การศึกษาเชิงวิเคราะห์แบบไปข้างหน้าและย้อนหลัง เรื่องการติดเชื้อที่เป็นสาเหตุของภาวะสมองอักเสบในประเทศไทย

นางสาวเบญจวรรณ สกุลสุจิราภา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอายุรศาสตร์ ภาควิชาอายุรศาสตร์
คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559

A Prospective and Retrospective Analytic Study of Infectious Cause of Encephalitis in Thailand

Miss Benjawan Skulsujirapa

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Medicine

Department of Medicine

Faculty of Medicine

Chulalongkorn University

Academic Year 2016

Thesis Title	A Prospective and Retrospective Analytic Study of Infectious Cause of Encephalitis in Thailand
By	Miss Benjawan Skulsujirapa
Field of Study	Medicine
Thesis Advisor	Assistant ProfessorOpass Putchareon, M.D.
Thesis Co-Advisor	Abhinbhen Saraya Wasontiwong, M.D.

Accepted by the Faculty of Medicine, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Medicine

(ProfessorSuttipong Wacharasindhu, M.D.)

THESIS COMMITTEE

.....Chairman

(ProfessorRoongroj Bhidayasiri, M.D.)

.....Thesis Advisor

(Assistant ProfessorOpass Putchareon, M.D.)

.....Thesis Co-Advisor

(Abhinbhen Saraya Wasontiwong, M.D.)

.....Examiner

(Pairoj Chattranukulchai, M.D.)

.....External Examiner

(Nirada Siriyakorn, M.D.)

เบญจวรรณ สกุลสุจิราภา : การศึกษาเชิงวิเคราะห์แบบไปข้างหน้าและย้อนหลัง เรื่องการติดเชื้อที่เป็นสาเหตุของภาวะสมองอักเสบในประเทศไทย (A Prospective and Retrospective Analytic Study of Infectious Cause of Encephalitis in Thailand) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. นพ.โอภาส พุทธเจริญ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: พญ.อภิญญ์เพ็ญ สาระยา วสันติวงศ์, 58 หน้า.

ที่มาของการวิจัย: ข้อมูลในอดีตของไข้สมองอักเสบที่เกิดจากการติดเชื้อในประเทศไทยพบว่าส่วนใหญ่เกิดจากเชื้อไวรัส เช่นเดียวกับข้อมูลส่วนใหญ่ที่พบทั่วโลก อย่างไรก็ตามข้อมูลในรายงานต่างมีสัดส่วนของไวรัสที่เป็นสาเหตุแตกต่างกันออกไป ส่วนใหญ่ที่พบเป็นสาเหตุหลักสามลำดับแรกคือ ไวรัสเจแปนนีส เอนเซปฟาลิติส, เอนเทอโรไวรัส, และเฮอร์ปีส์ไวรัส สาเหตุของไข้สมองอักเสบที่เกิดจากการติดเชื้อนี้เป็นที่ยอมรับกันทั่วไปว่ามีความแตกต่างกันขึ้นกับภูมิภาค, ฤดูกาล และมาตรการป้องกันการติดเชื้อที่หลากหลาย นอกจากนั้นยังพบว่ามีการเปลี่ยนแปลงทางพลวัตของเชื้อต่างๆอย่างต่อเนื่อง ข้อมูลปัจจุบันในแต่ละพื้นที่จึงมีความสำคัญในการพัฒนาลำดับชุดการตรวจหาสาเหตุที่เหมาะสมอันจะทำให้มีความคุ้มค่าต้นทุน-ประสิทธิผล

วัตถุประสงค์: เพื่อศึกษาสาเหตุของไข้สมองอักเสบในโรงพยาบาลตติยภูมิโดยการตรวจหาสาเหตุอย่างกว้างขวาง

วิธีการวิจัย: เก็บข้อมูลผู้ป่วยไข้สมองอักเสบที่ได้รับการรักษาแบบผู้ป่วยในในโรงพยาบาลจุฬาลงกรณ์ โรงพยาบาลตติยภูมิในกรุงเทพมหานคร โดยทำการศึกษาแบบไปข้างหน้าตั้งแต่เดือนพฤศจิกายน พ.ศ. 2559 ถึงเดือนมีนาคม พ.ศ. 2560 และการศึกษาย้อนหลังตั้งแต่เดือนมกราคม พ.ศ. 2557 ถึงเดือนตุลาคม พ.ศ. 2559 ทำการตรวจทางจุลชีววิทยา และน้ำเหลืองวิทยาตามลำดับขั้นตอน โดยจำแนกตามผลการตรวจน้ำไขสันหลังเบื้องต้นในครั้งแรก ในขั้นตอนแรกมีการตรวจหาเชื้อแบคทีเรีย, เชื้อรา, มัยโคแบคทีเรีย, และไวรัสที่พบเป็นสาเหตุของไข้สมองอักเสบได้บ่อย ในรายที่ผลการตรวจขั้นตอนแรกไม่พบสาเหตุจะได้รับการตรวจหาสาเหตุเพิ่มเติมเป็นขั้นตอน ในกรณีที่ยังไม่พบสาเหตุจะได้รับการตรวจด้วยวิธี family-wide polymerase chain reaction สำหรับไวรัส 9 วงศ์ที่เคยมีการรายงานว่าเป็นสาเหตุของไข้สมองอักเสบได้

ผลการศึกษา: ผู้ป่วย 52 รายเข้าร่วมในการศึกษา 27 ราย (ร้อยละ 51.9) ตรวจไม่พบสาเหตุ, 10 ราย (ร้อยละ 19.2) พบสาเหตุจากการติดเชื้อไวรัส, 3 ราย (ร้อยละ 5.8) พบสาเหตุจากการติดเชื้อแบคทีเรีย และ 12 ราย (ร้อยละ 23) พบสาเหตุจากความผิดปกติทางระบบภูมิคุ้มกัน พบการติดเชื้อไวรัสวาริเซลลา ซอสเตอร์ 4 ราย, ไวรัสเฮอร์ปีส์ ซิมเพล็กซ์ 3 ราย, ไซโตเมกาโลไวรัส 2 ราย, ไวรัสมีเซิลส์ (ไวรัสหัด) 1 ราย, เชื้อแบคทีเรีย *L. monocytogenes* 2 ราย และเชื้อแบคทีเรีย *S. agalactiae* 1 ราย ตรวจไม่พบมีการติดเชื้อจากไวรัสที่นำโดยแมลง และไวรัสก่อโรคอุบัติใหม่ มีผู้ป่วย 6 รายได้รับการวินิจฉัยเป็น anti-NMDA encephalitis ในจำนวนนี้ 3 รายมีการเคลื่อนไหวของช่องปากและกระพุ้งแก้มผิดปกติ ซึ่งในการศึกษานี้ไม่พบความผิดปกติเช่นนี้ในสมองอักเสบจากสาเหตุอื่น และมีเพียง 1 รายที่พบมีเนื้องอก teratoma ร่วมด้วย ผู้ป่วยที่มีการติดเชื้อไวรัสเอชไอวีและมีอาการแสดงของผื่นพบมีความสัมพันธ์กับการเกิดไข้สมองอักเสบจากไวรัส ผู้ป่วยที่มีไข้สมองอักเสบจากเชื้อไวรัสวาริเซลลา ซอสเตอร์ อาจไม่พบการกำเริบของผื่นในช่วงเดียวกับที่เริ่มมีอาการผิดปกติทางระบบประสาทได้ อาการ dysphasia มีความสัมพันธ์กับสมองอักเสบจากการติดเชื้อ, การเคลื่อนไหวผิดปกติมีความสัมพันธ์กับสมองอักเสบจากการติดเชื้อไวรัสและ anti-NMDA encephalitis, อาการอ่อนแรงสัมพันธ์กับสมองอักเสบจากเชื้อไวรัส และสมองอักเสบกลุ่มที่ไม่ทราบสาเหตุ การกรวดน้ำไขสันหลังพบว่า ในกลุ่มที่มีสมองอักเสบจากความผิดปกติทางระบบภูมิคุ้มกันมีแนวโน้มจะมีจำนวนเม็ดเลือดขาวและโปรตีนต่ำกว่ากลุ่มอื่นๆ

