. Patients taking AED had five times more chances to develop BTS (HR 5.29 95%CI 2.0-13.9 p=0.13).[28]

The cumulative hazard of BTS observed in our study is greater than that reported in the literature for epilepsy caused by causes other than NCC, in which 60% - 70% of patients respond to AEDs, effectively controlling their seizures.[184, 185] In those non-NCC patients, poor seizure control has been associated with factors such as early onset of

epilepsy, epilepsy of unknown cause, seizure semiology, higher numbers of seizures, epileptiform EEG activity, and intellectual disability.[186-188]

A strong predictor of BTS was to have an abnormal interictal EEG. Six of eight participants with abnormal EEG experienced BTS, with most occurring within the first 6 months of follow-up (5/6). Sub-analyses showed that abnormal EEG was strongly related with longer duration of disease; 7/8 had more than 36 months of disease (epilepsy) and all (8/8) had three or fewer bands on EITB. Sharma et al.[8] also report an abnormal EEG as a risk factor for BTS in NCC, and in non-NCC-related epilepsy, abnormal EEG has also been reported as a risk factor of BTS.[140]

The main predictor of BTS in our study was to have a seizure episode in the year prior to enrollment. These participants were three times more likely to have a breakthrough seizure than those who were seizure-free for more than 12 months. Having a history of frequent (ten or more) episodes of seizures before enrollment was also a strong predictor of BTS. It has been consistently described by the literature, in both NCC-related and non-NCC-related epilepsy, that several episodes of seizures is a potential risk factor for BTS.[96, 140-142]

Being female was associated with a higher risk of BTS in univariate analyses, while in the adjusted model lose their statistical significance when adjusted by number of previous seizure. The tendency for women to have more seizures has been previously reported in non-NCC patients. It has been consistently reported that progesterone has an antiepileptic effect, while estrogen increases the risk of seizures.[189, 190] Thus, menstruation has been reported as an attributable cause of seizures due to the association with progesterone levels. Also, a similar effect occurs during the ovulatory and peri-ovulatory phases due to an estrogen peak and progesterone withdrawal. Additionally, anovulatory periods involve a higher risk of seizure due to high ratio estrogen/ progesterone.[189-193] Sex has not been reported in the literature of calcified NCC and BTS.[28, 96, 142]

Consistent with other reports in the literature, our study did not find a relationship between the number of calcifications and an increased risk of BTS.[28, 194] This suggests that in patients with several cysts only one or a few lesions are epileptogenic or dominant. It argues against the hypothesis that direct calcium toxicity can be the causal effect of seizures in calcified NCC, because if seizures events were related to calcium toxicity, the epileptic activity should be proportionally related to the number of calcifications. However, we did not find this association present in our study, nor has it been reported in other studies.[28, 194] This finding can explain why in endemic areas we found a sizable proportion of asymptomatic subjects with brain calcifications due to NCC.[14, 18]

Patients with calcified NCC are frequently seronegative on EITB, since antibodies decay and disappear months or years after cyst death.[195, 196] In community-based studies conducted in endemic areas, it is common to find seronegative symptomatic and asymptomatic participants with calcified lesions compatible with NCC.[14, 18, 197] In our cohort, there was supporting evidence of NCC in 174/210 participants (159 were positive to EITB and 15 had history of antiparasitic treatment for NCC). The remaining 36 individuals had calcified images compatible with NCC calcifications confirmed by the study neuroradiologist, as cerebral calcified lesions due to NCC have a particular punctuated and rounded shape. The morphological characteristics of calcified lesions among those 36/210 patients without supporting evidence of NCC were similar to those of the 174/210 confirmed by serology and/or an antecedent of viable brain cysts. Also, as expected, these patients had a longer history of disease and fewer brain calcifications. Additionally, a sensitivity analysis excluding those 36 participants did not change the main results.

Contrary to the existing literature in PWE not related to NCC,[198] a family history of epilepsy did not represent a risk factor for BTS in this cohort of people with epilepsy and calcified NCC under AED treatment. Family history of seizure has a greater role in persons with epilepsy with a genetic basis.[199] While a genetic component for epilepsy caused by NCC has been suggested,[200-202] family history of epilepsy has not been consistently demonstrated as a risk factor for BTS in NCC.[28, 96, 142]

Prior use of cysticidal drugs had only a marginal protective effect. This protective role is consistent with literature showing the use of these drugs in viable NCC has a beneficial effect in seizure control in the long term.[25, 26, 203] However, most of this information came from non-randomized studies using historical controls, and the accuracy of results might be questionable.[71, 72, 204, 205] In contrast, the results of our BTS cohort study provides more robust support for this protective effect.

This study has limitations. The main limitation is a potential for recall bias among subjects with epilepsy and calcified NCC, as living with this chronic disease may have affected the numbers and type seizures reported at baseline. Another potential bias is that the only source of information for patients with generalized seizures is a close relative and/or witness that can only partially observe and describe the seizure episode. Thus, information bias could also be underestimating the number of events recorded in the cohort. This bias may occur in almost all population studies attempting to assess this covariable as a risk for BTS. The study physicians were not masked to the radiological or clinical information, thus they could had become more prone to identify seizure relapse in patients with many calcifications or with large calcifications, or with a history of history of severe or recurrent seizures. Thus this cohort may have overestimated the numbers of BTS. Additionally, we included patients attending a national referral center for neurological diseases in Peru. These represent a subgroup of subjects with NCC who may be more likely to have suboptimal seizure control, and because of this, selection bias may have been introduced affecting the representativeness of the results. 47 participants had censoring because they did stop their AED; however these patients were followed and the seizure events were also identified, thus, this group was composed by patients with informative censoring.

3.6 CONCLUSION

This prospective cohort provides important evidence that patients with calcified NCC and epilepsy provides key evidence for clinical management based on these other risk factors. Participants with abnormal interictal EEG should be considered in high risk of presenting with BTS in the short term. In addition, patients with calcification and epilepsy presenting with a history of more than ten seizures or with at least one seizure in the last year are at higher risk of BTS and should have close follow-up. Additionally, these findings may be extrapolated to other causes of epilepsy caused by brain calcifications or epilepsy due to other etiologies.[206]

3.7 ANNEXES

Figure 3.7.1 Flowchart to detail how the study cohort was assembled from the original cohort of patients with calcified NCC and neurological symptoms.
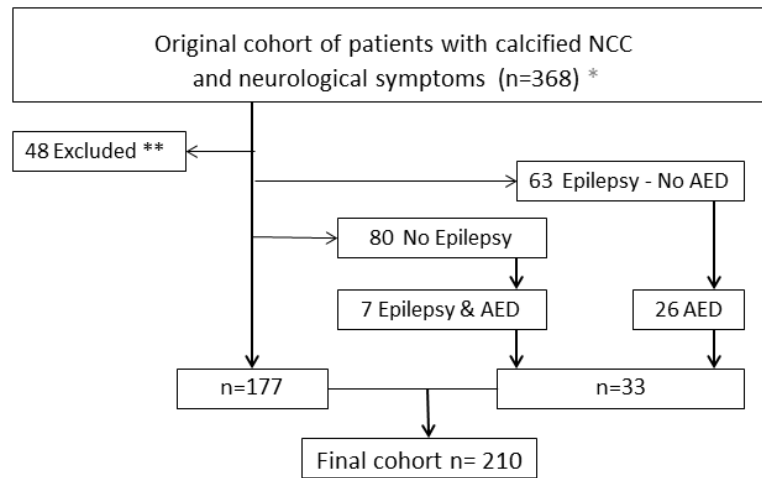
* Patients with calcified NCC are included if have diagnosis of epilepsy and are under AED
** Excluded (48): 9 original CT images were not found, 8 had elevated antigen levels, 21 had viable cysts, 1 had granuloma, 8 had not clear brain calcification, 1 left the study just after ICF signing

Figure 3.7.2. Kaplan-Meier survival curve of the study cohort. Dash marks represent censored observations. Confidence interval (CI)
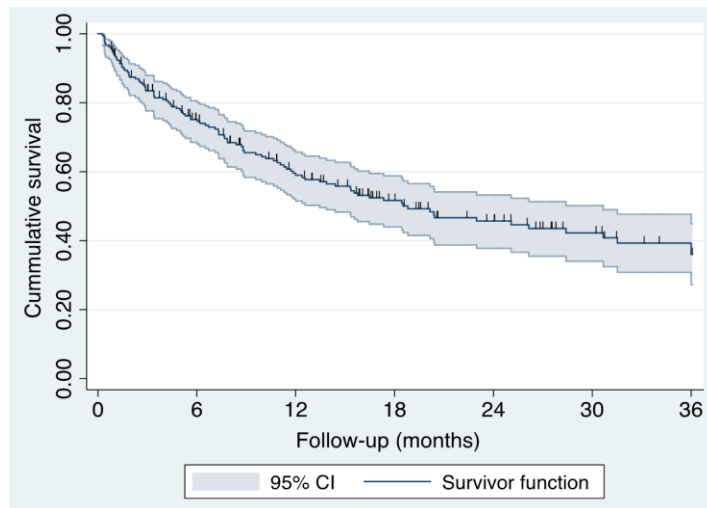
Figure 3.7.3 Kaplan-Meier survival curves of the entire study cohort by EEG.
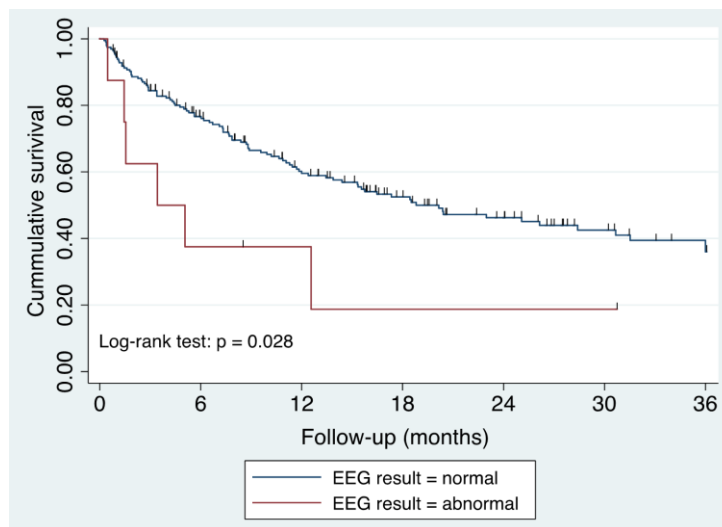
Figure 3.7.4 Kaplan-Meier survival curves of the study cohort after removing 8 participants with abnormal EEG (n=202).

A. Sex
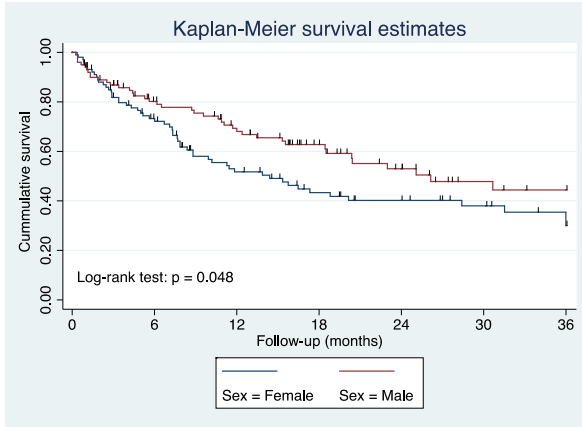
B. Seizure-free time
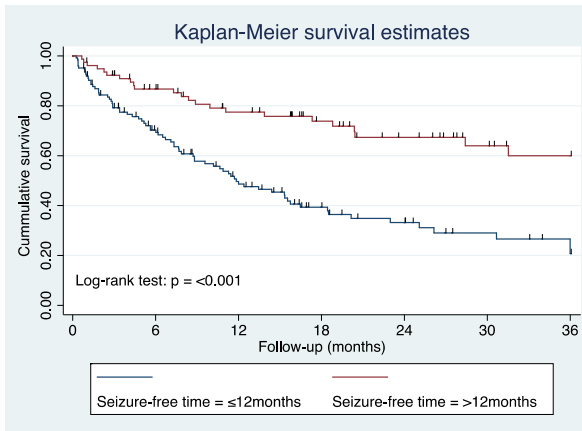
C. Number of previous seizures
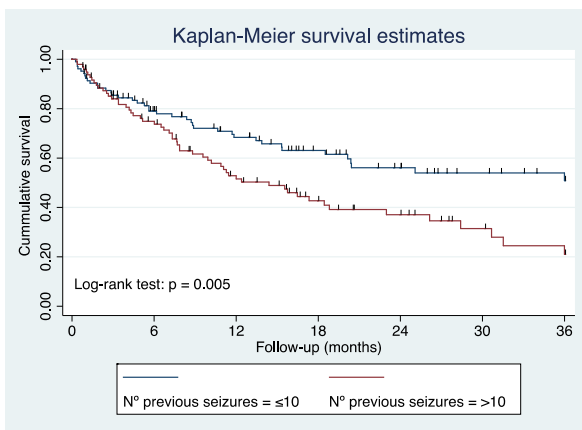
Table 3.7.1 Characteristics of the study cohort and comparisons between patients with and without breakthrough seizure during follow-up (N=210)

Variables	n (%)	Total (N=210)	Breakthrough seizure		p value
			Yes (N=103) n (%)	No (N=107) n (%)	
Age (years)					
	Mean ± SD	36.3 ± 13.1	35.3 ± 12.2	37.3 ± 13.9	0.275
	≤28 years	71 (33.8 %)	36 (35.0 %)	35 (32.7 %)	0.248
	29-40 years	73 (34.8 %)	40 (38.8 %)	33 (30.8 %)	
	>40 years	66 (31.4 %)	27 (26.2 %)	39 (36.5 %)	
Sex (male)		102 (48.6 %)	42 (40.8 %)	60 (56.1 %)	**0.027**
Previous APT (yes)		134 (63.8 %)	60 (58.3 %)	74 (69.2 %)	0.100
Seizure time before enrollment (months)					
	Median (IQR)	79 (36-181)	79 (31--178)	78 (41-200)	0.442
	>36 months	156 (74.3 %)	72 (69.9 %)	84 (78.5 %)	0.152
Seizure-free time before enrollment (months)					
	Median (IQR)	5.7 (1-22)	2.6 (1-12)	12.3 (2-36)	**<0.001**
	>12 months	78 (37.1)	23 (22.3)	55 (51.4)	**<0.001**
Number of previous seizures[a]					
	Median (IQR)	10 (3-33)	12 (4-48)	7 (3-24)	**0.005**
	>10 seizures	101 (48.3)	59 (57.8)	42 (39.3)	**0.007**
Family history of seizures		26 (12.4)	11(10.7)	15(14.0)	0.463
Generalized and/or focal seizures with generalization		172 (81.9 %)	91 (88.4 %)	81 (75.7 %)	**0.017**
EITB result ≥4 bands		159 (75.7 %)	73 (70.9 %)	86 (80.4 %)	0.109
EEG result (Abnormal)[b]		8 (3.9 %)	6 (5.9 %)	2 (2.0 %)	0.140
Number of calcifications					
	Median (range)	3 (1-118)	3 (1-118)	3 (1-74)	0.724
	Single calcification	70 (33.3 %)	35 (34.0 %)	35 (32.7 %)	0.978
	2 to 10 calcifications	89 (42.4 %)	43 (41.8 %)	46 (43.0 %)	
	>10 calcifications	51 (24.3 %)	25 (24.2 %)	26 (24.3 %)	
Perilesional Edema [c]		38(18.2 %)	25 (24.2 %)	13 (12.2 %)	**0.024**
Large calcifications [c]		13 (6.2%)	7 (6.9%)	6 (5.5)	0.694
Location of calcifications					
	Frontal lobe	164 (78.1 %)	83 (80.6 %)	85 (75.7 %)	0.393
	Parietal lobe	115 (54.8 %)	54 (52.4 %)	61 (51.7 %)	0.505
	Occipital lobe	83 (39.5 %)	44 (42.7 %)	39 (36.5 %)	0.353
	Temporal lobe	85 (40.5 %)	43 (41.8 %)	42 (39.3 %)	0.713

Standard deviation (SD), interquartile range (IQR), antiparasitic treatment (APT), enzyme-linked immunoelectrotransfer blot (EITB), electroencephalogram (EEG).
Bold values are considered statistically significant (p<0.05).
[a] One observation was missed. [b] Six observations were missed. [c] At least one calcified lesion

Table 3.7.2 Risk factors for seizure breakthrough in patients with calcified NCC and epilepsy under antiepileptic drug *

Variables		Univariate models		Adjusted model	
		HR (95%CI)	p value	HR (95% CI)	p value
Age	≤28 years	1.00			
	29-40 years	1.14 (0.72-1.83)	0.561		
	>40 years	0.75 (0.45-1.26)	0.280		
Sex	Male	1.00			
	Female	1.50 (1.00-2.25)	**0.049**		
Previous APT	Yes	1.00			
	No	1.31 (0.87-1.97)	0.194		
Seizure time	>36 months	1.00			
	≤36 months	1.33 (0.86-2.04)	0.195		
Seizure-free time before enrollment	>12 months	1.00		1.00	
	≤12 months	2.91 (1.82-4.66)	**<0.001**	2.69 (1.67-4.34)	**<0.001**
Number of previous seizures	≤10	1.00		1.00	
	>10	1.77 (1.18-2.66)	**0.006**	1.55 (1.02-2.33)	**0.038**
Family history of seizures	No	1.00			
	Yes	0.98 (0.53-1.85)	0.967		
Generalized and/or focal seizures with generalization	No	1.00			
	Yes	1.30 (0.71-2.38)	0.392		
EITB (+) ≥ 4bands	Positive	1.00			
	Negative	1.01 (0.63-1.62)	0.953		
Number of calcifications	Single	1.00			
	2 to 10	1.06 (0.67-1.69)	0.805		
	>10	1.08 (0.64-1.83)	0.762		
Perilesional Edema [c]	No	1.00			
	Yes	1.27 (0.79-2.03)	0.319		
Large calcifications [c]	No	1.00			
	Yes	1.08 (0.47-2.48)	0.852		
Location of calcifications	Frontal lobe	1.39 (0.82-2.34)	0.219		
	Parietal lobe	0.90 (0.60-1.34)	0.583		
	Occipital lobe	1.23 (0.82-1.83)	0.308		
	Temporal lobe	1.00 (0.67-1.49)	0.987		

*Patients with an abnormal EEG result were excluded for the analyses
Confidence interval (CI), antiparasitic treatment (APT), enzyme-linked immunoelectrotransfer blot (EITB), and electro encephalogram (EEG)
Bold p values are statistically significant
[c] At least one calcified lesion

3.8 SUPPLEMENTARY INFORMATION

3.8.1. Table. Kaplan-Meier Survival Probability Estimates

Time	Beg. Total	Fail	Net Lost	Survivor Function	Std. Error	[95% Conf. Int.]	
.26	202	1	0	0.9950	0.0049	0.9654	0.9993
.36	201	1	0	0.9901	0.0070	0.9610	0.9975
.39	200	3	0	0.9752	0.0109	0.9416	0.9896
.43	197	1	0	0.9703	0.0119	0.9351	0.9865
.66	196	1	0	0.9653	0.0129	0.9287	0.9833
.79	195	1	1	0.9604	0.0137	0.9224	0.9800
.85	193	1	0	0.9554	0.0145	0.9161	0.9766
.89	192	1	1	0.9504	0.0153	0.9099	0.9730
.95	190	1	0	0.9454	0.0160	0.9036	0.9694
.99	189	1	2	0.9404	0.0167	0.8975	0.9657
1.02	186	0	1	0.9404	0.0167	0.8975	0.9657
1.05	185	1	0	0.9354	0.0173	0.8913	0.9619
1.12	184	1	0	0.9303	0.0180	0.8851	0.9581
1.15	183	1	0	0.9252	0.0186	0.8790	0.9542
1.32	182	2	0	0.9150	0.0197	0.8668	0.9463
1.41	180	0	1	0.9150	0.0197	0.8668	0.9463
1.45	179	1	0	0.9099	0.0203	0.8608	0.9423
1.61	178	1	0	0.9048	0.0208	0.8548	0.9382
1.78	177	1	0	0.8997	0.0213	0.8488	0.9341
1.87	176	2	0	0.8895	0.0222	0.8370	0.9258
1.91	174	1	0	0.8844	0.0227	0.8311	0.9216
1.94	173	0	1	0.8844	0.0227	0.8311	0.9216
2.27	172	1	0	0.8792	0.0231	0.8252	0.9174
2.47	171	1	0	0.8741	0.0236	0.8193	0.9131
2.53	170	1	0	0.8689	0.0240	0.8135	0.9088
2.66	169	1	0	0.8638	0.0244	0.8076	0.9045
2.79	168	1	1	0.8586	0.0248	0.8018	0.9002
2.86	166	1	0	0.8535	0.0252	0.7960	0.8958
2.89	165	2	0	0.8431	0.0259	0.7844	0.8870
2.93	163	0	2	0.8431	0.0259	0.7844	0.8870
2.96	161	0	1	0.8431	0.0259	0.7844	0.8870
3.22	160	0	1	0.8431	0.0259	0.7844	0.8870
3.32	159	0	1	0.8431	0.0259	0.7844	0.8870
3.39	158	3	0	0.8271	0.0270	0.7665	0.8733
3.65	155	0	1	0.8271	0.0270	0.7665	0.8733
3.95	154	1	0	0.8217	0.0274	0.7605	0.8687
4.04	153	0	1	0.8217	0.0274	0.7605	0.8687
4.18	152	1	0	0.8163	0.0277	0.7545	0.8640
4.31	151	1	0	0.8109	0.0280	0.7486	0.8593
4.41	150	1	0	0.8055	0.0284	0.7426	0.8546
4.47	149	1	0	0.8001	0.0287	0.7367	0.8498
4.5	148	0	1	0.8001	0.0287	0.7367	0.8498
4.77	147	1	0	0.7947	0.0290	0.7307	0.8451
5	146	1	0	0.7892	0.0293	0.7247	0.8403
5.1	145	0	2	0.7892	0.0293	0.7247	0.8403
5.16	143	1	0	0.7837	0.0296	0.7187	0.8354
5.29	142	1	0	0.7782	0.0299	0.7127	0.8305
5.39	141	0	1	0.7782	0.0299	0.7127	0.8305
5.49	140	0	1	0.7782	0.0299	0.7127	0.8305
5.62	139	2	0	0.7670	0.0305	0.7005	0.8206
5.65	137	0	1	0.7670	0.0305	0.7005	0.8206
5.85	136	0	1	0.7670	0.0305	0.7005	0.8206
5.95	135	0	1	0.7670	0.0305	0.7005	0.8206
6.02	134	1	0	0.7613	0.0308	0.6943	0.8156
6.08	133	0	1	0.7613	0.0308	0.6943	0.8156
6.15	132	0	1	0.7613	0.0308	0.6943	0.8156
6.18	131	1	0	0.7555	0.0311	0.6879	0.8104
6.51	130	1	0	0.7497	0.0314	0.6816	0.8052
6.71	129	1	0	0.7438	0.0317	0.6754	0.8000
7.1	128	1	0	0.7380	0.0320	0.6691	0.7948
7.3	127	1	0	0.7322	0.0323	0.6628	0.7896

7.33	126	2	0	0.7206	0.0328	0.6504	0.7791
7.53	124	0	1	0.7206	0.0328	0.6504	0.7791
7.66	123	1	0	0.7147	0.0330	0.6441	0.7738
7.69	122	1	0	0.7089	0.0333	0.6379	0.7685
7.86	121	2	0	0.6972	0.0338	0.6254	0.7578
7.92	119	0	1	0.6972	0.0338	0.6254	0.7578
7.96	118	0	1	0.6972	0.0338	0.6254	0.7578
8.38	117	1	0	0.6912	0.0340	0.6191	0.7524
8.48	116	0	1	0.6912	0.0340	0.6191	0.7524
8.55	115	0	1	0.6912	0.0340	0.6191	0.7524
8.71	114	1	0	0.6851	0.0342	0.6127	0.7469
8.81	113	2	0	0.6730	0.0347	0.5999	0.7358
8.88	111	1	0	0.6670	0.0349	0.5935	0.7302
9.57	110	1	0	0.6609	0.0351	0.5871	0.7246
9.93	109	1	0	0.6548	0.0353	0.5808	0.7190
10.19	108	1	0	0.6488	0.0355	0.5744	0.7134
10.29	107	0	1	0.6488	0.0355	0.5744	0.7134
10.65	106	1	0	0.6426	0.0357	0.5681	0.7077
10.75	105	0	1	0.6426	0.0357	0.5681	0.7077
10.78	104	0	1	0.6426	0.0357	0.5681	0.7077
10.88	103	1	0	0.6364	0.0359	0.5615	0.7019
11.08	102	1	0	0.6302	0.0361	0.5550	0.6961
11.28	101	1	0	0.6239	0.0362	0.5486	0.6903
11.44	100	1	0	0.6177	0.0364	0.5421	0.6845
11.51	99	0	1	0.6177	0.0364	0.5421	0.6845
11.7	98	1	0	0.6114	0.0366	0.5356	0.6786
11.84	97	1	0	0.6051	0.0367	0.5290	0.6727
12	96	1	0	0.5988	0.0369	0.5226	0.6668
12.39	95	1	0	0.5925	0.0370	0.5161	0.6609
12.46	94	0	1	0.5925	0.0370	0.5161	0.6609
12.89	93	0	1	0.5925	0.0370	0.5161	0.6609
12.92	92	0	1	0.5925	0.0370	0.5161	0.6609
13.45	91	1	1	0.5860	0.0372	0.5094	0.6548
13.61	89	0	1	0.5860	0.0372	0.5094	0.6548
13.87	88	1	0	0.5793	0.0374	0.5025	0.6485
14.4	87	1	0	0.5726	0.0375	0.4956	0.6423
14.47	86	0	1	0.5726	0.0375	0.4956	0.6423
15.06	85	0	2	0.5726	0.0375	0.4956	0.6423
15.32	83	1	0	0.5657	0.0377	0.4885	0.6358
15.35	82	1	0	0.5588	0.0379	0.4814	0.6293
15.55	81	1	0	0.5519	0.0380	0.4743	0.6228
15.68	80	0	1	0.5519	0.0380	0.4743	0.6228
15.75	79	1	1	0.5450	0.0382	0.4671	0.6162
15.81	77	0	1	0.5450	0.0382	0.4671	0.6162
15.98	76	0	1	0.5450	0.0382	0.4671	0.6162
16.31	75	0	1	0.5450	0.0382	0.4671	0.6162
16.34	74	0	1	0.5450	0.0382	0.4671	0.6162
16.47	73	1	0	0.5375	0.0384	0.4594	0.6092
16.5	72	0	2	0.5375	0.0384	0.4594	0.6092
16.6	70	0	1	0.5375	0.0384	0.4594	0.6092
16.83	69	0	1	0.5375	0.0384	0.4594	0.6092
17	68	0	1	0.5375	0.0384	0.4594	0.6092
17.33	67	1	0	0.5295	0.0386	0.4510	0.6018
17.56	66	0	1	0.5295	0.0386	0.4510	0.6018
17.95	65	0	1	0.5295	0.0386	0.4510	0.6018
18.44	64	1	0	0.5212	0.0389	0.4423	0.5942
18.51	63	1	1	0.5129	0.0392	0.4337	0.5865
18.81	61	1	0	0.5045	0.0394	0.4250	0.5787
19.23	60	0	1	0.5045	0.0394	0.4250	0.5787
19.4	59	0	1	0.5045	0.0394	0.4250	0.5787
19.5	58	0	2	0.5045	0.0394	0.4250	0.5787
19.96	56	0	1	0.5045	0.0394	0.4250	0.5787
20.15	55	1	0	0.4953	0.0397	0.4153	0.5703
20.38	54	1	0	0.4862	0.0401	0.4057	0.5619
20.42	53	1	0	0.4770	0.0403	0.3961	0.5534
20.48	52	0	1	0.4770	0.0403	0.3961	0.5534
20.55	51	0	1	0.4770	0.0403	0.3961	0.5534
22.32	50	0	1	0.4770	0.0403	0.3961	0.5534
22.98	49	1	0	0.4673	0.0407	0.3859	0.5444
23.51	48	0	1	0.4673	0.0407	0.3859	0.5444

23.97	47	0	2		0.4673	0.0407	0.3859	0.5444
24	45	0	1		0.4673	0.0407	0.3859	0.5444
24.56	44	0	1		0.4673	0.0407	0.3859	0.5444
24.99	43	0	1		0.4673	0.0407	0.3859	0.5444
25.08	42	1	0		0.4561	0.0412	0.3740	0.5345
25.97	41	0	1		0.4561	0.0412	0.3740	0.5345
26.14	40	1	0		0.4447	0.0417	0.3618	0.5242
26.5	39	0	1		0.4447	0.0417	0.3618	0.5242
26.76	38	0	1		0.4447	0.0417	0.3618	0.5242
26.93	37	0	1		0.4447	0.0417	0.3618	0.5242
27.42	36	0	1		0.4447	0.0417	0.3618	0.5242
27.48	35	0	1		0.4447	0.0417	0.3618	0.5242
27.72	34	0	1		0.4447	0.0417	0.3618	0.5242
28.14	33	0	1		0.4447	0.0417	0.3618	0.5242
28.41	32	1	0		0.4308	0.0427	0.3464	0.5124
30.15	31	0	1		0.4308	0.0427	0.3464	0.5124
30.51	30	0	1		0.4308	0.0427	0.3464	0.5124
30.67	29	1	0		0.4160	0.0437	0.3298	0.4998
31.4	28	0	1		0.4160	0.0437	0.3298	0.4998
31.53	27	1	0		0.4006	0.0447	0.3128	0.4866
33.07	26	0	1		0.4006	0.0447	0.3128	0.4866
33.99	25	0	1		0.4006	0.0447	0.3128	0.4866
36	24	2	22		0.3672	0.0468	0.2765	0.4581

3.8.2 Figures: Plots of observed-versus-predicted survival curves

A. Sex
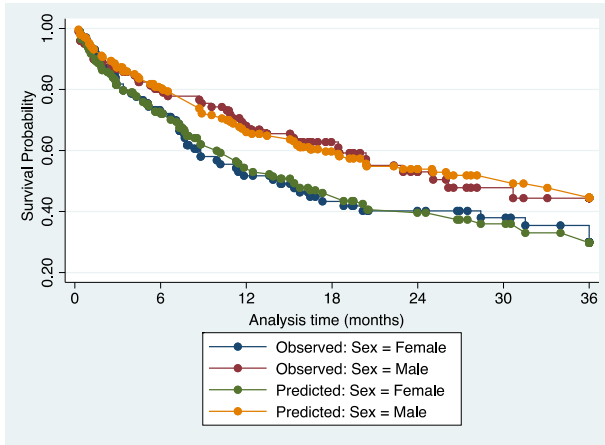
B. Seizure-free time
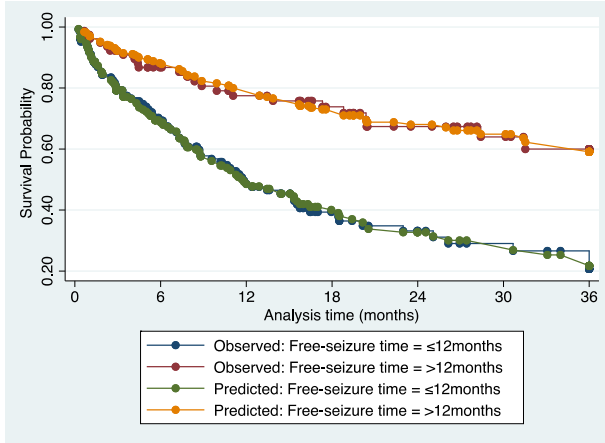
C. Number of previous seizures
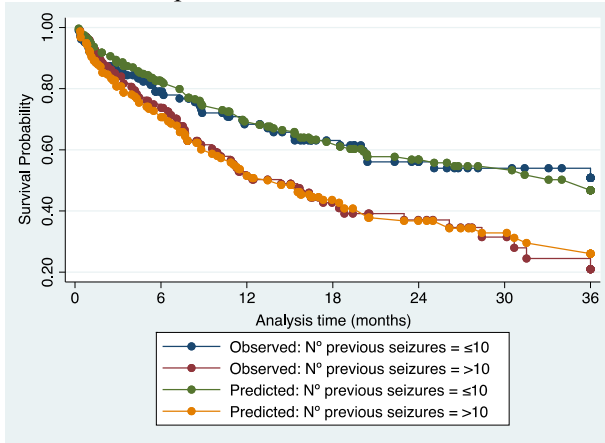
CHAPTER FOUR

– RESEARCH PAPER 03 –

SEIZURE RELAPSE AFTER ANTIEPILEPTIC DRUGS WITHDRAWAL IN PATIENTS WITH CALCIFIED NEUROCYSTICERCOSIS AND EPILEPSY

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Keywords:

Neurocysticercosis, cysticercosis, *Taenia* solium, calcification, epilepsy, seizure, antiepileptic withdrawal, Perú.