สรุป: ในกลุ่มไข้สมองอักเสบที่เกิดจากการติดเชื้อ พบว่าเกิดจากการติดเชื้อไวรัสกลุ่มเฮอร์ปีส์มากที่สุด ซึ่งใกล้เคียงกับรายงานในกลุ่มประเทศพัฒนาแล้ว ในการศึกษานี้ไม่พบว่ามีการติดเชื้อจากไวรัสก่อโรคอุบัติใหม่ หนึ่งในสี่พบว่ามีสมองอักเสบจากความผิดปกติทางระบบภูมิคุ้มกัน โดยพบว่ามีสัดส่วนของ anti-NMDA encephalitis ที่สัมพันธ์กับเนื้องอก teratoma ในสัดส่วนที่ต่ำกว่ารายงานจากประเทศตะวันตก ควรนึกถึงภาวะไข้สมองอักเสบจากความผิดปกติทางระบบภูมิคุ้มกันไว้ในการวินิจฉัยแยกโรคเสมอในผู้ป่วยที่มาด้วยอาการไข้สมองอักเสบ

ภาควิชา อายุรศาสตร์ ลายมือชื่อนิสิต .....

สาขาวิชา อายุรศาสตร์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5874041030 : MAJOR MEDICINE

KEYWORDS: ENCEPHALITIS / INFECTIOUS ENCEPHALITIS / AUTOIMMUNE ENCEPHALITIS / PARANEOPLASTIC ENCEPHALITIS

BENJAWAN SKULSUJIRAPA: A Prospective and Retrospective Analytic Study of Infectious Cause of Encephalitis in Thailand. ADVISOR: ASST. PROF.OPASS PUTCHAREON, M.D., CO-ADVISOR: ABHINBHEN SARAYA WASONTIWONG, M.D., 58 pp.

Background: Previous reports of infectious encephalitis in Thailand showed viruses as major pathogens similar to worldwide data. Major viruses in studies varied among Japanese encephalitis, enteroviruses and herpesviruses. Infectious etiologies vary by regions, seasons and preventive strategies done. Dynamic change of pathogen is believed to occur continually. Local data in each region is important to develop an algorithm of investigations for the cost-effectiveness.

Objectives: To study the etiology of encephalitis in a tertiary-care hospital using extensive tests

Methods: This is a prospective study of patients with encephalitis between November 2016 to March 2017 and a retrospective review of the clinical data and prospective analysis of archived samples of patients with encephalitis who were admitted to the King Chulalongkorn Memorial hospital, a tertiary hospital in Bangkok, from January 2014 to October 2016. Microbiological and serological studies were done according to an algorithm based on initial cerebrospinal fluid analysis. Initial tests were for bacteria, fungus, mycobacterium and commonly prevalent viruses. In cases that initial results yielded negative findings, further testing for infectious etiology was done by stepwise approach. 9 family-wide polymerase chain reaction of viruses was performed to assess for infectious etiology.

Results: Fifty-two patients were enrolled. Twenty-seven (51.9%) patients had no etiology identified. Three patients (5.8%) had bacterial etiology, 10 (19.2%) had viral etiology, and 12 (23%) had immune-mediated encephalitis. Varicella zoster virus was identified in 4 cases, HSV in 3 cases, CMV in 2 cases, measles in 1 case, *L. monocytogenes* in 2 cases and *S. agalactiae* in 1 case. No arbovirus nor emerging viral pathogens were identified. Six patients had anti-NMDA encephalitis, 3 cases had orobuccal dyskinesia, which was found only in anti-NMDA encephalitis in our study. Only 1 out of 6 patients was found to have teratoma. Baseline characteristic of HIV infection and the presence of skin rash were associated with viral etiology. Patients with VZV encephalitis might not have active skin lesion at the onset of neurological symptoms. Dysphasia was associated with infectious etiology, abnormal movement was associated with viral etiology and anti-NMDA encephalitis, motor weakness was associated with viral and unknown etiology. Cerebrospinal fluid profile of the immune-mediated encephalitis had the lowest number of white blood cells and protein. All patients survived at 7 days after admission.

Conclusion: Infection caused by herpesviruses was the most prevalent viral etiology, similar to studies from most developed countries. Emerging viral pathogens were not detected to cause encephalitis in this study. A quarter of patients presenting with acute encephalitis in this study had immune-mediated encephalitis. Fewer ratio of anti-NMDA encephalitis patients with teratomas than in western case series. Autoimmune and paraneoplastic encephalitis should be kept in the differential diagnosis in patients with acute encephalitis.

Department:	Medicine	Student's Signature	.....
Field of Study:	Medicine	Advisor's Signature	.....
Academic Year:	2016	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

This research was supported by grants from the Government National Science and Technology Development Agency.

We thank our colleagues from King Chulalongkorn Memorial Hospital and the Neuroscience Center for Research and Development & WHO--CC for Research and Training on Viral Zoonoses, Bangkok, Thailand who provided insight and expertise that greatly assisted the research, although they may not agree with all of the interpretations/conclusions of this paper.

We thank

Assistant Professor Opass Putcharoen, M.D., M.Sc. (Thesis Advisor)

Abhinbhen Saraya Wasontiwong, M.D., M.Sc. (Thesis Co-advisor)

Professor Thiravat Hemachudha, M.D., FACP

Supaporn Wacharapluesadee, Ph.D.