4.1 ABSTRACT

4.1.1 Introduction. Neurocysticercosis is an infection of the human central nervous system by the cystic larval stage of the tapeworm *Taenia solium.* As part of their natural life cycle or subsequent to antihelmintic treatment, parenchymal brain cysts degenerate and resolve, either disappearing or leaving a small, calcified lesion in the host brain. Some of these patients will present seizures and subsequently epilepsy, needing symptomatic treatment with antiepileptic drugs (AED). After an appropriate seizure free period, these patients are candidates for AED withdrawal. Risk factors for seizure relapse after AED withdrawal in calcified NCC have not been adequately assessed. Departing from a cohort of patients with calcified NCC and epilepsy under AED treatment (n=202), a subgroup analysis was performed to evaluate the incidence rate and factors associated with seizure recurrence in patients who withdraw their AED treatment (n=62).

4.1.2 Methods. This cohort study assessed data from participants of the parent cohort who withdrew their AED treatment. In the parent cohort, participants had a detailed history and clinical examination. During the follow-up, the study team carefully recorded dates and characteristics of seizure episodes, seizure-free periods, and date and reason of AED discontinuation. Patients were monitored by telephone every two-weeks and also were asked to attend a clinical visit at the study site every 3 months. They were also instructed to immediately report to the study team any seizure-related event. The study physician and neurologist confirmed the information and classified seizures according to the ILAE guidelines. Descriptive and survival analysis were performed using Kaplan-Meier estimator and Cox regression.

4.1.3 Results. Sixty two participants who withdrew AED were included in this observational cohort. Their mean age of participants was 38 years and there were slightly more females than males (34/28). Throughout a mean follow up time of 17.3 months, 17 (27.4%) patients presented with seizure relapses. The cumulative survival incidence of seizure relapse was 41.4% along a maximum of 36 months of follow-up. Nearly half of

this observational cohort (31/62) had less than 2 years seizure-free before AED withdrawal, and almost all patients who had seizure relapses (15/17) belong to this group of participants. Cox regression models of the entire cohort showed that the main risk factors were having seizures in the last two years before AED withdrawal (HR 9.21 95%IC 2.22-38.04 p= 0.002). We only selected this subgroup of 31 participants for subsequent analysis. We found that having a history of ten or more seizures and having had at least a generalized seizure or focal seizure with generalization show a trend of increased risk of seizure relapse but without statistical significance. Participants prone to withdraw the AED are young people, participants with history of only focal seizures, free of seizures in the prior year to enrollment, and participants with a weak serum reaction to EITB-WB.

4.1.4 Conclusion. We recommend that patients with calcified NCC achieve at least 2 years seizure-free and adhere to standard guidelines to be considered ready for AED withdrawal. Additionally, patients with a history of ten or more seizures, generalized seizures, or focal seizures with generalization should consider continuing AED treatment.

4.2 INTRODUCTION

Neurocysticercosis (NCC), defined as the infection of the central nervous system (CNS) by the larval stage of the zoonotic tapeworm *Taenia solium*, remains a major challenge in public health due to associated secondary epilepsy.[1-3] It has been consistently demonstrated that NCC is one of the leading cause of acquired epilepsy in endemic countries,[5-8] and likely worldwide; in fact, approximately 80% of the disease burden caused by epilepsy is located in developing countries.[4, 207]

T. solium larvae lodge in the brain parenchyma as viable cysts. At the end of their natural life cycle or subsequent to antiparasitic treatment, these cysts degenerate and a portion of these lesions progress to a residual parenchymal calcified lesion.[13] Cerebral calcifications persist in the host brain permanently, and in endemic areas, calcifications have been consistently associated with seizures in population and hospital-based studies.[5-8, 208]

There is consensus in the literature that an individualized approach to the discontinuation of AED in PWE is needed considering the main risk factors:[209, 210] older age at seizure onset, longer duration of epilepsy disease, abnormal neurological examination or psychiatric findings, abnormal EEG findings, increased number of seizures during AED therapy or history of status epilepticus, greater number of AED used during treatment, and failure in previous withdrawal attempts.[148, 211, 212] Additionally, gradual AED withdrawal is also recommended.[145, 210, 213, 214]

Recommendations for AED withdrawal in patients with epilepsy and NCC have never been systematically assessed and are mostly based on expert opinion.[215] Most studies in NCC that report risk factors for seizure relapse after AED withdrawal have been done in NCC patients with mixed NCC (viable, degenerating, and/or calcified) [29, 41] and in patients with a single enhancing lesion.[152-156, 166] Risk factors in these studies, besides the presence of residual calcifications, are having recurrent or multiple seizures,

abnormal interictal EEG, and seizures events that occurred under AED treatment. In calcified NCC, only three small studies have reported seizure relapse after AED withdrawal and risk factors have been poorly assessed.[30, 31]

In a cohort of patients with epilepsy due to calcified NCC under AED drug (n=202) we found that control of seizures is suboptimal, survival analysis showed that only 36% remains free of seizure after a follow-up of 3 years. Main risk factors for breakthrough seizure were having had a seizure within one year before enrollment, a history of ten or more seizures, and having an abnormal inter-ictal EEG (Chapter 3). This current study was performed in patients belonging to this cohort in whom AED treatment was interrupted (n=62) and is aimed to evaluate the incidence rate of seizure relapse after AED withdrawal and its associated risk factors.

4.3 MATERIAL AND METHODS

4.3.1 Study Design. This is a prospective cohort study to explore risk factors for incidence of seizure recurrence and associated risk factors in epileptic patients with calcified NCC who withdrew their AED treatment.

4.3.2 Study Population and selection criteria. Departing from a cohort study of approximately 202 patients with calcified NCC under AED treatment, we identified a sub-group of 62 patients who had withdrawn their AED treatment in order to evaluate their seizure relapse and risk factors. Selection criteria for the parent cohort included: male or female participants between 12-75 years of age, epilepsy secondary to NCC, and calcified NCC as determined by CT scan. Participants with abnormal paroxistic EEG, viable or degenerating NCC lesions, positive parasite antigen test, intracranial hypertension, hydrocephalus, or focal neurological deficits were excluded.

4.3.3 Study Activities. We included patients with calcified NCC under AED who were already under follow-up as part of a cohort who did stop their AED treatment. In the parent cohort, participants had a detailed history and clinical examination where they provided clinical information regarding seizure history (months since first seizure, seizure-free months before enrollment, number of previous seizures, description of seizures, and family history of seizures). All this information was updated considering the day when the participant withdrew their AED as the date of enrollment. Also we considered previously recorded information such as previous courses of antiparasitic treatment as well as topographic location and size of calcified lesions in brain CT scan images. At the time of enrollment in this cohort (patients who stopped their AED treatment), we did not perform an additional EEG, serum EITB,[55] or ELISA for antigen detection.[183] We used the results obtained for these tests at entry to the parent cohort for our analysis.

4.3.4 Follow-up. We carefully recorded dates and characteristics of seizure episodes, seizure-free periods, and day and reason of AED discontinuation. Patients in this cohort were closely followed to monitor seizure relapse from AED discontinuation for 36 month after enrollment (or until November 2017). They were asked to visit the clinical site every 3 months for follow up and instructed to immediately report to the study team any event compatible with a seizure. They were also monitored by telephone every 2 weeks by the study team. A diagnosis of seizures was initially made by interview with the patient or witnesses of the event; the information was verified by the study physician and study neurologist. All seizure events were classified according the 2010 ILAE guideline.[105] The new epilepsy classification according to ILAE 2017[106] did not change the seizure classification made in this study. The attending neurologist was responsible for medical care if participants presented with seizure activity during the follow-up. The study team provided the attending neurologist with all clinical information and test results obtained as part of this study.

4.3.5 Outcome Assessment. Our main outcome was the presence of seizure relapses after AED withdrawal. Seizure dates were recorded to assess the cumulative incidence, incidence rate, and to perform a time-to-event analysis, which considered the time period since AED withdrawal until event (seizure relapse), lost to follow-up, or censoring, to calculate the survival curve free of seizure and the hazard ratio.

4.3.6 Covariates. We evaluated the following covariates: age in years (continuous variable and also as categories using tertiles), sex (female/male), previous antiparasitic treatment for viable neurocysticercosis (yes/no), months since first seizure (discrete variable and also as dichotomous using >36 months), seizure-free months before enrollment (discrete variable and also as dichotomous variable using >24 months), number of previous seizures (discrete variable and also dichotomous variable using >10 seizures), family (first-degree relatives) history of seizures (yes/no), a history of type of seizures (focal seizures /generalized seizure or focal seizure with generalization), EITB result (≤3 reactive antibody bands/≥4 reactive bands), tapering at AED withdrawal (yes/no), number of calcifications (discrete variable and also as categories 1/2-10/>10),

and topographic location of calcifications according to cerebral lobes: frontal/other, parietal/other, occipital/other, and temporal/other. (Table 4.7.1) All variables involving time measurement were considered in relation to the date of AED withdrawal.

4.3.7 Statistical analysis. To characterize the population, descriptive information was presented using summary statistics. Categorical variables were expressed as proportions, whereas continuous and discrete variables were expressed using mean ± standard deviation or median and ranges respectively. Overall frequency of seizure relapse was reported with 95% confidence intervals, and compared by covariates using two-sample parametric or non-parametric tests as appropriate to variable. For the survival analysis the following criteria were applied: event - seizure reported by the patient and confirmed by the study physician , time origin - date when patient completed AED withdrawal, time metric – duration of follow-up in months up to event, administrative censoring (36 months of follow-up or predefined date: November 30, 2017), re-starting of AED, or lost to follow-up. We employed bivariate Cox regression analysis to evaluate hazard risk. We also used the survival Kaplan-Meier estimator and log-rank test method to assess cumulative event-free survival curve with confidence interval of 95%. Predicted survival functions after Cox regression were also plotted. Additionally, we explored variables associated with AED withdrawal using bivariate analysis and multivariate analysis with a log-binomial regression model. All statistical analyses were done using Stata/IC 15.0 (Stata Corp, College Station, TX, US).

4.3.8 Human subjects rights protection. The study was reviewed and approved by the UPCH IRB and by the IRB of the National Institute of Neurological Sciences both in Lima, Peru. All study personnel who had contact with subjects or data, completed a course on human subject protection.

4.4 RESULTS

From 202 participants with epilepsy, calcified NCC and AED treatment in the parent cohort, 62 patients stopped their antiepileptic treatment and were included in this study. This prospective cohort was carried out from July 2012 to November 2017. The main characteristics of these 62 patients are presented in Table 4.7.1. The mean age of patients was 38.1 ± 13.2 years, and the proportion of females was slightly higher (54.8%, 34/62) than males. Thirty-nine (62.9%, 39/62) patients had a history of antiparasitic treatment for NCC, the median time with seizures was 96 months (IQR: 53-195), the median number of previous seizures was 9 (IQR: 3-21), the median seizure-free time before enrollment was 24 months (IQR: 6-59), and most cases (72.6% [45/62]) reported at least one generalized seizure or a focal seizure with generalization. Seven patients (11.3% [7/62]) had a family history of seizures, and ten patients (16.1% [10/62]) had an EITB result ≥4 bands. The participants had a median of four (IQR: 1-9) calcifications, and lesions were more frequently located in the frontal lobes (77.4%) than in other lobes.

In a mean follow up time of 17.3 months after AED withdrawal, 17 (27.4% ± 13.2) patients presented with seizure relapses. The strongest risk factor for seizure relapse was the length of time free of seizures at the time of AED discontinuation. Only 6.5% (2/31) of people who were seizure free for more than 2 years presented a seizure relapse, compared to 48.4% (15/31) of people who had at least one seizure in the previous 2 years before AED withdrawal ($p<0.001$). Participants with a history of ten or more seizures had more seizure relapses (40.7% [11/27] versus 17.1% [6/35], $p=0.039$). A shorter time of disease (≤ 36 months) was a marginal risk factor for seizure relapse (57.1% [4/7] versus 23.6% [13/55], $p=0.061$). Other characteristics were not significantly different between patients who presented seizure relapses or not during the study (Table 4.7.1).

Survival analysis showed 17 patients who had seizure relapse in a total analysis time of 1,071.3 months at risk with a median time per subject of 17.52 months (IQR: 7.9-

20.9 months), to an incidence rate of 15.9 events per 1,000 patient-month. The Kaplan-Meier estimator showed that the probability of being seizure-free after 3 years of AED withdrawal is was 58.7% (95% CI: 38.3%-74.31%) (Figure 4.7.1 and Supplementary Information 4.8.1).

The standard of care recommends at least 24 months seizure-free to retire the AED; in our cohort, 15 out of 17 events were observed in participants with less than 24 months free of seizures (Figure 4.7.2), and the time-to-event evaluation using univariate Cox analysis showed that these participants had 9.2 times the risk of having an seizure relapse (HR 9.21, 95% IC 2.22-38.04, p=0.002). This group, participants with less than two years free of seizures include 31 out of 62 patients of the entire cohort (Table 4.7.1).

These 31 participants with less than 24 months free of seizures did not presented an additional statistically significant risk factor, probably due to small number of participants. Nevertheless, we found that having a history of ten or more seizures before AED increased the risk of seizure relapse (HR 1.96, 95%IC 0.66-5.79, p=0.220). Also, participants with a history of partial seizures with generalization or generalized seizures had a higher risk of having a seizure relapse, but without statistical significance (HR 2.19, 95%IC 0.48-9.88, p=0.306, Table 4.7.2).

None of the other variables: age, sex, antecedent of antiparasitic treatment, family antecedent of seizure, a positive cysticercosis EITB-LLGP serology ($\geq$ 4 bands), number or location of calcifications, age of onset of epilepsy, gradual tapering of AED or time with seizures were associated with a higher risk of new seizures after AED withdrawal.

We evaluated variables related to AED withdrawal (Tables 4.7.3 and 4.7.4). From the 202 participants in the main cohort, 62 of them stopped treatment. We compared these participants who ended AED treatment (due to medical indication [42], self-decision [18], and unknown causes [2]) with those who continued medication (140). We found, in the adjusted model, that the participants who were seizure-free for more than one year were 1.6 times more likely to withdraw from the antiepileptic treatment

(1.60 95%IC 1.05-2.39, p=0.014). On the other hand, patients who were older than 28 years (29y-40y RR 0.56 95%IC 0.33-0.92, p=0.024 and >40y RR 0.69 95%IC 0.43-1.12, p=0.138), had a history of generalized seizures or focal seizure with generalization (RR 0.59 IC95% 0.38-0.91, p=0.019), or had more than three bands in their EITB-WB result (RR 0.56 IC95% 0.32-1.00, p=0.052), were less likely to stop their antiepileptic treatment.

4.5 DISSCUSSION

Our study demonstrates that a significant proportion of patients with epilepsy secondary to NCC who withdraw AED therapy will have seizure relapses, mostly due to premature interruption of AEDs. Over a median follow-up period time of 17.5 months, the cumulative incidence of seizure relapse was 27.4% (17/62). The incidence rate was 15.9 events per 1,000 patient-months, and survival analysis showed a cumulative hazard of seizure relapse of 42.3% in a maximum of 36 months of follow up.

Half of our cohort (31 patients) stopped their AED treatment before achieving 2 years seizure-free, and almost all cases with seizure relapses (15 out of 17, (88.2%) occurred in these patients. Withdrawing AEDs before 24 months seizure free was associated with a 9 times higher risk of seizure relapse on univariate time-to-event analysis (HR 9.21 95%IC 2.22-38.04, p=002), making it evident that the standard recommendation of 2 years seizure free before discontinuing AED therapy should be appropriately followed in patients with calcified NCC. In this high risk subgroup (individuals who withdrew AEDS before achieving 2 years seizure free), there was around twice the risk for relapse if they had a history of ten or more seizures before AED withdrawal and a history of partial seizures with generalization or generalized seizures, although none of these associations reached statistical significance.