Sininat Petcharat, B.Sc.

for assistance with the research and comments that greatly improved the manuscript.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Background

Encephalitis is a condition in which there is an inflammation of the brain parenchyma along with clinical symptoms and signs of functional abnormality of the brain. Clinical syndromes are comprised of 1. alteration of consciousness, 2. behavioral change, 3. focal abnormal function of the brain, or 4. seizures. Encephalitis can be caused by infection, or inflammation by noninfectious etiology such as autoimmune or paraneoplastic conditions. In cases of infectious etiology, viruses are the most common cause, for example, the herpes viruses, enteroviruses or flaviviruses. Encephalitis caused by infection is important because it has epidemic potential and high mortality and morbidity rates. The diagnosis of encephalitis requires detailed history and physical examination, blood and cerebrospinal fluid analysis, and neuroimaging tests. Not many facilities in Thailand can do extensive laboratory investigation required to correctly diagnose encephalitis.

Etiology of encephalitis is largely unknown, ranges from 32-75% (1-5). Previous studies showed infectious etiology accounted for approximately one-fourth of all encephalitis incidences (1). Most studies showed viral etiology as the most common pathogens, with herpes simplex virus-1 (HSV-1) the leading cause of sporadic encephalitis in developed world (6). Endemic encephalitis etiology varies by regions, seasons, and preventive measures. Moreover, the dymanics of pathogens is believed to change continually over time.

Infectious encephalitis has epidemic potential. Knowing the pathogens not only aids in treatment but also has epidemiological benefit. Also because half of emerging human pathogenic viruses reported during the past decades were first recognized in

patients presented with encephalitis (7), study of encephalitis is a good surveillance for emerging infectious pathogens.

Due to limitation of investigations in the past and accessibility of investigations in different regions of each country and financial support, it may be assumed that infectious etiology has been underestimated, especially in low-income countries. Local data in each region is important to develop an algorithm of investigations for the cost-effectiveness.

Purpose and Benefit

The purpose of this research is to identify the infectious etiology of encephalitis by performing extensive investigations compared to the routine tests done. We believe that doing additional tests may allow us to detect more infectious etiology. As a result of this, we have devised an algorithm of tests and treatment which can be generalized for practitioners in Thailand.

Research Questions

A. Primary Research Question

Null hypotheses: The infectious etiology of encephalitis that can be detected by microbiological culture and molecular study of cerebrospinal fluid equals 25 percent.

Alternative hypotheses: The infectious etiology of encephalitis that can be detected by culture and molecular study of cerebrospinal fluid does not equal 25 percent.

B. Secondary Research Question

Clinical and laboratody findings related to each type of infection

Conceptual Framework

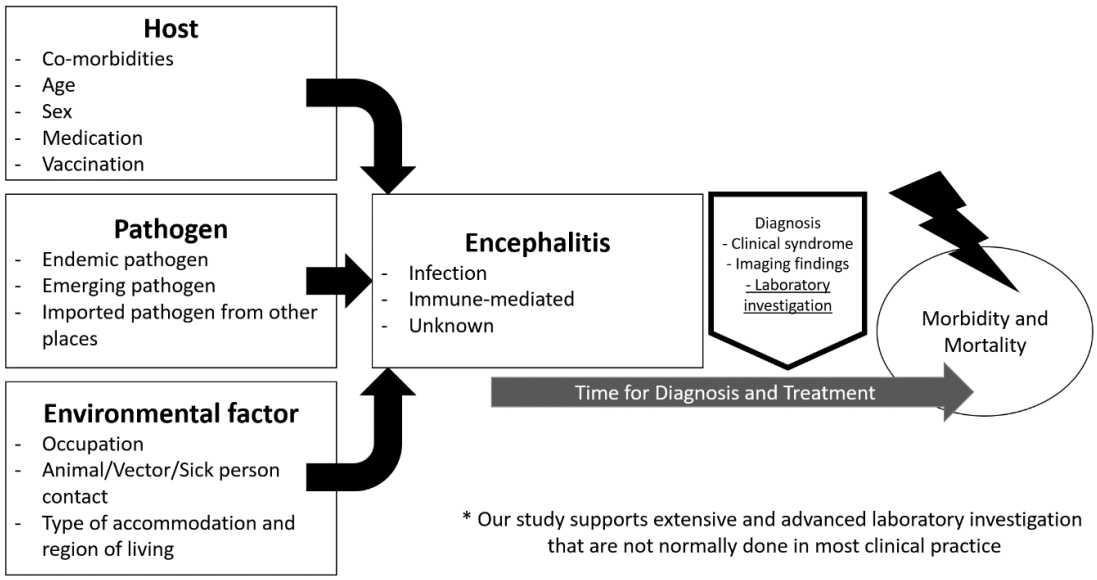

Figure 1. Conceptual framework of the study

Assumption

Patients who were enrolled in this study represent encephalitic patients in Thailand, with King Chulalongkorn Memorial hospital being a tertiary-care hospital in Bangkok with referrals from every regions of Thailand. This study focuses on viral pathogens due to high probability of underdetection in previous studies because of difficulty in specimen processing and unavailability of tests, especially in smaller healthcare facilities.

Operational Definition

A. Encephalitis was defined as having

1. Fever

2. Clinical findings show inflammation of the brain parenchyma; alteration of consciousness, altered sensorium, focal neurological deficits, and seizures

3. Abnormal investigations (at least one)

a) abnormal brain imaging compatible with encephalitis

b) abnormal cerebrospinal fluid profile

c) evidence of pathogen or abnormal immunity related to encephalitis in cerebrospinal fluid or serum

B. Meningoencephalitis was defined as having encephalitis with symptoms and/or signs of meningeal irritation which are

1. Photophobia

2. Positive nuchal rigidity and/or Kernig's sign and/or Brudzinski's sign

C. Fever is defined as core temperature equals or more than 38 degree Celcius (8-10).

Research Design

Observational prospective and retrospective analytic study

Study design

Patients with clinical evidence of encephalitis who attended the King Chulalongkorn Memorial hospital, a tertiary referral hospital in Bangkok, Thailand, were prospectively and retrospectively studied. The prospective part of the study was conducted from November 2016 to March 2017. After the study was approved by the KCMH ethics committee, the researcher personally contacted all of the physicians working in the internal medicine department and informed them of the study. Every three months, the researcher reminded the physicians about the study. Written informed consent was obtained from all patients. If the patients were impaired or underage, then the consent was obtained from a family member, parent or guardian. Only patients with clinical evidence of encephalitis with or without meningitis were enrolled into the study.

The retrospective part of the study was conducted from January 2014 to October 2016. The researcher reviewed all encephalitis data from the hospital's

database regardless of its cause and archived samples of encephalitis patients were analyzed for 9 family-wide polymerase chain reaction (PCR) of viruses. The ICD-10 code for encephalitis was used to search the hospital medical database.

For the prospective part of the study, each enrolled subject, 2 tubes of 3 ml EDTA blood and 2 tubes of 3 ml clotted blood was obtained by venepuncture. Hemoculture (Bactec®) was obtained in 2 sets of specimens. In cases without contraindication to nasal swab, nasal swab was also obtained by inserting a dry polyester swab gently into the nostril, and then placing it in 2 ml of viral transport medium (VTM). Cerebrospinal fluid was obtained by lumbar puncture and 10-15 ml was collected in glass sterile container. All inoculated medium was kept at 4 °C until transportation to laboratory.

Routine laboratory testing included complete blood count (CBC), biochemical panel –blood urea nitrogen (BUN), creatinine, and alanine transferase (ALT). Other diagnostic tests included chest X-ray, rapid NS1 antigen assay, dengue ELISA IgM/IgG, anti-HIV, Gram's stain, acid fast bacilli stain, cryptococcal antigen, culture of bacteria/mycobacteria/fungus, PCR for *Mycobacterium tuberculosis* complex were done as ordered by the attending physician. All reference diagnostic tests were performed at the Department of Microbiology, Faculty of Medicine, Chulalongkorn University except viral studies and multiplex PCR for bacterial meningitis which were performed at the WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University.

Total nucleic acid was extracted directly from CSF or serum specimens (0.2-1.0 mL) by Boom's technique using a commercial available extraction kit (bioMrieux, France). The commercially available real-time PCR assays were used for detection of HSV1-6, Pan-enterovirus, JE virus, Dengue virus, Zika virus, West Nile virus and Adenovirus. The real-time PCR for detection of Nipah virus, Tick-Borne Encephalitis Virus, Chandipura virus and Thogoto virus were performed using the in-house protocols according to the published literatures (11-14). PREDICT family-wide RT-PCR assays were used to detect known or novel virus in the family enterovirus, Seadornavirus,

Paramyxovirus, Arenavirus, Flavivirus, Alpgavirus, Henipavirus, Phlebovirus, and Rhabdovirus(15)

CHAPTER 2
LITERATURE REVIEW

Encephalitis is an inflammation of the brain structures: neurons, vessels or glial cells. However, a consensual definition of the syndrome is difficult to obtain, and it is even more difficult to define encephalitis due a specific agent. Most viruses can be responsible for infectious encephalitis, but the number of encephalitis cases is very limited with regards of the incidence of benign infections from these pathogens. Viruses responsible for encephalitis can be animal-borne, vector-borne or human-to-human transmitted, they can infect preferentially immunocompetent or immunosuppressed patients, and some of them have demonstrated their epidemic potential. Herpes simplex encephalitis is recognized worldwide as the most frequent infectious encephalitis, and the only one with a validated specific treatment. Encephalitis following some viral infections such as measles or rabies can be prevented by vaccination. Unfortunately, effective treatment currently lacks for most encephalitic viral agents identified so far (6).

Etiology of encephalitis is largely unknown, ranges from 32-75% (1-5). Previous studies showed infectious etiology accounted for approximately one-fourth of all encephalitis incidences (1). Most studies showed viral etiology as the most common pathogens, with herpes simplex virus-1 (HSV-1) the leading cause of sporadic encephalitis in developed world (6). Endemic encephalitis etiology varies by regions, seasons, and preventive measures. Moreover, the dymanics of pathogens is believed to change continually over time.

Granerod J and Crowcroft NS reviewed literature to study the epidemiology of encephalitis (16). They found the most common cause of infectious encephalitis was viruses, with an incidence 3.5-5.4 per 100,000 patient-year affecting all ages with a predilection for pediatric population and male. Encephalitis occurred worldwide for some pathogens e.g. herpesviruses, but some caused encephalitis only in specific regions e.g. arboviruses. Although definite epidemiological trends were evident, it was

difficult to make generalisations as few population-based studies exist. Most cases were not reported to health authorities, and many possible pathogens were implicated but in most cases a cause was never found. They concluded that better understanding of the epidemiology of encephalitis would pave the way for better prevention and control strategies of this devastating disease.

The largest study that has been a landmark study in encephalitis etiology was carried out in California, the United States, during June 1998 to December 2000 by Glaser CA et al (1). There were 334 patients enrolled, 25 percent were categorized into infectious etiology. Confirmed and probable viral etiology was made in 31 (9%) patients, bacterial etiology in 9 (3%) patients, parasitic etiology in 2 (1%) patients. Possible infectious etiology was made in 41 (12%) patients, noninfectious etiology of encephalitis in 32 (10%) patients, infection at other body sites than central nervous system in 11 patients (3%). For most patients in this study (208 patients, 62%) the etiology was unknown. The leading cause of definitive infectious encephalitis in this study was herpesviruses and enteroviruses, but they only accounted for 5% of cases while some previous reports identified HSV-1 in 10-20% of cases.

Olivia KJ and Dazzak P reviewed literature for 1,415 human pathogenic viruses and found that 77 viruses were emerging infectious pathogen at that time. In 49% of the emerging viruses, the patients presented encephalitis or other severe neurological involvement. Moreover 89% was emerging viral zoonosis (7).

Stahl JP and Mailles A reviewed literature to describe new features on the epidemiology of encephalitis worldwide (17). They found that Rabies caused one of the most severe types of encephalitis as it was lethal in all cases, and it was endemic in some countries. It was thought that the virus had been eradicated in Western Europe, but it re-emerged in Greece and Italy. Physicians should be aware of this diagnosis in the case of severe encephalitis. Some viruses (Powassan, Nipah, and Hendra) were becoming endemic in some new parts of the world (USA and Australia). Because of their severity, they were healthcare concerns in those countries and for travelers (e.g. in Asia). Also a new concept that herpes simplex virus was suspected to be a trigger for

autoimmune encephalitis. They stated that encephalitis is a good marker for the detection of emerging infections and new findings about the relationship between herpes simplex virus encephalitis and autoimmune encephalitis open a new concept for a better management of patients.

Venkatesan A described more about autoimmune causes of encephalitis (18). Type-A gamma-aminobutyric acid ($GABA_a$) receptor antibodies have been recently identified in encephalitis with refractory seizures, whereas the roles of antibodies to the glycine receptor and dipeptidyl peptidase-like protein 6 have been defined in progressive encephalomyelitis with rigidity and myoclonus. Findings in the US cases of encephalomyelitis presenting with acute flaccid paralysis raised the possibility that enterovirus D68, a common respiratory pathogen, may cause central nervous system disease. Mortality from acute encephalitis occurs in about 10% of cases, with a large proportion of survivors suffering from cognitive or physical disability. In addition to delay in institution of appropriate antiviral or immune therapy, several potentially reversible factors associated with poor prognosis have been identified, including cerebral edema, status epilepticus, and thrombocytopenia.