In contrast, only 6.5% (2/31) of patients who had been seizure-free in the previous 2 years after antiparasitic withdrawal presented with a seizure relapse. This low proportion is strikingly different from the scarce reports of seizure relapse after AED withdrawal in calcified NCC. Only three studies evaluated this risk prospectively.[29-31] The cumulative incidence of seizure relapse was over 80% in these studies in the first year of follow-up after AED withdrawal (83% [25/30],[30] 90.9% [10/11][29] and 100% [8/8] [31]). Many potential reasons could contribute to this evident discrepancy. Unlike prior studies, our cases were systematically included as part of a much larger cohort and likely

included cases with milder epilepsies (the extremely high rates of seizure relapse in older studies suggest a bias towards more severe clinical disease). We also ruled out viable or degenerating NCC lesions using MRI and CT, as well as the serological assays of choice (LLGP-EITB for antibody detection, and monoclonal antibody-based ELISA for the detection of circulating parasite antigen). Finally, we had a very close follow up of cohort patients, likely encouraging better compliance with drug treatment and risk avoidance. In these well-defined patients with pure calcified NCC who have achieved 2 years free of seizures, adding additional months or years of AED treatment may not be indicated and conversely may unnecessarily expose them to adverse events, impact in their quality of life, and cause significant direct and indirect economical costs.

In other epilepsies, the cumulative incidence of seizure relapse in adults who withdraw their AED after 2 years free of seizures are higher than our results and ranges from 15% to 32% and from 29% to 43% at 1 or 2 years of follow-up after AED withdrawal respectively.[143, 147, 149-151, 216] On the other hand, studies in patients with single enhancing lesions (SEL) with 2 years free of seizure presented similarly low cumulative incidence of seizure relapses at 12 to 18 months of follow-up (12% or less [5.8% 3/51,[153] 11.5% 30/26,[155] and 12% 13/108[156]]).

The standard approach to AED tapering considers at least 2 years seizure free,[145, 209, 210, 213, 214] in addition to assessing the most common causes of seizure relapse. This risk factors include older age at seizure onset, longer duration of epilepsy disease, abnormal neurological or psychiatric findings, abnormal EEG findings, increased number of seizures during AED therapy, history of status epilepticus, or greater number of AEDs used during treatment and failure in previous withdrawal attempts.[148, 211, 212]

As in the parent cohort of patients under AED treatment, having a history of ten or more prior seizure episodes seems to be a predictor of seizure relapse. In the univariate model, people with this condition have almost double the chance of having a seizure relapse in the following 3 years after AED withdrawal but without statistical significance. This variable was not evaluated in the previous three studies in calcified NCC published

by Del Brutto et al.[29, 30] and Narayana.[31]   However, in patients with epilepsy caused by other etiologies than NCC, it has been consistently reported that having had multiple seizure events is a prognostic factor for seizure relapse after AED withdrawal, probably because epileptogenic circuits have been already established.[145]

Patients having a history of generalized seizures or focal seizures with generalization also had almost two-times the chance of having a seizure relapse in the following 3 years in univariate time to event analysis but without statistical significance, probably due to the small sample size. A history of generalized seizures, in patients with epilepsy caused by other pathologies than NCC, has also been consistently reported as risk factor for seizure relapse in the literature.[217]

Having received antiparasitic treatment was not associated with increased risks of seizure relapse after AED withdrawal. This finding is consistent with previous studies in calcified NCC published by Del Brutto et al.,[30] and Nash et al.[28] Data from the overall parent cohort (Chapter 3), as well as other studies, are consistent with this finding, supporting the fact that antiparasitic treatment does not result in an increased risk of seizure events in the long term.[25, 26, 71, 72, 203-205]

A number of variables were not associated with the risk of seizure relapses, including age, sex, strength of the antibody response, and less intuitively, breakthrough seizures, and number or location of parasitic brain lesions. Seizure events during the AED treatment has been consistently pointed out as risk factor for seizure relapse after AED withdrawal however in this cohort this variable was not a risk factor for seizure recurrence.

Variables related to AED withdrawal in our cohort under AED treatment were also evaluated. Identification of these variables may help physicians and patients make more refined decisions on whether or not to stop antiepileptic treatment. We found four variables related to continuation of the antiepileptic drug, and all of them were associated with disease consciousness. Patients were more likely to continue AED treatment if they

were older (>28 years), had at least one seizure one year before enrollment (in the parent cohort), had a history of generalized seizure or focal seizure with generalization, and had strong positive test results from the EITB-Western Blot (more than three bands). Interestingly, the number of calcifications or the number of previous seizures was not related to the decision for antiepileptic treatment withdrawal.

Our study has some evident weaknesses, in particular the small number of participants that affects the statistical significance and precluded the use of a multivariate model of analysis. Since clinical patient care was managed by the attending neurologists and was not part of the observational cohort procedures, variable criteria were applied to indicate AED withdrawal, in some cases without EEG tracings before tapering drug coverage. Seizure characterization was likely suboptimal, based on patient interviews and interviews with relatives and witnesses without video-EEG support. We did not perform a new serology before AED withdrawal. Thus, our analysis of this variable only considered the serology result at entry to the preceding cohort. Our study cohort incorporated patients systematically included from a larger cohort of patients with calcified NCC as they withdrew AEDs (increasing their representativity) but at the same time, the parent cohort was assembled in a neurological reference center, and as such, they may not be representative of all patients with NCC. Finally, the quality of our seizure data may be affected by recall bias, indirect source of information (witnesses of seizures with generalization), and misclassification arising from the fact that the study physician was not masked to the radiological or clinical information and may therefore have over- or under-diagnosed seizure relapses.

AED withdrawal has several benefits including prevention of side effects such as cognitive impairment or possible teratogenic effects, improvement of quality of life, reduction of direct and indirect economic costs, reduction of stigma, and elimination of potential drug interaction.[143] Nevertheless, AED withdrawal can result in seizure relapse.[144] In patients with calcified NCC, gradual withdrawal of AEDs should be considered only in patients with at least 2 years free of seizures. Some authors advocate

for early AED withdrawal,[218, 219] According to our data, this advice is evidently contraindicated for patients with calcified NCC and epilepsy.

4.6 CONCLUSION

Patients with calcified NCC and epilepsy should follow the standard of care recommendation and complete at least 2 years free of epilepsy before gradual AED retirement. On the other hand, adding further months or years of AED treatment after 2 years free of seizures may not be indicated given the low likelihood of seizure relapse, less than 10% (2/31), and additional treatment could expose those patients to undesired adverse events, negative effect in their quality of life, and cause unnecessary economic burden.

4.7 ANNEXES

TABLES

Table 4.7.1. Demographic, clinical and neuroimaging characteristics of the study cohort (N=62), and bivariate analysis by seizure relapse.

Characteristics	n (%)	Seizure relapse		
		Yes (N=17) n (%)	No (N=45) n (%)	*p value*
Age (years) Mean ± SD	38.1 ± 13.2	37.8 ± 13.3	38.2 ± 13.3	0.914
Sex (Female)	34 (54.8%)	10 (58.8%)	24 (53.3%)	0.698
Previous APT (yes)	39 (62.9%)	10 (58.8%)	29 (64.4%)	0.683

Seizure time (months)
  Median (IQR)
  ≤36 months
	96 (53-195)
	7 (11.3%)
		144 (54-218)
		4 (23.5%)
			87 (53-181)
			3 (6.7%)
				0.734
				0.061

Number of previous seizures
  Median (IQR)
  >10 seizures
	9 (3-21)
	27 (43.6%)
		16 (8-51)
		11 (64.7%)
			6 (3-16)
			16 (35.6%)
				0.045
				0.039

Seizure-free time before enrollment (months)
  Median (IQR)
  >24 months
	24 (6-59)
	31 (50.0%)
		10 (3-16)
		2 (11.4%)
			40 (18-61)
			29 (64.4%)
				0.002
				<0.001

Family history of seizures	7 (11.3%)	3 (17.65%)	4 (8.9%)	0.331
Generalized and/or focal partial seizures with generalization	45 (72.6%)	14 (82.4%)	31 (68.9%)	0.289
EITB result ≥4 bands*	10 (16.1%)	2 (11.1%)	8 (17.8%)	0.566
AED withdrawal with gradual taper (yes)[b]	23 (43.4%)	6 (40.0%)	17 (44.7%)	0.754

Number of calcifications
  Median (IQR)
  Single calcifications
  2 to 10 calcifications
  >10 calcifications
	4 (1-9)
	19 (30.7%)
	29 (46.7%)
	14 (22.6%)
		3 (1-10)
		5 (29.4%)
		8 (47.6%)
		4 (23.5%)
			4 (1-9)
			14 (31.1%)
			21 (46.7%)
			10 (22.2%)
				0.597
				0.989

Location of calcifications
  Frontal lobe
  Parietal lobe
  Occipital lobe
  Temporal lobe
	48 (77.4%)
	35 (56.5%)
	20 (32.3%)
	29 (46.8%)
		13 (76.5%)
		12 (70.6%)
		4 (23.5%)
		8 (47.1%)
			35 (77.8%)
			23 (51.1%)
			16 (35.6%)
			21 (46.7%)
				0.913
				0.168
				0.366
				0.978

Abbreviations: Standard deviation (SD), antiparasitic treatment (APT), interquartile range (IQR), enzyme-like immunoelectrotransfer blot (EITB), electroencephalogram (EEG), antiepileptic drug (AED), not determined (ND).
Data at the time of AED withdraw is presented, except in cases marked with (*) where data come from the time of entry to the parent cohort
Bold values are considered statistically significant (*p*<0.05).
[a]Three observations were missed
[b]Nine observations were missed

Table 4.7.2 Descriptive and Univariate Cox proportional-hazard regression model to assess potential risk factors for seizure relapse during follow-up in patients who did not achieve two years seizure-free time before withdrew their AED.

Variables		Number of Patients (n=31)	Number of Events (Total=15)	Univariate models	
				Hazard ratio (95% CI)	*p value*
Age					
	≤28 years	12	6 (50.0)	1.00	
	29-40 years	10	4 (40.0)	0.60 (0.16-2.21)	0.455
	>40 years	9	5 (55.6)	1.11 (0.33-3.74)	0.856
Sex					
	Male	11	6 (54.6)	1.00	
	Female	20	9 (45.0)	0.88 (0.31-2.4)	0.815
Previous APT					
	Yes	16	8 (50.0)	1.00	
	No	15	7 (46.7)	0.83 (0.30-2.30)	0.722
Seizure time					
	>36 months	24	11 (45.8)	1.00	
	≤36 months	7	4 (57.1)	1.65 (0.52-5.21)	0.392
Number of previous seizures					
	≤10 seizures	14	5 (35.7)	1.00	
	>10 seizures	17	10 (58.2)	1.96 (0.66-5.79)	0.220
Family history of seizures					
	No	27	13 (48.2)	1.00	
	Yes	4	2 (50.0)	0.76 (0.17-3.38)	0.719
Generalized and/or focal seizures with generalization					
	No	7	2 (28.6)	1.00	
	Yes	24	13 (54.2)	2.19 (0.48-9.88)	0.306
EITB positive to ≥ 4bands*					
	Positive	25	13 (52.0)	1.00	
	Negative	6	2 (33.3)	1.57 (0.35-7.02)	0.556
AED withdrawal with gradual taper[a]					
	Yes	12	6 (50.0)	1.00	
	No	18	9 (50.0)	1.28 (0.45-2.62)	0.641
Number of calcifications					
	Single calcifications	7	5 (71.4)	1.00	
	2 to 10 calcifications	18	6 (33.3)	0.61 (0.19-2.02)	0.421
	>10 calcifications	6	4 (66.7)	1.76(0.45-6.79)	0.410
Location of calcifications					
	Frontal lobe	27	12 (44.4)	1.01 (0.28-3.65)	0.983
	Parietal lobe	19	10 (52.6)	2.13 (0.88-6.52)	0.203
	Occipital lobe	10	4 (40.0)	0.82 (0.26-2.59)	0.735
	Temporal lobe	15	8 (53.3)	1.69 (0.60-4.78)	0.319

Abbreviations: Standard deviation (SD), antiparasitic treatment (APT), interquartile range (IQR), enzyme-like immunoelectrotransfer blot (EITB), antiepileptic drug (AED), not determined (ND).
Data at the time of AED withdraw is presented, except in cases marked with (*) where data come from the time of entry to the parent cohort
[a]One observations were missed

Table 4.7.3. Demographic, clinical and neuroimaging characteristics of the patients belonging to the parent study cohort (N=202), and bivariate analysis by AED withdrawal.

Variables	AED Withdrawal		*p* value
	Yes (N=62) n (%)	No (N=140) n (%)	
Age (years)			
Mean ± SD	36.0 ± 13.2	36.7 ± 29	0.774
≤28 years	25 (40.3 %)	42 (30.0 %)	0.211
29-40 years	17 (27.42 %)	55 (39.3 %)	
>40 years	20 (32.3 %)	43 (30.7 %)	
Sex (male)	28 (45.2 %)	72 (51.4 %)	0.411
Previous APT (yes)	39 (62.9 %)	93 (66.43 %)	0.627
Seizure time prior to stopping AED (months)			
Median (IQR)	68 (26-178)	81 (37-181)	0.470
>36 months	44 (71.0 %)	105(75.0 %)	0.548
Seizure-free time prior to stopping AED (months)			
Median (IQR)	12 (2-37)	5 (1-20)	**<0.019**
>12 months	31 (50.0)	47 (33.6)	**<0.027**
Number of previous seizures[a]			
Median (IQR)	9 (3-21)	11 (3-41)	0.386
>10 seizures	27 (43.6)	70 (50.2)	0.372
Family history of seizures	7(11.3)	19(13.6)	0.655
Generalized and/or focal seizures with generalization	46 (74.2 %)	119 (85.0 %)	0.067
EITB result ≥4 bands	10 (16.3 %)	40 (28.6 %)	0.059
Perilesional Edema[c]	12 (32.4 %)	50 (30.3 %)	0.800
Large calcifications[c]	4 (33.3 %)	58 (30.5 %)	0.838
Number of calcifications			
Median (range)	4 (1-19)	2 (1-12)	0.973
Single calcification	19 (30.7 %)	47 (33.6 %)	0.659
2 to 10 calcifications	29 (46.8 %)	56 (40.0 %)	
>10 calcifications	14 (22.6 %)	37 (26.4 %)	
Location of calcifications			
Frontal lobe	48 (77.4 %)	112 (80.0 %)	0.677
Parietal lobe	35 (56.5 %)	76 (54.3 %)	0.775
Occipital lobe	20 (32.3 %)	61 (43.6 %)	0.130
Temporal lobe	29 (46.8 %)	54 (38.6 %)	0.274

Standard deviation (SD), interquartile range (IQR), antiparasitic treatment (APT), enzyme-linked immunoelectrotransfer blot (EITB).
Bold values are considered statistically significant (*p*<0.05).
[a] One observation was missed.
[c] At least one calcified lesion

Table 4.7.4 Baseline risk factors for antiepileptic drug withdrawal in patients with calcified NCC and epilepsy (n=202)

Variables		Univariate models		Adjusted model	
		RR (95%CI)	p value	RR (95% CI)	p value
Age	≤28 years	1.00		1.00	
	29-40 years	0.63 (0.38-1.06)	0.084	0.56 (0.33-0.92)	**0.024**
	>40 years	0.85 (0.52-1.37)	0.507	0.69 (0.43-1.12)	0.138
Sex	Female	1.00			
	Male	0.84 (0.55-1.27)	0.413		
Previous APT	No	1.00			
	Yes	0.90 (0.58-1.38)	0.625		
Seizure time	≤36 months	1.00			
	>36 months	0.87 (0.55-1.36)	0.542		
Seizure-free time before enrollment	≤12 months	1.00		1.00	
	>12 months	1.60 (1.05-2.39)	**0.026**	1.66 (1.11-2.49)	**0.014**
Number of previous seizures[a]	≤10	1.00			
	>10	0.83 (0.54-1.26)	0.374		
Family history of seizures	No	1.00			
	Yes	0.86 (0.44-1.68)	0.663		
Generalized and/or focal seizures with generalization	No	1.00		1.00	
	Yes	0.64 (0.41-1.00)	0.052	0.59 (0.38-0.91)	**0.019**
EITB (+) ≥ 4bands	Negative	1.00			
	Positive	0.58 (0.32-1.06)	0.078	0.56 (0.32-1.00)	**0.052**
Number of calcifications	Single	1.00			
	2 to 10	1.18 (0.73-1.91)	0.489		
	>10	0.95 (0.53-1.71)	0.874		
Perilesional Edema [c]	No	1.00			
	Yes	1.07 (0.63-1.80)	0.798		
Large calcifications [c]	No	1.00			
	Yes	1.09 (0.48-2.50)	0.835		
Location of calcifications					
	Frontal lobe	0.90 (0.55-1.47)	0.673		
	Parietal lobe	1.06 (0.70-1.62)	0.776		
	Occipital lobe	0.71 (0.45-1.12)	0.140		
	Temporal lobe	1.26 (0.83-1.90)	0.272		

*Patients with an abnormal EEG result were excluded for the analyses

Confidence interval (CI), antiparasitic treatment (APT), enzyme-linked immunoelectrotransfer blot (EITB), and electro encephalogram (EEG).