In Thailand, the epidemiological data on encephalitis was scant. The data before 2000s was derived from series or case reports. One study of pediatric patients in Bangkok between 1996 and 1998 identified viral agents in 26 patients from 40 patients(19). Dengue virus was identified in 8 patients, Japanese encephalitis virus in 6 patients, herpes simplex virus in 4 patients, human herpes virus type 6 in 3 patients, mumps in 2 patients, enterovirus in 1 patient, varicella-zoster virus in 1 patient, and Rabies virus in 1 patient. Between 1970s and 1980s there were 1,500 to 2,500 cases of Japanese encephalitis virus reported annually(20). Routine infant vaccination for Japanese encephalitis has been introduced since 2001. During 2002 and 2008 reported cases of Japanese encephalitis to the national registry decreased four- to eight-fold from earlier decades (21). The largest study of encephalitis in Thailand was done in 2003-2005 in 5 hospitals in Bangkok and 2 hospitals in Hat-Yai (21, 22). Among 149 patients with acute encephalitis 60% were under 18 years of age and almost half met

the definition of meningoencephalitis. The three most common confirmed or probable infectious agents were Japanese encephalitis virus (21 patients, 14%), enteroviruses (6 patients, 4%), and *Orientia tsutsugamushi* (6 patients, 4%).

The infectious etiology of encephalitis data in Thailand differed from other studies in Asia. A study of 127 encephalitis patients from Taiwan in 2000 and 2001 showed herpesviruses, BK virus and arboviruses as most common infectious agents (23). A study of 152 patients from India in 2007 showed enteroviruses and *Flavivirus* were more common than herpesviruses (24). While a study of 99 patients in Cambodia between 1999 and 2000 showed *Streptococcus*, BK virus, Ebstein-Barr virus and *Cryptococcus* were the common cause of encephalitis in that area (25).

A recent study in Thailand by Saraya A et al including 103 patients with encephalitis and/or myelitis between 2010 and 2012 from a tertiary hospital in Bangkok and referral centers from 17 hospital in Thailand, identified 25 (24.3%) patients in infectious etiology (26). Among infectious agents HSV-1 was the most common, found in 6 (5.8%) patients, followed by varicella-zoster virus in 4(3.9%) patients, Japanese encephalitis virus in 3 (2.9%) patients. Immune-mediated encephalitis was identified in 25 (24%) patients.

In summary encephalitis is a clinical syndrome with high morbidity and mortality. Management and outcome depend on early accurate diagnosis which is difficult because most of encephalitis cases the etiology is unknown. This unknown etiology was partly due to technological and financial limitation to investigate, and partly due to the tendency of underreport and underinvestigation. The current data on encephalitis is incomplete especially in developing countries. The study of encephalitis will provide more information on the changing epidemiology of pathogens in infectious encephalitis and for the previously unknown etiology, with new laboratory technique the pathogen may be detected.

CHAPTER 3
RESEARCH METHODOLOGY

Study population

A. Prospective study (November 1st, 2016 to March 31st, 2017)

1. Inclusion Criteria

a) Any patient ≥ 15 years old

b) Initial clinical diagnosis of encephalitis with or without meningitis

c) Admitted to King Chulalongkorn Memorial hospital

2. Exclusion Criteria

a) Pregnant women

b) Patients contraindicated to lumbar puncture (i.e., has infection of the skin and soft tissue overlying the area intended for lumbar puncture, and suspected to have different intracranial pressure between supretentorial and infratentorial compartment of the brain such as midline shift, loss of suprachiasmatic and basilar cistern, space-occupying lesions in posterior fossa, loss of the superior cerebellar cistern, loss of quadrigeminal plate cistern)

c) Patients with high risk to lumbar puncture (platelets less than 40,000 /μL and/or coagulopathy)

B. Retrospective study (January 1st, 2014 to October 31st , 2016)

1. Inclusion criteria

a) Any patient ≥ 15 years old

b) Admitted to King Chulalongkorn Memorial hospital

c) Had a diagnosis of encephalitis by ICD-10 G04 Acute disseminated encephalitis and encephalomyelitis, unspecified or G05 Encephalitis, myelitis and encephalomyelitis in diseases classified elsewhere

2. Exclusion criteria

a) Pregnant women

Sample Size Determination

N = number of patients enrolled

Formula :

$$N = Z^2_{\alpha} pq / r^2$$

α = Probability of type I error = 0.05

p = Proportion of infection in encephalitis = 0.25[1]

q= Proportion of non-infection in encephalitis = 0.75

Maximum tolerable error for the prevalence estimate = 0.1

N = 72

Methods

A. Prospective Study (figure 2.)

1. The researcher explained to the patient and/or the guardian purposes and benefits of this study, methods, risk and also answered to questions the patient and/or the guardian might have before obtaining the consent.

2. After the patient was enrolled, physical exam was done.

3. Encephalitis patient was treated according to standard practice.

4. Cerebrospinal fluid volume 10-15 mL was obtained by lumbar puncture was collected in glass sterile container. Tests were done in algorithmic manner. (Figure) All inoculated medium was kept at 4 °C until transportation to laboratory.

5. Venepuncture was done by registered nurses, physicians or medical students under attending physicians supervision. For this study, blood sample was

obtained in two 3-mL clot blood tube and two 3-mL EDTA blood tube. Hemoculture for aerobic bacteria was done using 10 mL of blood in each of the two bottles (Bactec).

6. Routine laboratory testing included complete blood count (CBC), biochemical panel –blood urea nitrogen (BUN), creatinine, and alanine transferase (ALT). Other diagnostic tests included chest X-ray, rapid NS1 antigen assay, dengue ELISA IgM/IgG, anti-HIV, Gram's stain, acid fast bacilli stain, cryptococcal antigen, culture of bacteria/mycobacteria/fungus, PCR for *Mycobacterium tuberculosis* complex were done as ordered by the attending physician. All reference diagnostic tests were performed at the Department of Microbiology, Faculty of Medicine, Chulalongkorn University except viral studies and multiplex PCR for bacterial meningitis which were performed at the WHO Collaborating Centre for Research and Training on Viral Zoonoses, Faculty of Medicine, Chulalongkorn University.

7. In cases with platelets more than 100,000/mL and no contraindication to nasal swab, nasal swab was also obtained by a dry polyester swab gently into the nostril, and then placing it in 2 ml of viral transport medium (VTM).

8. Total nucleic acid was extracted directly from CSF or serum specimens (0.2-1.0 mL) by Boom's technique using a commercial available extraction kit (bioMrieux, France). The commercially available real-time PCR assays were used for detection of HSV1-6, Pan-enterovirus, JE virus, Dengue virus, Zika virus, West Nile virus and Adenovirus. The real-time PCR for detection of Nipah virus, Tick-Borne Encephalitis Virus, Chandipura virus and Thogoto virus were performed using the in-house protocols according to the published literatures[5-8]. PREDICT family-wide RT-PCR assays were used to detect known or novel virus in the family enterovirus, Seadornavirus, Paramyxovirus, Arenavirus, Flavivirus, Alphavirus, Henipavirus, Phlebovirus, and Rhabdovirus[9]

9. Second blood collection was collected 2-3 weeks later for further tests in case the primary specimens yielded negative results for etiology of encephalitis.

10. The researcher contacted the attending physician and/or the patient after obtaining the results of tests at every step. Data was recorded in case record form.

B. Retrospective Study

1. The researcher searched the King Chulalongkorn Memorial hospital database for inpatient records of encephalitic patients age ≥ 15 years old.

2. Clinical and laboratory data of the patients were reviewed by the researcher. Data was recorded in case record form. Patients with unknown etiology of encephalitis were identified.

3. Archived cerebrospinal fluid and blood samples of encephalitic patients with unknown etiology was performed. Total nucleic acid was extracted directly from CSF or serum specimens (0.2-1.0 mL) by Boom's technique using a commercial available extraction kit (bioMrieux, France). PREDICT family-wide RT-PCR assays were used to detect known or novel virus in the family enterovirus, Seadornavirus, Paramyxovirus, Arenavirus, Flavivirus, Alphavirus, Henipavirus, Phlebovirus, and Rhabdovirus[9]

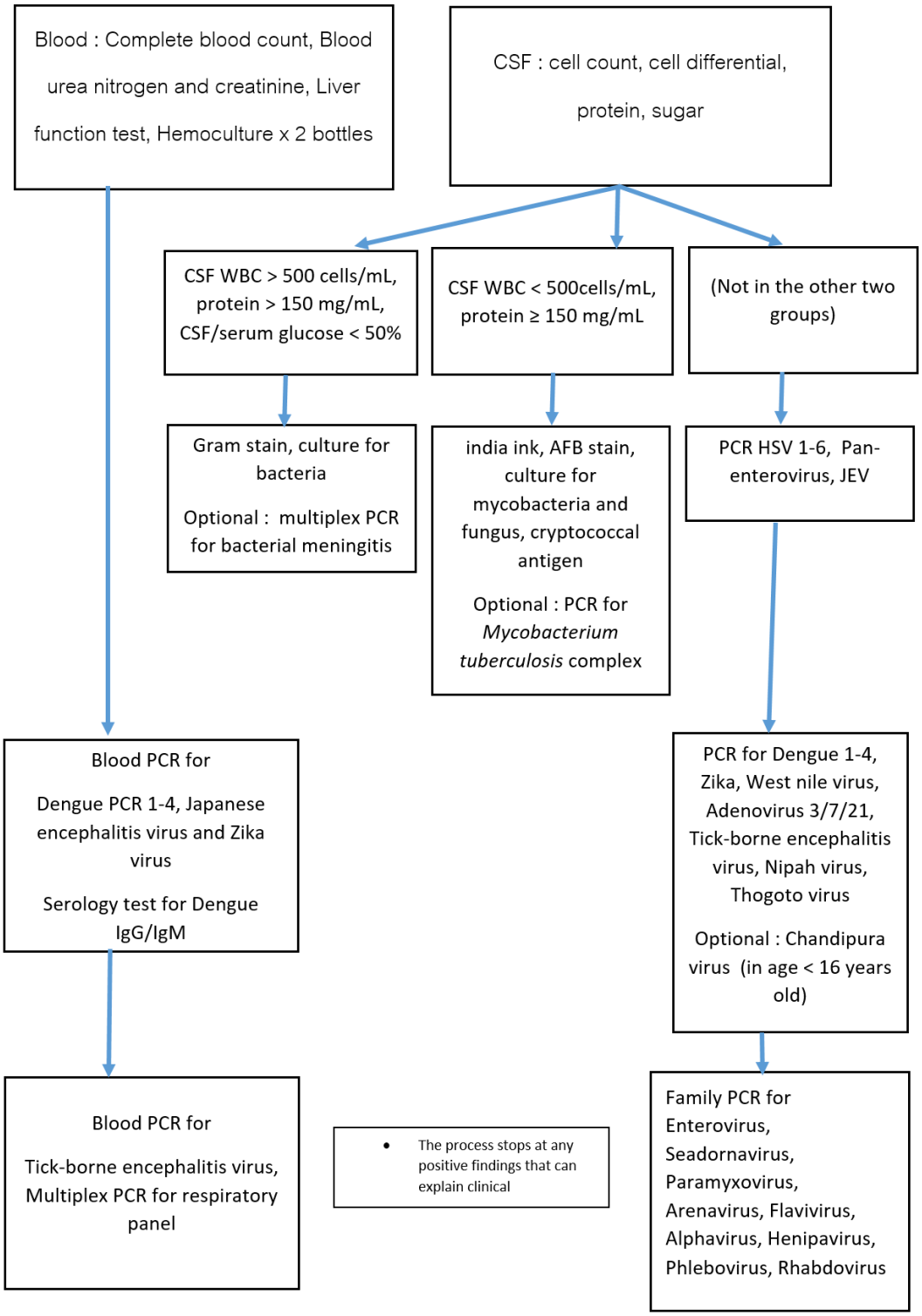

Figure 2. Prospective study algorithm of investigations; the process stops at any positive findings that can explain the clinical findings.

Statistical analysis

The data was analyzed by Statistical Package for Social Sciences (SPSS) Version 22 for Windows. Descriptive data was described as frequency and percentage. For 3-way comparison and 4-way comparison between the groups (infections: bacterial and viral, noninfectious, unknown etiology) with regards to demographic, clinical, and laboratory features. Categorical data was analyzed by the Chi-square (χ^2) or Fisher's exact test. Continuous data with normal distribution was analyzed by One-way ANOVA, data with abnormal distribution but with same distribution across categories was analyzed by Kruskall-Wallis test. All tests are 2-sided with *P* value equal or less than 0.05 as significant.

CHAPTER 4
RESULTS

Characteristics of the Study Patients

We enrolled 52 patients, including cases who met the case definition of encephalitis in 43 patients and meningoencephalitis in 9 patients.