Bold p values are statistically significant

[a] One observation was missed.

[c] At least one calcified lesion

Figure 4.7.1 Kaplan-Meier plot of seizure relapse in patients who stopped their AED

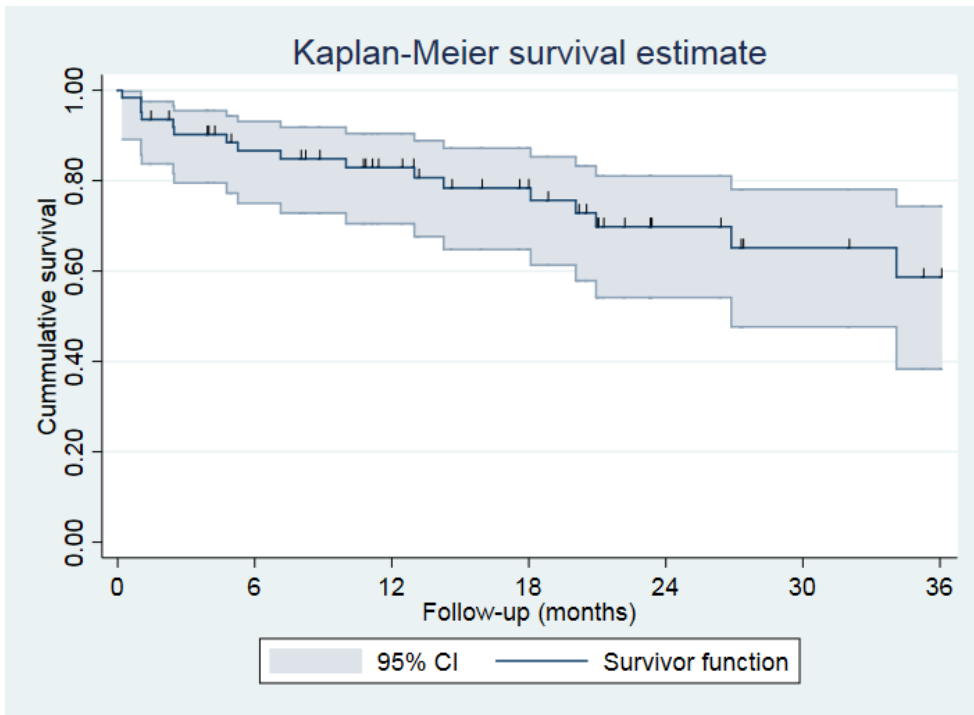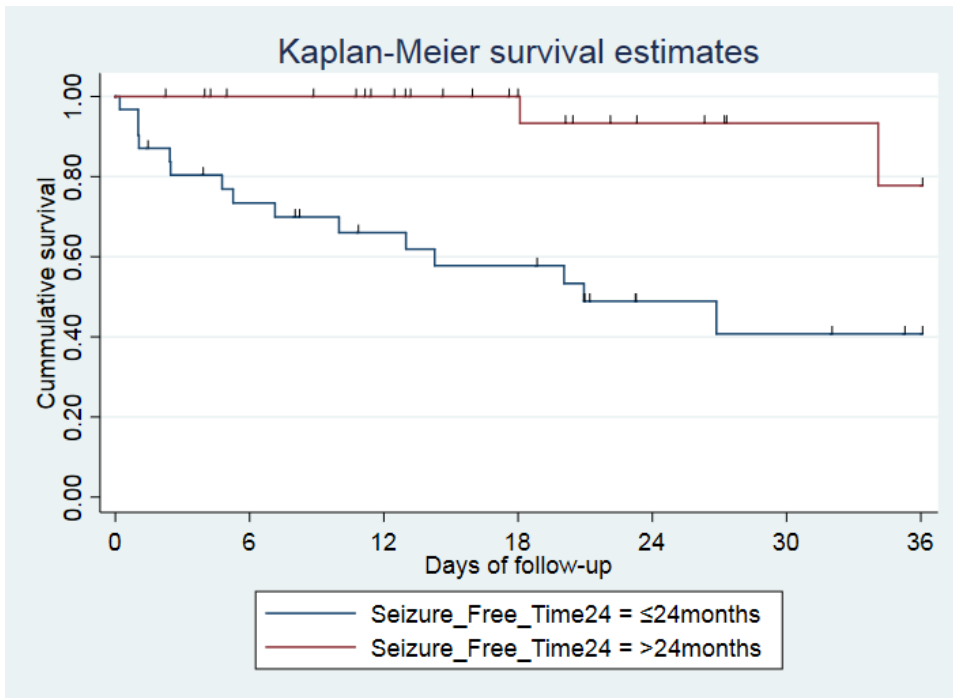Figure 4.7.2 Survival curve by seizure-free time before AED withdrawal

Supplementary Information

4.8.1 Table Kaplan-Meier Survival Probability Estimates

Time	Beg. Total	Fail	Net Lost	Survivor Function	Std. Error	[95% Conf. Int.]	
.1973	62	1	0	0.9839	0.0160	0.8910	0.9977
1.019	61	2	0	0.9516	0.0273	0.8574	0.9841
1.052	59	1	0	0.9355	0.0312	0.8372	0.9753
1.381	58	0	1	0.9355	0.0312	0.8372	0.9753
2.17	57	0	1	0.9355	0.0312	0.8372	0.9753
2.433	56	1	0	0.9188	0.0348	0.8158	0.9654
2.466	55	1	0	0.9021	0.0380	0.7949	0.9548
3.847	54	0	1	0.9021	0.0380	0.7949	0.9548
3.912	53	0	1	0.9021	0.0380	0.7949	0.9548
4.175	52	0	1	0.9021	0.0380	0.7949	0.9548
4.767	51	1	0	0.8844	0.0412	0.7725	0.9432
4.899	50	0	1	0.8844	0.0412	0.7725	0.9432
5.26	49	1	0	0.8663	0.0441	0.7501	0.9309
7.134	48	1	0	0.8483	0.0467	0.7282	0.9182
7.956	47	0	1	0.8483	0.0467	0.7282	0.9182
8.153	46	0	1	0.8483	0.0467	0.7282	0.9182
8.778	45	0	1	0.8483	0.0467	0.7282	0.9182
9.995	44	1	0	0.8290	0.0495	0.7047	0.9044
10.68	43	0	1	0.8290	0.0495	0.7047	0.9044
10.78	42	0	1	0.8290	0.0495	0.7047	0.9044
11.08	41	0	1	0.8290	0.0495	0.7047	0.9044
11.34	40	0	1	0.8290	0.0495	0.7047	0.9044
12.39	39	0	1	0.8290	0.0495	0.7047	0.9044
12.89	38	0	1	0.8290	0.0495	0.7047	0.9044
12.99	37	1	0	0.8066	0.0530	0.6762	0.8886
13.12	36	0	1	0.8066	0.0530	0.6762	0.8886
14.27	35	1	0	0.7836	0.0563	0.6477	0.8720
14.56	34	0	1	0.7836	0.0563	0.6477	0.8720
15.88	33	0	1	0.7836	0.0563	0.6477	0.8720
17.52	32	0	2	0.7836	0.0563	0.6477	0.8720
17.92	30	0	1	0.7836	0.0563	0.6477	0.8720
18.08	29	1	0	0.7565	0.0605	0.6132	0.8529
18.77	28	0	1	0.7565	0.0605	0.6132	0.8529
20.05	27	1	0	0.7285	0.0644	0.5785	0.8325
20.12	26	0	1	0.7285	0.0644	0.5785	0.8325
20.45	25	0	1	0.7285	0.0644	0.5785	0.8325
20.94	24	1	1	0.6982	0.0685	0.5414	0.8102
20.98	22	0	1	0.6982	0.0685	0.5414	0.8102
21.21	21	0	1	0.6982	0.0685	0.5414	0.8102
22.13	20	0	1	0.6982	0.0685	0.5414	0.8102
23.24	19	0	1	0.6982	0.0685	0.5414	0.8102
23.28	18	0	1	0.6982	0.0685	0.5414	0.8102
23.31	17	0	1	0.6982	0.0685	0.5414	0.8102
26.33	16	0	1	0.6982	0.0685	0.5414	0.8102
26.86	15	1	0	0.6516	0.0781	0.4764	0.7808
27.22	14	0	1	0.6516	0.0781	0.4764	0.7808
27.32	13	0	2	0.6516	0.0781	0.4764	0.7808
31.96	11	0	1	0.6516	0.0781	0.4764	0.7808
34.09	10	1	0	0.5865	0.0936	0.3832	0.7431
35.21	9	0	1	0.5865	0.0936	0.3832	0.7431
36	8	0	8	0.5865	0.0936	0.3832	0.7431

4.8.2 Table Cox proportional-hazard regression models to assess potential risk factors for seizure relapse after AED withdrawal in patients with calcified NCC (Entire cohort n=62)

Variables	Univariate models		Multivariate model[a]	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Age				
≤28 years	1.00			
29-40 years	0.49 (0.14-1.79)	0.282		
>40 years	1.31 (0.44-3.94)	0.624		
Sex				
Male	1.00			
Female	1.16 (0.44-3.05)	0.762		
Previous APT				
Yes	1.00			
No	1.29 (0.51-3.28)	0.588		
Seizure time				
>36 months	1.00		1.00	
≤36 months	3.37 (1.09-10.39)	0.034	2.68 (0.79-9.13)	0.115
Seizure-free time before enrollment				
>24 months	1.00		1.00	
≤24 months	9.21 (2.22-38.04)	0.002	5.98 (1.27-28.20)	0.024
Number of previous seizures				
≤10 seizures	1.00		1.00	
>10 seizures	2.30 (0.88-5.96)	0.088	2.96 (1.04-8.40)	0.041
Family history of seizures				
No	1.00			
Yes	1.26 (0.36-4.38)	0.715		
Generalized and/or focal seizures with generalization				
No	1.00		1.00	
Yes	2.57 (0.73-9.05)	0.141	2.7 (0.73-10.63)	0.134
EITB positive to ≥ 4bands				
Positive	1.00			
Negative	1.53 (0.35-6.66)	0.574		
AED withdrawal with gradual taper[b]				
Yes	1.00			
No	1.55 (0.57-424)	0.383		
Number of calcifications				
Single calcifications	1.00			
2 to 10 calcifications	1.14 (0.38-3.40)	0.820		
>10 calcifications	1.28 (0.34-4.80)	0.713		
Location of calcifications				
Frontal lobe	1.07 (0.35-3.27)	0.899		
Parietal lobe	2.39 (0.88-6.52)	0.089	2.80 (0.73-10.63)	0.134
Occipital lobe	0.69 (0.22-2.09)	0.508		
Temporal lobe	1.03 (0.41-2.62)	0.949		

Abbreviations: Confidence intervals (CI), antiparasitic treatment (APT), enzyme-linked immunoelectrotransfer blot (EITB), antiepileptic drug (AED)
[a]Multivariate model was fitted using covariates with a *p* <0.200 from Wald test in univariate analysis.
[b]Three observations were missed

4.8.3 Table. Schoenfeld's residual test to assess the proportional hazards assumption for variables included in the multivariate Cox regression model.

Variables	Multivariate model 1[a]	
	Rho	*p* value
Seizure time (≤36 months)	0.0152	0.955
Number of previous seizures (>10)	0.1121	0.667
Seizure-free time before enrollment (≤24 months)	-0.4975	0.050
Generalized and/or partial seizures with secondary generalization	0.1644	0.525
Calcified lesions in temporal lobe	0.2896	0.175
Global test		0.279

4.8.4 Figure. Survival curve by number of prior seizures before AED withdrawal
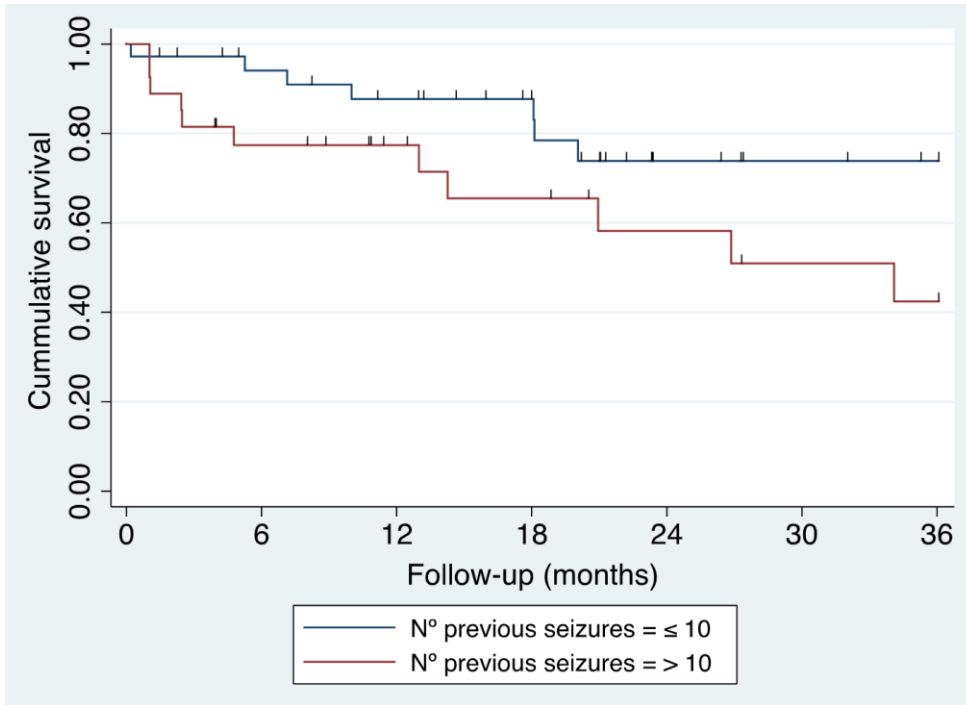
4.8.5 Figure. Survival curve by time of disease (seizures) AED withdrawal
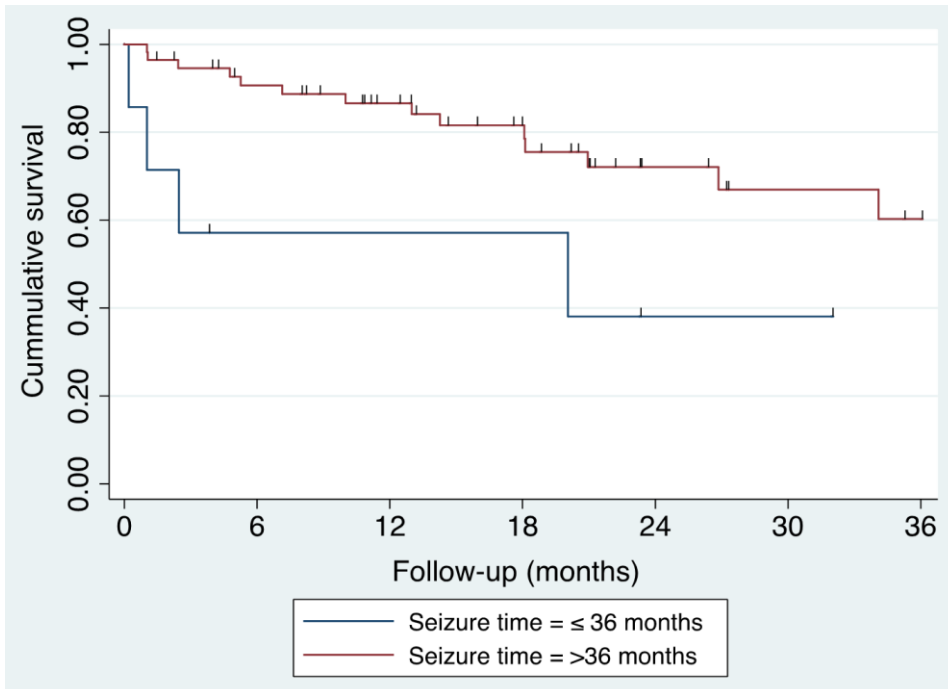
4.8.6 Figure. Observed versus predicted survival curves plot for each of the variables included in the multivariate Cox proportional-hazard regression model in the entire cohort