Fifteen patientes were enrolled in prospective study, 37 patientes were enrolled in retrospective study. In retrospective unknown etiology group, archived specimen of cerebrospinal fluid and serum were available only in 10 out of 20 patients. All yieded negative result by family-wide PCR of 9 virus family (figure 3)

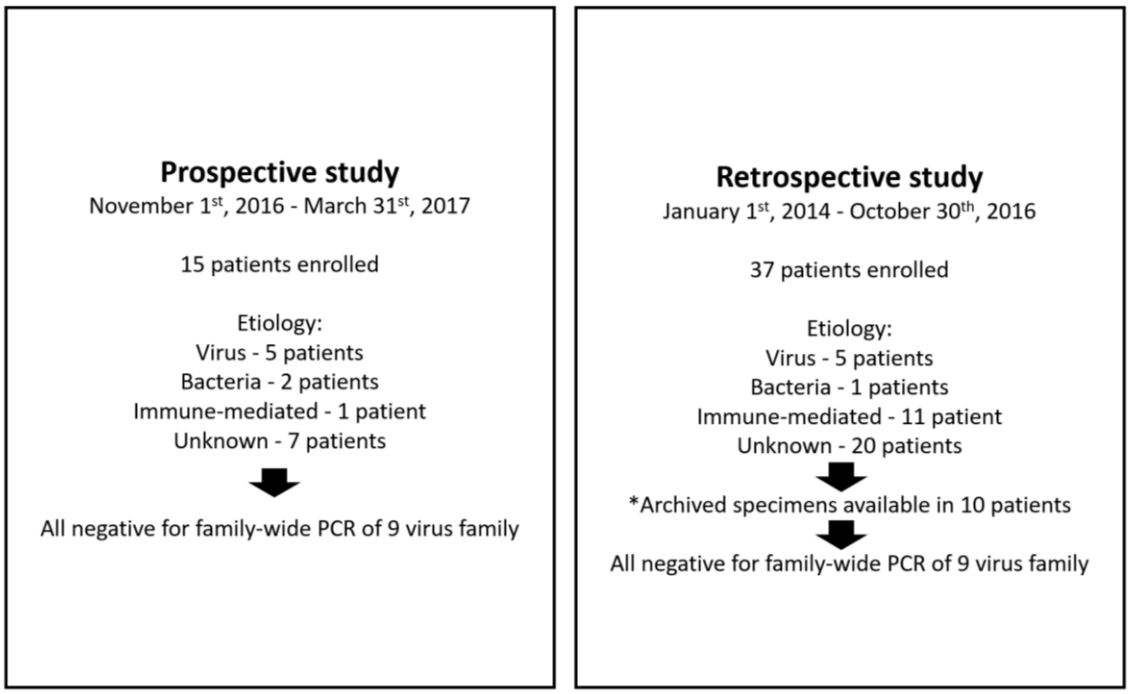

Figure 3. Summary of cases in prospective and restrospective study.

Cerebrospinal pleocytosis found for 32 patients. Neuroimaging was done in 44 patients and abnormalities were found in 30 patients. April and May had highest incidence of admission with average case per month of 7 (figure 4).

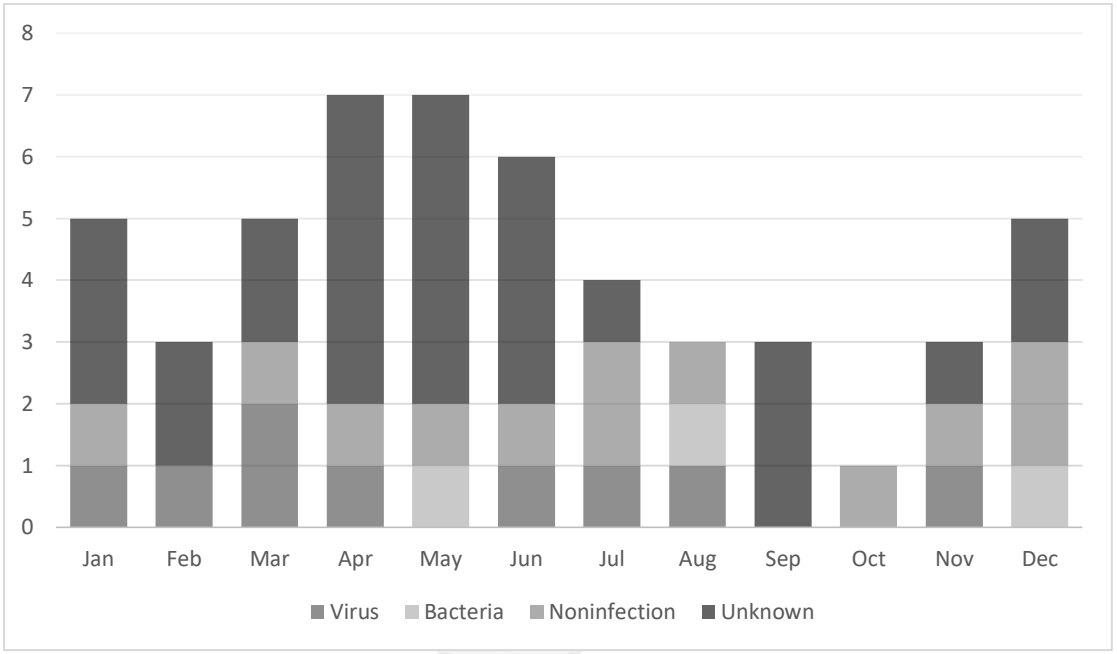

Figure 4. Month of admission, average case/month-year

Ten patients had viral etiology, 3 patients had bacterial etiology, 12 patients had noninfectious etiology, and 27 patients had unknown etiology (figure 5).