A. Seizure-free time until AED withdrawal
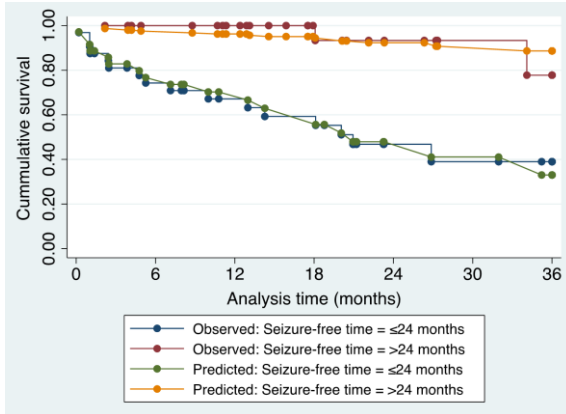
B. Time with disease
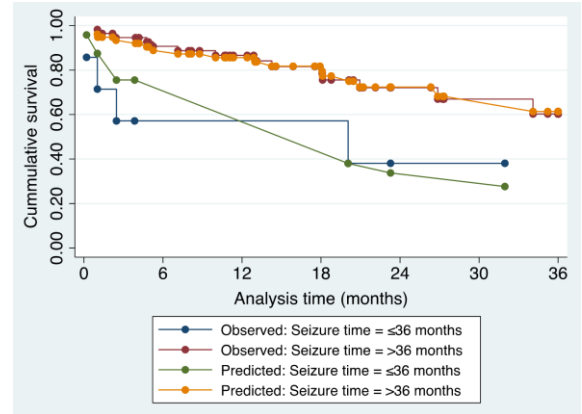
C. Number of previous seizures
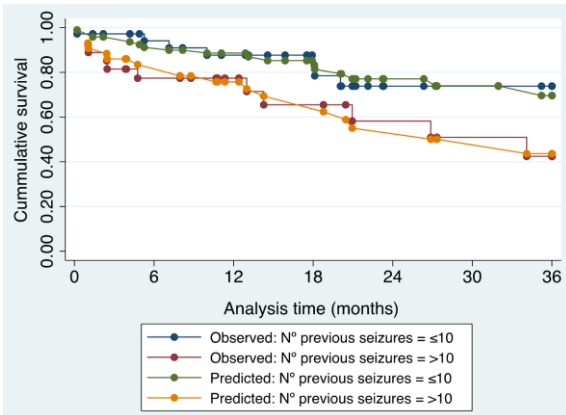
D. Seizures with generalization
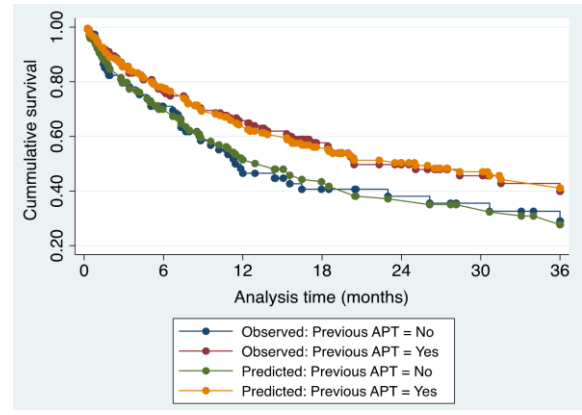
E. Lesions in parietal lobes
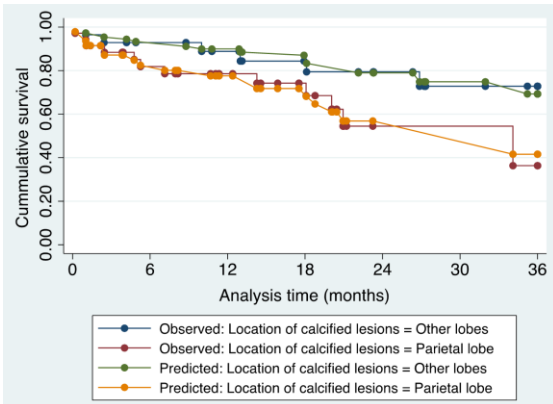
CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

Neurocysticercosis is still considered a major cause of acquired epilepsy in endemic countries, consistently around 30% of the secondary epilepsy is attributable to *T. solium* cysticercosis.[5, 6, 8] NCC-associated epilepsy is a public health problem in developed countries mainly due to travel and migration.[12, 220, 221]. According the WHO almost 80% of the people with epilepsy live in low and middle income countries, thus NCC is one of the leading contributors of secondary epilepsy worldwide.[222] Additionally, the *T. solium* zoonosis is the foodborne parasite causing the highest global burden and resulting in a total of 28,000 deaths annually and 2.8 million disability-adjusted life-years (DALYs).[223]

Parasites contained in viable cysts in the brain parenchyma eventually die due to natural life cycle or because of antiparasitic treatment; some of the cysts resolve, but others persist as small calcified lesions.[164] Some of these patients with calcified scars will develop acute seizure and epilepsy. There is abundant evidence in the literature supporting the hypothesis that cysts that evolve to calcified lesion are more prone to experience seizures and, subsequently develop epilepsy.[5]

Understanding the cumulative incidence and risk factors for calcification (study 1), subsequent breakthrough seizure during AED treatment (study 2), and seizure relapse after AED withdrawal (study 3) would provide important information to implement an individualized treatment approach. It would also potentially decrease the calcification process (study 1) and offer a better management of epilepsy, thus reducing the burden of the disease caused by epilepsy due to calcified NCC (Study 2 and 3).

5.1 SUMMARY OF MAJOR FINDINGS

5.1.1 Paper 1.

The first study showed that 37.8% (188/497) of viable brain parenchymal cysts result in residual calcification one year after antiparasitic treatment. The risk factors for calcification at cyst level are large size of cysts ($\geq$14 mm of diameter) and cysts with perilesional edema prior to antiparasitic treatment. At the patient level, we found as risk factors: longer duration of disease (more than 24 months of epilepsy), mild immunologic reaction as measured by EITB before antiparasitic treatment (less than four parasite proteins recognized by antibodies), use of an enhanced doses of ABZ (1.5 times), use of lower corticosteroid doses ($\leq$6 mg/d), and lack of antiparasitic re-treatment after the first round of treatment among those with incomplete cyst cure. Thus, use of combined antiparasitic drugs, early antiparasitic re-treatment, and use of enhanced doses of corticosteroids would reduce or avoid calcification after antiparasitic treatment and potentially reduce future seizure recurrence.

5.1.2 Paper 2.

This cohort study involved 210 participants with epilepsy and calcified NCC. Almost 50% (103/210) presented with BTS during the follow-up. The Kaplan-Meier estimator showed that only 36% (95% CI: 27.2%-44.9%) remained free of seizures during a maximum of 3 years follow-up. Most of the events occurred within the first 2 years of follow-up (94/103). We found that having an abnormal interictal EEG was a main predictor of BTS (6 out of 8), and most the events were observed within the first 6 months (5 out of 6). Given the very high risk of these eight participants, they were excluded from further analysis. In the univariate and multivariate analysis we found two main predictors of BTS: having a history of ten or more seizures (HR: 2.91 p<0.001 and HR: 2.69 p<0.001 respectively) and having at least one seizure event within the previous year prior to enrollment (HR: 1.77, p=0.006 and HR 1.55 p= 0.038 respectively).

5.1.3 Paper 3

This cohort was composed of 62 participants who withdrew their AED. Seventeen (24.7%) participants presented with seizure relapses; the cumulative incidence was 41.4% along a maximum follow up of 36 months. Half of this cohort (31/62) had less than 2 years of seizure free before AED withdrawal, and almost all patients who experience seizure relapse belong to this group (15/17). Cox regression models showed that having seizures in the last 2 years before AED withdrawal had nine times more risk of seizure relapse (HR 9.21 95%IC 2.22-38.04 p= 0.002). Further analysis of only these participants (seizure-free ≤24 months) found that having a history of ten or more seizure and at least one generalized seizure or focal seizure with generalization show a trend of increased risk of seizure relapse but without statistical significance.

5.2 STUDY LIMITATIONS

5.2.1 Study 1

The main weaknesses of this study were the limitations inherent in the neuroimaging techniques and the loss to follow-up. MRI is the standard test for evaluating viable parenchymal cysts, and CT scan is the best technique to identify parenchymal calcified lesions.[37, 47, 49, 224] However, the sensitivity and specificity of these neuroimaging techniques are suboptimal. Additionally some of the participants in the original trials may have been evaluated using different neuroimaging machines, and this may have introduced a measurement bias. However, since patients come from three randomized clinical studies, this is likely a non-differential bias and did not significantly alter the results observed in this study.

Another limitation is the loss to follow-up. However, the number lost to follow-up were minimal (8.5%, 18/212) and, due to the internal validation of the original studies, these losses were probably of a non-differential nature.

5.2.2 Study 2

The main study limitation of this study was a potential recall bias, due to long history of seizure among participants in this cohort. As a consequence, recall bias may have affected the numbers and type (description) of seizures documented at baseline.[225, 226] Also in cases of generalized seizures we obtain the information from a close relative and/or witness who could only partially observe and describe the seizure episode. Thus information bias could have caused an underestimation of the number of events in the cohort.

Additionally, the study team was not masked to the radiological or clinical information. Thus some conditions, such as many or large calcifications or history

of severe or recurrent seizures may have resulted in an overestimate of the numbers of BTS. We included patients attending a national referral center for neurological diseases in Peru. Therefore they represent a selected proportion of subjects with NCC, and most likely those with suboptimal seizure control, and because of this, selection bias may have been introduced affecting the representativeness of the results. Those censored due to stopping AED may induce informative censoring.

5.2.1 Study 3

The main weakness of this cohort is the small numbers of participants which would affect the statistical significance and did not allow us to perform a multivariate model analysis. We did not perform an EEG before AED withdrawal because patient care was not part of the observational study. Additionally, we had no video-EEG support, in order to have a solid evidence of seizure characterization. As in the previous study, we included in this study patients who seeked medical care at the INCN (Instituto Nacional de Ciencias Neurologicas), a national referral center for neurological disease in Peru. In this sense, a lack of representativeness of the study population is anticipated. And, finally as in the parent cohort, seizure recall, the use of witnesses of the event as an indirect source of information, and the fact that the study physician was not masked to the radiological or clinical information and may be potential bias in our study.

5.3 RECOMMENDATIONS FOR FUTURE RESEARCH

5.3.1 Study 1

There is strong evidence that brain calcifications are associated with epilepsy in NCC.[23, 121, 124] Avoiding or reducing the calcification process could potentially reduce the risk of seizures in the long term. This cohort could serve to evaluate if a greater proportion of calcification is associated with more seizure relapse in the long term. Also we can evaluate serological status (number of reactive bands at EITB Western blot) decay over time and see if it correlates with the proportion of remaining calcified lesions or further seizure events. Our cohort presented with 38% calcified lesions at one year after antiparasitic treatment; we can take advantage of this cohort to explore if new calcified lesions appear in the following years as was suggested by Das and collaborators,[24] which could mean that the process of calcification of each cyst follows a different time pattern in each individual.

According to our findings, there are two additional interventions in patients with viable parenchymal NCC that could potentially reduce the risk of calcification: (1) the use of a higher-dose of corticosteroid regimen and (2) early re-treatment with antiparasitic drugs. These studies could initially be evaluated in animal models for further implementation in human studies. Additional interventions that are suggested by the literature such as use of etidronate[227, 228] and osteopontine [229, 230] could also be evaluated.

5.3.1 Study 2

There is a lack of studies that explore the precipitating (trigger) factor(s) for breakthrough seizures in patients with calcified NCC. This has been extensively studied in causes of epilepsy but not in patients with calcified NCC under AED treatment. In order to provide evidence–based AED management to

patients with calcified NCC, we would like to develop a prospective, controlled study comparing AED regimes. We were surprised to find that number or location of calcification was not related with BTS. This opens additional lines of research that should be explored such as genetic factor that can affect the epileptic threshold or biomolecular marker that are involved in seizure an epilepsy in this patients. Abnormal interictal EEG is a main biomarker for early BTS. Thus, studies in prolonged EEG and/or video EEG could be more informative predictors of BTS. With the results from larger, multicenter studies a tests and recommendations could be developed to help physicians provide better care for patients with epilepsy due to calcified NCC.[140] Since calcified NCC is a chronic condition, it can serve as a model to evaluate structural and functional brain changes, such as the hippocampal sclerosis[16, 205] or thinning of the cerebral cortex and its relationship with new convulsive events.[231, 232]

5.3.1 Study 3

Our findings support the recommendation that patients with calcified NCC and epilepsy should maintain their AED treatment until completion of at least 2 years free of seizures. The estimated relapse rate in these patients is less than 10% (2/31) in the 3 years of follow up after AED withdrawal. Future studies should evaluate in patients with calcified NCC that already achieved at least 2 years free of seizures, if an extra year or 2 years of antiepileptic treatment really offers a significant decrease of seizure relapse in the following years, as suggested by many authors in epilepsy due to other causes than NCC.[145, 209, 210, 213, 214] A larger cohort should be conducted to confirm potential risk factors such as having a history of ten or more seizure, having less than 36 months with seizures, and having had at least a generalized seizure or focal with generalization. Also abnormal electrical activity at EGG (extended tracing and/or video-EEG) should be evaluated as a risk factor before AED withdrawal.[148, 211, 212]. Also to explore precipitating (trigger) factor of seizure relapse in calcified NCC patients without AED treatment has not been still studied. Finally, as in the study 2, larger and multicentric studies can provide enough information to develop monogram that

individualize the medical care and recommendation for people with epilepsy due to calcified NCC who stop their AED.[140]

5.4 POLICY IMPLICATIONS

Our findings can impact directly the management of patients with calcified NCC. According to our results from study 1, use of combined antiparasitic drugs, albendazole plus praziquantel, results in fewer calcifications compared to increased doses of ABZ,[77, 172, 173] and use of enhanced doses of corticosteroids versus the standard doses during the antiparasitic treatment and weeks after[78] not only improved the control for seizure in the first weeks of antiparasitic treatment, as demonstrated by Garcia and collaborators, but decreases the proportion of calcified cysts. Finally, we can recommend considering an early antiparasitic re-treatment regardless the efficacy of the initial antiparasitic course in order to reduce the proportion of calcification. If these interventions reduce the proportion of calcified lesions we can possibly also reduce the subsequent burden of seizures due to NCC.

Studies 2 and 3 are focused on patients with established NCC calcified lesions, who will remain with this condition for their entire life. Some of these patients will have recurrent, and in a few cases, intractable epilepsy. As a chronic condition there are some structural consequences such as hippocampal sclerosis or cortical thickness that can worsen their epilepsy.[16, 205, 231, 232]

Seizures and epilepsy have an important impact in neurobiological, cognitive, psychosocial consequences, and economical aspects.[233] Identifying and implementing interventions to reduce the calcification scars could potentially improve the prognosis the secondary epilepsy (Study 1). Also identifying risk factors for breakthrough seizures may improve antiepileptic care in patients with calcified NCC (Study 2). Finally, consistent criteria for AED withdrawal (Study 3) will not only reduce morbidity and mortality caused by this disease but will also improve the quality of life, reduce stigma and lower associated direct and indirect cost for patients and health systems.

CHAPTER SIX

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CHAPTER SEVEN

CURRICULUM VITAE

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Associate Investigator at Center for Global Health
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Education/Training

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Universidad Peruana Cayetano Heredia. Lima, Perú	BS	2001	Human Medicine
Universidad Peruana Cayetano Heredia. Lima, Perú	MD	2001	Human Medicine
Tropical Medicine Institute Alexander von Humboldt, Universidad Peruana Cayetano Heredia. Lima, Perú	MSc	2012	Control of Infectious Diseases
Johns Hopkins School of Public Health. Baltimore, MD	MPH	2013	Public Health
Johns Hopkins School of Public Health. Baltimore, MD	PhD(c)	2013 –	International Health

A. Personal Statement

My participation in the Cysticercosis Working Group in the last 18 years and the permanent training in research activities have been fruitful where I acted as associate investigator and responsible for design, monitoring and ethics and regulatory-related issues of clinical and pre-clinical research studies. I have been member of the IRB of the US Naval Medical Research Center Detachment in Peru for three years. After of my medical degree I have completed two Master degrees, one in Infectious Diseases Control at Universidad Peruana Cayetano Heredia and the second one in Public Health at Johns Hopkins School of Public Health (JHSPH). Currently I am PhD candidate in the International Health department (Global Disease Epidemiology and Control program) at JHSPH. Also I am PI of a current NIH-IRID grant in animal models involving naturally infected pigs (1R01AI116456-01).

B. Positions and Honors

Positions and Employment

2001 – Pres.   Associate Investigator and Bioethics Officer, Cysticercosis Unit, Universidad Peruana Cayetano Heredia - Instituto Nacional de Ciencias Neurologicas, Lima, Peru.

2014 – Pres.	Associate Investigator at Center for Global Health, Universidad Peruana Cayetano Heredia, Lima, Peru.
2016 – 2017	WHO, Diagnosis and Treatment Guidelines for Taenia solium Neurocysticercosis, Member of the Sistematic Review group.
2008 – 2010	Chief Project Officer Caritas Ayaviri – Puno
2006 – 2010	IRB Member, Naval Medical Research Center Detachment. Peru
2001 – 2002	Attending Physician: Centro Medico "Sagrada Familia", Lima Peru.
2000 – 2001	Fellow in, Internal Medicine, Surgery, Pediatrics at Hospital Nacional Cayetano Heredia, Lima Peru and Gynecology at Hospital Arzobispo Loayza Lima, Peru.

Award

| 2012 | Ruth Rice Puffer Fund Scholarship for International Students Johns Hopkins School of Public Health |

C. Contribution to Science

1. Most of my contributions to science have been as part of the Cysticercosis Working Group. Probably the main contribution is related to the development of better antiparasitic treatment for patients with neurocysticercosis. As part of my medical degree dissertation, while still in medical school, I began working on research testing a short treatment for neurocysticercosis (Bustos 2006). After that, I collaborated with the idea to improve the treatment efficacy with a new approach: the combined antiparasitic treatment with albendazole plus praziquantel. I lead the pre-clinical studies in naturally infected pigs with promising results (Gonzalez 20012). These results were strong enough to get funds to develop two clinical trials; the first one to evaluate pharmacokinetics aspects (Garcia 2011) and the second one to evaluate the efficacy of the treatment in a multicentric clinical trial (Garcia 2014). The results have re-defined the standard of care of this disease.

   - **Bustos JA**, Pretell EJ, Llanos-Zavalaga F, Gilman RH, Del Brutto OH, Garcia HH; for The Cysticercosis Working Group in Peru. Efficacy of a 3-day course of albendazole treatment in patients with a single neurocysticercosis cyst. Clin Neurol Neurosurg. 2006 Feb;108(2):193-4.
   - Gonzalez AE, **Bustos JA**, Jimenez JA, Rodriguez ML, Gilman RH, Garcia HH; Cysticercosis Working Group in Peru. Efficacy of diverse antiparasitic treatments for cysticercosis in the pig model. Am J Trop Med Hyg. 2012 Aug;87(2):292-6.
   - Garcia HH, Lescano AG, Lanchote VL, Pretell EJ, Gonzales I, **Bustos JA**, Takayanagui OM, Bonato PS, Horton J, Saavedra H; Pharmacokinetics of combined treatment with praziquantel and albendazole in neurocysticercosis. British Journal of Clinical Pharmacology; 2011; 72:77-84. PMCID 3141188
   - Garcia HH, Gonzales I, Lescano AG, **Bustos JA,** Zimic M, Gavidia M, Rodriguez L., Najar E., et al. Efficacy of combined antiparasitic therapy with praziquantel and albendazole for neurocysticercosis: a double-blind, randomised controlled trial. Lancet Infectious Diseases, 2014 Jul; 14:687-695.

2. As part of my dissertation for a master program in Control of Infectious Diseases. I developed a study to evaluate the usefulness of a new ELISA test to detect antigens of a *T.solium* tapeworm (CoAg-ELISA) after antiparasitic treatment. The study provided evidence that CoAg-ELISA is useful to evaluate treatment failure in taeniasis. Early

assessment at day 15 would detect treatment failure before patients become infective. This is an important piece of information for control program of taeniasis/cysticercosis.

- **Bustos JA**, Rodriguez S, Jimenez JA, Moyano LM, Castillo Y, Ayvar V, Allan JC, Craig PS, Gonzalez AE, Gilman RH, Tsang VC, Garcia HH; Cysticercosis Working Group in Peru. Detection of Taenia solium taeniasis coproantigen is an early indicator of treatment failure for taeniasis. Clin Vaccine Immunol. 2012 Apr;19(4):570-3.

3. Calcified lesions are the most common cerebral finding of NCC because they accumulate in the brain and are a measure of prior infections. It is agreed that even after a successful anti-parasitic treatment, the remaining calcified scars persist, acting as foci of relapsing inflammation and seizures. I have participated in studies to characterize this phenomenon and currently I am conducting a study in animal models to evaluate the role of bisphosphonates as an option to avoid or reduce the calcification process and their consequences.

- **Bustos JA**, Garcia HH, Dorregaray R, Naranjo M, Pretell EJ, Gonzalez AE, Gilman RH; for The Cysticercosis Working Group in Peru. Detection of muscle calcifications by thigh CT scan in neurocysticercosis patients. Trans R Soc Trop Med Hyg. 2005 Oct;99(10):775-9.
- **Bustos JA**, Montoya E, Garcia HH; Cysticercosis Working Group in Peru. Residual brain calcifications in neurocysticercosis. Am J Trop Med Hyg. 2008 Mar;78(3):363.
- Nash TE, Pretell EJ, Lescano AG, **Bustos JA**, Gilman RH, Gonzalez AE, Garcia HH; Cysticercosis Working Group in Peru. Perilesional brain oedema and seizure activity in patients with calcified NCC: a prospective cohort and nested case-control study. Lancet Neurol. 2008 Dec;7(12):1099-105.
- 1R01AI116456-01, NIH-NIAID, (**PI: Bustos, JA**), 4/01/2015 - 03/31/2020, "Use of bisphosphonates to reduce the calcification process in animal models of cysticercosis"

4. In a shared effort with Dr. Garcia and Katherine Sota, a UPCH medical student, we developed the idea of a new animal model for neurocysticercosis and epilepsy using sheep. With this proposal, Ms. Sota got a small intramural grant for the initial steps to evaluate the feasibility of experimental intracranial infection with oncospheres of *T. solium*. In 4 out of 15 sheep of 4 to 8 months of age. Direct microscopic observation and histological evaluation with H-E staining confirmed that the cyst corresponds to cyst or degenerating larval stage of *T. solium*. This exploratory experiment provided the proof-of-concept that intracranial experimental infection with *T. solium* post-oncospheres could cause neurocysticercosis in the sheep model.

5. WHO Consultant. In August 2016, I was asked to conduct systematic reviews for the drafting of "WHO Diagnosis and Treatment Guidelines for *Taenia solium* Neurocysticercosis (NCC)" and provided the report for consideration by the Guideline Review Committee. These systematic reviews covered twelve PICO Questions. The guideline was targeted at health care providers working at a first or second level facility or at district level including basic outpatient and inpatient services. It was also aimed to be used by the policy makers, health care planners and program managers in government

and development and international agencies. These guidelines will also be useful for academicians and researchers to inform teaching and research agendas.

D. Complete List of Published Work

Peer-reviewed publications (in chronological order, n=30)

1. Arroyo G, **Bustos JA**, Lescano AG, Saavedra H, Rodriguez S, Pretell EJ, Takayanagui OM, Horton J, Gonzalez AE, Gilman RH, Garcia HH; Cysticercosis Working Group in Peru. Albendazole sulfoxide plasma levels and efficacy of antiparasitic treatment in patients with parenchymal neurocysticercosis. Clin Infect Dis. 2019 Nov 13;69(11):1996-2002.

2. **Bustos JA**, Ninaquispe BE, Rodriguez S, Castillo Y, Yang SY, Gilman RH, DornyP, Gabriël S, García HH, Gonzalez AE, For The Cysticercosis Working Group In Peru. Performance of a Sandwich Antigen-Detection ELISA for the Diagnosis of Porcine Taenia solium Cysticercosis. Am J Trop Med Hyg. 2019 Mar;100(3):604-608.

3. Duque KR, Escalaya AL, Zapata W, Burneo JG, **Bustos JA**, Gonzales I, Saavedra H, Pretell EJ, Garcia HH; Cysticercosis Working Group in Peru. Clinical topography relationship in patients with parenchymal neurocysticercosis and seizures. Epilepsy Res. 2018 Sep;145:145-152.

4. Herrick JA, Maharathi B, Kim JS, Abundis GG, Garg A, Gonzales I, Saavedra H, **Bustos JA**, Garcia HH, Loeb JA; Cysticercosis Working Group in Peru. Inflammation is a key risk factor for persistent seizures in neurocysticercosis. Ann Clin Transl Neurol. 2018 Apr 10;5(5):630-639.

5. Garcia HH, Castillo Y, Gonzales I, **Bustos JA**, Saavedra H, Del Brutto OH, Wilkins PP, Gonzalez AE, Gilman RH; Cysticercosis Working Group in Peru. Low sensitivity and frequent cross-reactions in commercially available antibody detection ELISA assays for Taenia solium cysticercosis. Trop Med Int Health. 2018 Jan;23(1):101-105.

6. Arroyo G, Rodriguez S, Lescano AG, Alroy KA, **Bustos JA**, Santivañez S, Gonzales I, Saavedra H, Pretell EJ, Gonzalez AE, Gilman RH, Tsang VCW, Garcia HH; Cysticercosis Working Group in Peru . Antibody Banding Patterns of the Enzyme-Linked Immunoelectrotransfer Blot and Brain Imaging Findings in Patients With Neurocysticercosis. Clin Infect Dis. 2018 Jan 6;66(2):282-288.

7. **Bustos JA**, García HH, Del Brutto OH. Reproducibility of Diagnostic Criteria for Ventricular Neurocysticercosis. Am J Trop Med Hyg. 2017 Dec;97(6):1953-1954.

8. **Bustos JA**, García HH, Del Brutto OH. Reliability of Diagnostic Criteria for Neurocysticercosis for Patients with Ventricular Cystic Lesions or Granulomas: A systematic review. Am J Trop Med Hyg. 2017 Sep;97(3):653-657.

9. Cangalaya C, **Bustos JA**, Calcina J, Vargas-Calla A, Mamani J, Suarez D, Arroyo G, Gonzalez AE, Chacaltana J, Guerra-Giraldez C, Mahanty S, Nash TE, García HH; Cysticercosis Working Group in Peru. Radiological evolution of porcine neurocysticercosis after combined antiparasitic treatment with praziquantel and albendazole. PLoS Negl Trop Dis. 2017 Jun 2;11(6):e0005624.

10. Nash TE, **Bustos JA**, Garcia HH; Cysticercosis Working Group in Perú. Disease Centered Around Calcified Taenia solium Granuloma. Trends Parasitol. 2017 Jan;33(1):65-73.

11. Cangalaya C, **Bustos JA**, Calcina J, Vargas-Calla A, Suarez D, Gonzalez AE, Chacaltana J, Guerra-Giraldez C, Mahanty S, Nash TE, García HH; Cysticercosis Working Group in Peru. Perilesional Inflammation in Neurocysticercosis -Relationship Between Contrast-Enhanced Magnetic Resonance Imaging, Evans Blue Staining and Histopathology in the Pig Model. PLoS Negl Trop Dis. 2016 Jul 26;10(7):e0004869.

12. **Bustos JA**, García HH, Del Brutto OH. Antiepileptic drug therapy and recommendations for withdrawal in patients with seizures and epilepsy due to neurocysticercosis. Expert Rev Neurother. 2016 Sep;16(9):1079-85.

13. Garcia HH, Lescano AG, Gonzales I, **Bustos JA**, Pretell EJ, Horton J, Saavedra H, Gonzalez AE, Gilman RH; Cysticercosis Working Group in Peru. Cysticidal Efficacy of Combined Treatment With Praziquantel and Albendazole for Parenchymal Brain Cysticercosis. Clin Infect Dis. 2016 Jun 1;62(11):1375-9.

14. Gonzalez AE, **Bustos JA**, Garcia HH, Rodriguez S, Zimic M, Castillo Y, Praet N, Gabriël S, Gilman RH, Dorny P; Cysticercosis Working Group in Peru. Successful Antiparasitic Treatment for Cysticercosis is Associated with a Fast and Marked Reduction of Circulating Antigen Levels in a Naturally Infected Pig Model. Am J Trop Med Hyg. 2015 Dec;93(6):1305-10.

15. Garcia HH, Gonzales I, **Bustos JA**, Saavedra H, Gavidia M, Rodriguez L, Najar E, Umeres H, Pretell EJ. Combined antiparasitic treatment for neurocysticercosis
- Authors' reply. Lancet Infect Dis. 2015 Mar;15(3):266-7.

16. Garcia HH, Gonzales I, Lescano AG, **Bustos JA**, Pretell EJ, Saavedra H, Nash TE; Cysticercosis Working Group in Peru. Enhanced steroid dosing reduces seizures during antiparasitic treatment for cysticercosis and early after. Epilepsia. 2014 Sep;55(9):1452-9.

17. Garcia HH, Gonzales I, Lescano AG, **Bustos JA**, Zimic M, Escalante D, Saavedra H, Gavidia M, Rodriguez L, Najar E, Umeres H, Pretell EJ; Cysticercosis Working Group in Peru. Efficacy of combined antiparasitic therapy with praziquantel and albendazole for neurocysticercosis: a double-blind, randomised controlled trial. Lancet Infect Dis. 2014 Aug;14(8):687-695.

18. Bruno E, Bartoloni A, Zammarchi L, Strohmeyer M, Bartalesi F, **Bustos JA**, Santivañez S, García HH, Nicoletti A; COHEMI Project Study Group. Epilepsy and neurocysticercosis in Latin America: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2013 Oct 31;7(10):e2480.

19. Gonzalez AE, **Bustos JA**, Jimenez JA, Rodriguez ML, Gilman RH, Garcia HH; Cysticercosis Working Group in Peru. Efficacy of diverse antiparasitic treatments for cysticercosis in the pig model. Am J Trop Med Hyg. 2012 Aug;87(2):292-6.

20. **Bustos JA**, Rodriguez S, Jimenez JA, Moyano LM, Castillo Y, Ayvar V, Allan JC, Craig PS, Gonzalez AE, Gilman RH, Tsang VC, Garcia HH; Cysticercosis Working Group in Peru. Detection of Taenia solium taeniasis coproantigen is an early indicator of treatment failure for taeniasis. Clin Vaccine Immunol. 2012 Apr;19(4):570-3.

21. Wallin MT, Pretell EJ, **Bustos JA**, Caballero M, Alfaro M, Kane R, Wilken J, Sullivan C, Fratto T, Garcia HH. Cognitive changes and quality of life in neurocysticercosis: a longitudinal study. PLoS Negl Trop Dis. 2012 Jan;6(1):e1493.

22. Garcia HH, Lescano AG, Lanchote VL, Pretell EJ, Gonzales I, **Bustos JA**, Takayanagui OM, Bonato PS, Horton J, Saavedra H, Gonzalez AE, Gilman RH; Cysticercosis Working Group in Peru. Pharmacokinetics of combined treatment with praziquantel and albendazole in neurocysticercosis. Br J Clin Pharmacol. 2011 Jul;72(1):77-84.

23. Nash TE, Pretell EJ, Lescano AG, **Bustos JA**, Gilman RH, Gonzalez AE, Garcia HH; Cysticercosis Working Group in Peru. Perilesional brain oedema and seizure activity in patients with calcified neurocysticercosis: a prospective cohort and nested case-control study. Lancet Neurol. 2008 Dec;7(12):1099-105.

24. **Bustos JA**, Montoya E, Garcia HH; Cysticercosis Working Group in Peru. Residual brain calcifications in neurocysticercosis. Am J Trop Med Hyg. 2008. Mar;78(3):363.

25: Chero JC, Saito M, **Bustos JA**, Blanco EM, Gonzalvez G, Garcia HH; Cysticercosis Working Group in Peru. Hymenolepis nana infection: symptoms and response to nitazoxanide in field conditions. Trans R Soc Trop Med Hyg. 2007 Feb;101(2):203-5.

26. Prasad S, MacGregor RR, Tebas P, Rodriguez LB, **Bustos JA**, White AC Jr. Management of potential neurocysticercosis in patients with HIV infection. Clin Infect Dis. 2006 Feb 15;42(4):e30-4.

27. **Bustos JA**, Pretell EJ, Llanos-Zavalaga F, Gilman RH, Del Brutto OH, Garcia HH; Cysticercosis Working Group in Peru. Efficacy of a 3-day course of albendazole treatment in patients with a single neurocysticercosis cyst. Clin Neurol Neurosurg. 2006 Feb;108(2):193-4.

28. Huisa BN, Menacho LA, Rodriguez S, **Bustos JA**, Gilman RH, Tsang VC, Gonzalez AE, García HH; Cysticercosis Working Group in Perú. Taeniasis and cysticercosis in housemaids working in affluent neighborhoods in Lima, Peru. Am J Trop Med Hyg.
2005 Sep;73(3):496-500.

29: **Bustos JA**, Garcia HH, Dorregaray R, Naranjo M, Pretell EJ, Gonzalez AE, Gilman RH; Cysticercosis Working Group in Peru. Detection of muscle calcifications by thigh CT scan in neurocysticercosis patients. Trans R Soc Trop Med Hyg. 2005 Oct;99(10):775-9.

30: Pretell EJ, Martinot C Jr, Garcia HH, Alvarado M, **Bustos JA**, Martinot C; Cysticercosis Working Group in Peru. Differential diagnosis between cerebral tuberculosis and neurocysticercosis by magnetic resonance spectroscopy. J Comput Assist Tomogr. 2005 Jan-Feb;29(1):112-4.

Meeting Abstract presentation (last five years)
1. Gianfranco Arroyo, Andres G. Lescano, **Javier A. Bustos**, Isidro Gonzales, Herbert Saavedra, Javier E. Pretell, Armando E. Gonzalez, Robert H. Gilman, Hector H. Garcia. Relationship between plasma levels of albendazole sulfoxide and antiparasitic efficacy in the treatment of neurocysticercosis. Oral presentation. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

2. Luz M. Toribio Salazar, Miryam de los Angeles, Clive J. Shiff, **Javier A. Bustos**, Armando Gonzalez, Bob Gilman, Hector Hugo Garcia, For the neurocysticercosis working group of Peru.

Cell-Free DNA (cfDNA) in Urine as a Novel Diagnosis for Human Neurocysticercosis. Poster session. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

3. Paul D. Jewell, Annette Abraham, Veronika Schmidt, Kevin G. Buell, **Javier A. Bustos**, Hector H. Garcia, Matthew A. Dixon, Martin Walker, Maria-Gloria Basanez, Andrea Winkler.Treatment of individuals living with neurocysticercosis and HIV/AIDS: a scoping review. Poster session. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

4. **Javier A. Bustos**, Laura Baquedano, Gianfranco Arroyo, Juan F. Calcina, Ana Vargas-Calla, Castillo Erick, Juan Chacaltana, Armando E. Gonzalez, Robert H. Gilman, Hector H. Garcia. Characterization of the Calcification Process of Brain Cysts in Pigs with Neurocysticercosis. Poster session. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

5. Gianfranco Arroyo, **Javier A. Bustos**, Laura Baquedano, Juan F. Calcina, Ana M. Vargas-Calla, Nancy Chile, Juan Chacaltana, Manuela Verastegui, Robert H. Gilman, Armando E. Gonzalez, Hector H. Garcia. A novel experimental pig model for neurocysticercosis via carotid Taenia solium oncosphere infection: Determination of the minimal infective dose and histopathological characterization. Oral presentation. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

6 .Hannah E. Steinberg, Paul Russo, Andrea Diestra, Cusi Ferradas, Maritza Calderon, Jeroen Bok, Linda Chanamé Pinedo, Deanna Zhu, Gaston Valencia, Cesar Ramal, **Javier A. Bustos**, Natalie M. Bowman, Lance Liotta, Alessandra Luchini, Robert H. Gilman. Diagnosis of Toxoplasmic Encephalitis in Persons Living with HIV in Urine. Poster session. ASTMH 67th Annual Meeting; 2018 October 28 - November 1; New Orleans, LA.

7 .Jesica A. Herrick, Anjali Garg, Jin Suh Kim, Biswajit Maharathi, Gerardo Gomez Abundis, Isidro Gonzales, Herbert Saavedra, **Javier A. Bustos**, Hector H. Garcia, Jeffery A. Loeb. Inflammation is a key risk factor for refractory seizures in patients with neurocysticercosis. Poster session. ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

8. Gianfranco Arroyo , Silvia Rodriguez , Andres G. Lescano , Karen A. Alroy , **Javier A. Bustos**, Saul Santivañez , Isidro Gonzales , Herbert Saavedra , Javier Pretell , Armando E. Gonzalez , Robert H. Gilman , Victor C. Tsang , Hector H. Garcia. Banding patterns of the enzyme-linked immunoelectrotransfer blot (eitb) and brain imaging findings in patients with neurocysticercosis. Poster session ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

9. Yesica Santos, Yesenia Castillo, Luz Toribio, Cindy Espinoza, Kevin Martel , Adriana Paredes, Cristina Guerra-Giraldez , Yagahira Castro-Sesquen , Isidro Gonzales , Herbert Saavedra , **Javier A. Bustos,** Theodore E. Nash , Hector H. Garcia1 , For the Cysticercosis Working Group in Peru. Standardization of a direct ELISA using monoclonal antibodies for the detection of parasite antigen in urine samples of patients with neurocysticercosis. Oral presentation. ASTMH 66th Annual Meeting; 2017; November 5-9; Baltimore, MD.

10. Gianfranco Arroyo, Andres G. Lescano, Juan F. Calcina, **Javier A. Bustos,** Teresa Lopez-Urbina, Silvia Rodriguez, Luis A. GomezPuerta, Seth O'Neal, Robert H. Gilman, Victor C. Tsang, Hector H. Garcia, Armando E. Gonzalez. Banding patterns of the enzyme-linked

immunoelectrotransfer blot (EITB) correlate with the infection status in porcine cysticercosis. Oral presentation. ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

11. Agueda Perez, Luz Toribio, Cindy Espinoza, Kevin Martel, Yagahira Castro-Sesquen, **Javier A. Bustos,** Theodore E. Nash, Hector H. Garcia, For the Cysticercosis Working Group in Peru. A novel magnetic particle-based approach for the purification and concentration of monoclonal antibodies from cell culture supernatant. Poster presentation.. ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

12. Percy M. Vilchez Barreto, Seth O'Neal, Ricardo GamboaMorán, Claudio Muro-Ecca, Luz-María Moyano, Michelle Beam, **Javier a. Bustos**, Sarah Gabriel, Pierre Dorny, Hector H Garcia for the Cysticercosis Working Group in Peru. Field based screening for circulating antigen in urine samples for the detection of severe forms of neurocysticercosis. Poster session. ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

13. Gino Castillo, Lizbeth Fustamante, Ana Delgado, Rogger Carmen, Maria del Carmen Ferrufino, **Javier A. Bustos**, Robert Gilman. Antiparasitic treatment in novel rat model for neurocysticercosis. Poster presentation. Poster session. ASTMH 66th Annual Meeting; 2017; November 5 – 9; Baltimore, MD.

14. Kevin R. Duque, **Javier A. Bustos**, Isidro Gonzales, Herbert Saavedra, Javier Pretell, Hector H. Garcia, for the Cysticercosis Working Group in Peru. Risk factors for seizure recurrence after successful antiparasitic treatment in parenchymal neurocysticercosis. Poster session. ASTMH 65th Annual Meeting; 2016; November 13-17; Atlanta, GA.

15. Jaeson S. Calla-Choque, Miguel A. Orrego, Pamela L. Pacheco- Rivera, **Javier A. Bustos**, Hector H. Garcia, Siddhartha Mahanty, Theodore Nash, Cristina Guerra-Giraldez, Cysticercosis Working Group in Peru. CDNA library from Taenia Solium racemose cyst: a novel tool for transcriptomics in neurocysticercosis. Poster session. ASTMH 65th Annual Meeting; 2016; November 13 – 17; Atlanta, GA.

16. Jesica A. Herrick, Anjali Garg, **Javier A. Bustos**, Hector H. Garcia, Jeffrey A. Loeb, Cysticercosis Working Group in Peru. Risk factors for refractory epilepsy development in neurocysticercosis. Poster session. ASTMH 65th Annual Meeting; 2016; November 13-17; Atlanta, GA.

17. Gianfranco Arroyo, Luis Antonio Gomez-Puerta, **Javier A. Bustos**, Robert Gilman, Hector Garcia, Armando Gonzalez. Use efficacy of a single-dose of oxfendazole at three different formulations against the larval stage of Taenia Solium in naturally infected pigs. Poster session. ASTMH 65th Annual Meeting; 2016; November 13 – 17; Atlanta, GA.

18. Luz Maria Moyano Vidal, Ricardo Gamboa, Percy Vilchez, Viterbo Ayvar, Ruth Atto, Exgar Oliva, Silvia Rodriguez, Seth O´Neal, **Javier A. Bustos**, Armando E. Gonzales, Robert H. Gilman, Victor C.w Tsang, Hector H. García, For The Cysticercosis Working Group of Peru. Efficacy of single doses of praziquantel 5-10 mg/kg for taeniasis under controlled conditions in rural communities of the northern coast of Peru. Oral presentation. ASTMH 65th Annual Meeting; 2016; November 13 – 17; Atlanta, GA.

19. Kevin R. Duque, **Javier A. Bustos**, Isidro Gonzales, Javier Pretell, Hector H. Garcia, for The Cysticercosis Working Group in Peru. Pathogenesis of seizures in neurocysticercosis: from

cysticercotic lesions to seizure semiology. Oral session. ASTMH 65th Annual Meeting; 2016; November 13 – 17; Atlanta, GA.

20. Luz M. Moyano, Percy Vilchez, Fernando Urizar, Exgar Oliva, Viterbo Ayvar, Javier A. Bustos, Hector H. Garcia, For the Cysticercosis Working Group of Peru. Association between edema peri-calcification and epileptic seizures a case-control study in endemic area for Cysticercosis in the northern coastal of Peru. Oral presentation. ASTMH 64th Annual Meeting; 2015; October 25 – 29; Philadelphia, PA.

21. Katherine A. Sota, **Javier A. Bustos**, Carmen Taquiri, Armando E. Gonzalez, Robert H. Gilman, Juan Jimenez, Isidro Gonzales, Hector H. Garcia, for the Cysticercosis Working Group in Peru. Prevalence and factors associated with intestinal taeniasis in patients with cysticercosis and their relatives. Scientific session. Oral presentation. ASTMH 64th Annual Meeting; 2015; October 25 – 29; Philadelphia, PA.

22. Carla Marcia Cangalaya, **Javier Bustos**, Juan Calcina, Ana Vargas, Armando Emico Gonzalez, Cristina Guerra Giraldez, Siddhartha Mahanty, Teodhore Nash4, Hugo Hector Garcia2, Cysticercosis Working Group in Peru. Correlation of Evans Blue staining and gadolinium enhancement in magnetic resonance imaging of pigs naturally infected with Taenia Solium. Poster session. ASTMH 64th Annual Meeting; 2015; October 25 – 29; Philadelphia, PA.

23. Carla Cangalaya, Cristina Guerra-Giraldez, **Javier A. Bustos,** Armando E Gonzalez, Siddhartha Mahanty, Theodore E Nash, Hector H Garcia, Cysticercosis Working Group in Peru. Three-dimensional distribution of Taenia solium cysts in porcine neurocysticercosis. Oral session. ASTMH 64th Annual Meeting; 2015; October 25 – 29; Philadelphia, PA.

E. Languages

Spanish:	Native speaker
English:	Fluent
Portuguese:	Reading / Listening
Italian:	Reading / Listening

F. Research Support
Ongoing Research Support
1R01AI116456-01   (BUSTOS, Javier A.)   March 2015 – Feb 2020
NIAID-NIH
Title: Use of bisphosphonates to reduce the calcification process in animal models of cysticercosis
- The aim of the study is to standardize an experimental model of cysticercosis infection and calcification in animal model and use it to evaluate whether bisphosphonates can interrupt the process of residual calcification.
Role: Principal Investigator

1U01NS086974-01A1   (GARCIA, Hector H.)   Aug 2015 – Jul 2020
NINDS-NIH
Title: Combined Albendazole and Praziquantel in Subarachnoid NCC, CCC, Lead application
- Clinical trial to evaluate whether the addition of PZQ during the initial 15 days of treatment of

SANCC improves the efficacy of the standard of care ABZ regimen in terms of a higher proportion of patients demonstrating disappearance of their SANCC lesions as well as an increased percent reduction of parasite volume.
Role: Medical Monitor

2D43TW001140-16   (GARCIA, Hector H.)   Jul 2016 – Jun 2021
FIC – NIH
Title: Training in Infectious Diseases in Peru – Time for Capacity Strengthening in Clinical Research
- The program will enroll 50 medium-term mentored post-graduate and pre-graduate students in a Diploma course (10 per year), 25 mentored Master's degree students (five per year), and up to seven PhD students including four enrolled in the UPCH PhD program.
Role: Collaborator

U19AI129909   (GARCIA, Hector H.)   Apr 2017 – Mar 2022
Title: Improving Diagnostic and Management Tools for NCC (Peru-JHU TMRC Program)
- Program grant to develop improved tools in key required areas for the diagnosis or monitoring of human cysticercosis
Role: Investigator

1R01NS103623-01A1   (O'NEAL, Seth E.)   Jul 2018 – Jun 2023
Title: Urine Screening for early detection of subarachnoid Neurocysticercosis
-      This proposal evaluates urine screening for early detection of the most severe form of infection in order to improve clinical outcomes.
Role: Medical Monitor

1R01AI143553-01   (ARROYO, Gianfranco)   Mar/2019 – Feb/2023
NIAID- NIH
Title: Alternative therapeutic approaches for the control of brain inflammation secondary to anthelmintic therapy in neurocysticercosis using a novel experimental pig model
- This proposal aims to optimize a novel experimental pig model for NCC, and to use this model to assess alternative therapy approaches using two drugs: etanercept and methotrexate as potential corticosteroid-sparing/replacing drugs for use in NCC.
Role: Co-Investigator

Completed Research Support
R21 NS094976-01   (GARCIA, Hector H.)   Jul 2016 – Dec 2017
NIH-NINDS
"Characterization of hippocampal sclerosis in individuals with calcified neurocysticercosis"
This project will explore hippocampal compromise in individuals with resolved neurocysticercosis to assess the relationship between NCC and hippocampal atrophy
Role: Medical Monitor

Grant 23981   Cysticercosis Working Group in Peru   April 2003 to 2014
Bill and Melind a Gates Foundation
A Demonstration Project to Eliminate Cysticercosis in Peru
Seven-years project to demonstrate feasibility of elimination in a defined endemic area
Role: Medical Monitor

RO1 NS054805 01   (GARCIA, Hector H.)   04/01/2006 - 2013

NINDS – NIH
Antiparasitic Therapy for Neurocysticercosis: Phase II/III Study on Safety and Efficacy of Combined Treatment with Praziquantel and Albendazole. To determinate if a 10-day combined regime of PZQ and ABZ improves the parasiticidal efficacy of ABZ alone at the same dose.
Role: Medical Monitor & Bioethics Assurance Officer

05-I-N124   Theodore Nash (PI)   2005 – 2010
NIAID- NIH (intramural research)
Increased Dosing of Corticosteroids in the Treatment of Intraparenchymal Neurocysticercosis
Role: Medical Monitor & Bioethics Assurance Officer