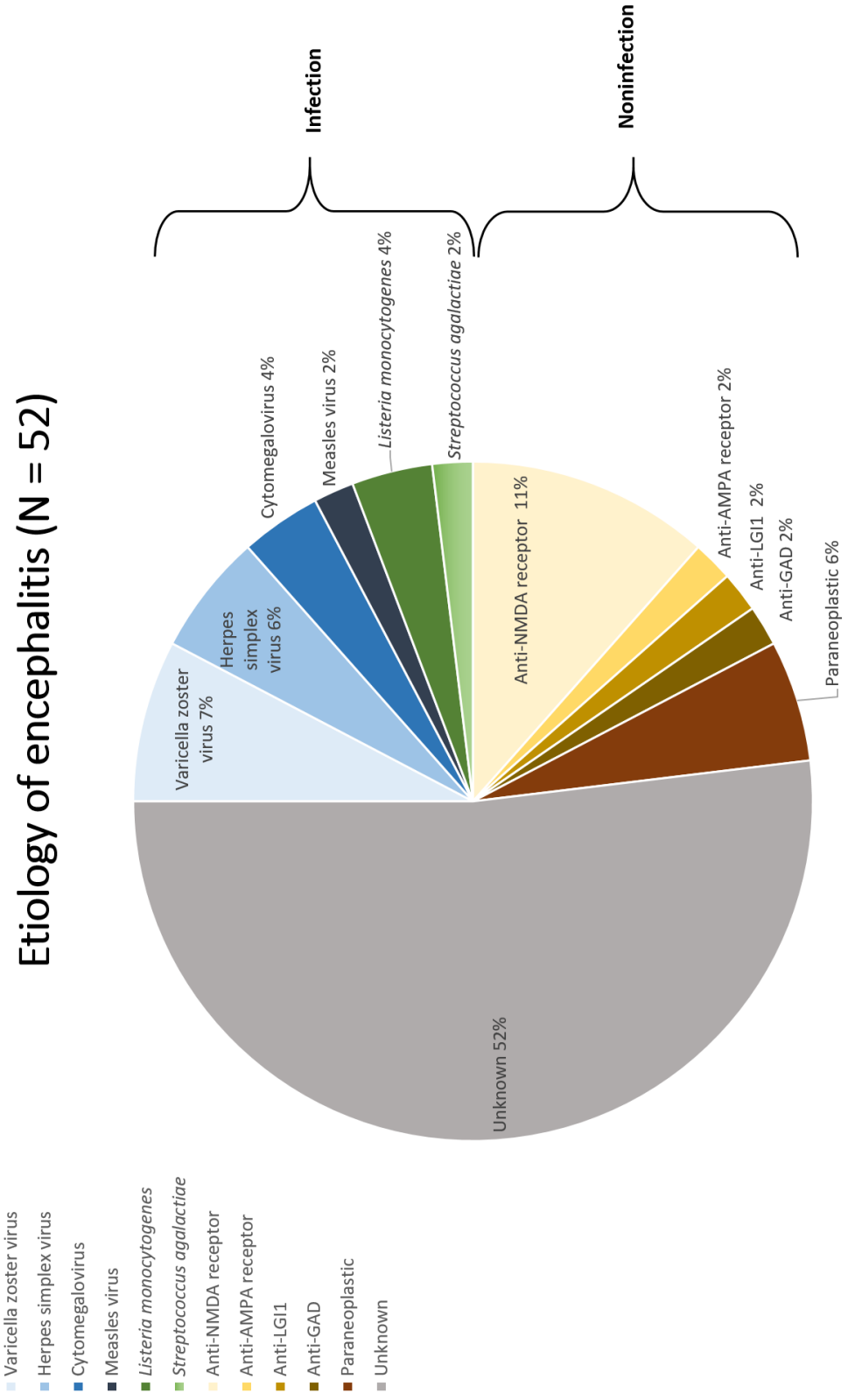

Figure 5. Etiology of encephalitis in this study.

Thirty-one (59.6%) patients were females. Forty-nine patients were Thai, one was Cambodian, one was Myanmar, and one was Indian. Median age was 51.5 (16-89) years. Twenty-four (46.2%) patients had comorbidities (table 1). The most common comorbidity was diabetes mellitus which was found in 9 (17.3%) patients, followed by solid organ malignancy in 5 (9.6%) patients, autoimmune disease in 4 (7.7%) patients, HIV infection in 4 (7.7%) patients, alcohol abuse in 3 (5.8%) patients, long-term immunosuppressive therapy (prednisolone 15 mg/day for systemic lupus erythematosous, methotrexate 5 mg/week for rheumatoid arthritis) in 2 (3.8%) patients, chronic liver disease in 1 (1.9%) patient and hematologic malignancy in 2 (3.8%) patients. Average duration of hospital stay was 26.9 (2-74) days. Prodrome symptoms (table 2) before the onset of neurological symptoms were present in 29 (55.7%) patients, with fever being the most common (24 patients, 46.1%), followed by headache in 15 (28.8%) patients. Average duration of prodrome was 8.8 (1-224) days. Duration from neuro onset to peak was 47.6 (0-1095) days. The most common neurological presentation was behavioral change in 25 (48.1%) patients, followed by psychomotor retardation in 24 (46.2%) patients, worsening headache or neck stiffness in 19 (36.5%) patients, motor weakness in 16 (30.8%) patients, seizures in 15 (28.8%) patients, sensory symptoms in 4 (7.7%) patients, hallucination in 4 (7.7%) patients, autonomic dysfunction in 3 (5.8%) patients, abnormal movement in 5 (9.6%) patients and dysphasia in 1 (1.9%) patient.

Physical neurological examination (table 3) revealed cranial nerve palsy in 13 (25.0%, 3 missing data) patients, motor weakness in 19 (36.5%, 3 missing data) patients, sensory abnormality in 4 (7.7%, 22 missing data) patients, hyperreflexia in 22 (42.3%, 4 missing data) patients and meningeal irritation sign in 9 (17.3%, 4 missing data) patients.

Table 1. Demographic characteristics of patients with encephalitis with an infectious, noninfectious, or unknown etiology.

Characteristic	Etiology group				4-group analysis *p*	3-group analysis *p*
	Infectious (13)		Noninfectious (12)	Unknown (27)		
	Bacteria (3)	Virus (10)				
Female	1 (33%)	5 (50%)	9 (75%)	17 (59%)	0.489	0.340
Age, median years (range)	52 (32-58)	57 (18-89)	55 (16-86)	49 (19-84)	0.424	0.479
Comorbidities	1 (33%)	7 (70%)	5 (42%)	11 (41%)	0.407	0.437
-Diabetes mellitus	0	4 (40%)	2 (17%)	3 (11%)	0.046	0.117
-Chronic liver diseases	0	0	0	1 (4%)	0.815	0.624
-Chronic kidney diseases	0	0	0	0	-	-
-HIV	0	3 (30%)	0	1 (4%)	0.031	0.051
-Hematologic malignancy	0	1 (10%)	1 (8%)	0 (0%)	0.411	0.324
-Solid organ malignancy	1 (33%)	0	0 (0%)	4 (15%)	0.163	0.338
-Autoimmune diseases	0	1 (10%)	2 (17%)	1 (4%)	0.514	0.374
-On long-term immunosuppressive drugs	0	0	2 (17%)	0 (0%)	0.074	0.031
-Alcohol abuse	1 (33%)	0	0	2 (7%)	0.129	0.620

Table 2. Clinical prodrome and neurological presenting symptoms of patients with encephalitis with an infectious, noninfectious, or unknown etiology.

Clinical course	Etiology group				4-group analysis *p*	3-group analysis *p*
	Infectious (13)		Noninfectious (12)	Unknown (27)		
	Bacteria (3)	Virus (10)				
Prodrome symptoms	3	7	5	14	0.277	0.174
Fever	3 (100%)	5 (50%)	2 (17%)	14 (52%)	0.044	0.055
Myalgia	1 (33%)	1 (10%)	0 (0%)	4 (15%)	0.352	0.361
Headache	2 (67%)	5 (50%)	1 (8%)	7 (26%)	0.077	0.038
Nausea/vomiting	1 (33%)	2 (20%)	0 (0%)	5 (18%)	0.349	0.226
Diarrhea	1 (33%)	2 (20%)	0 (0%)	1 (4%)	0.089	0.051
Respiratory tract symptoms	0 (0%)	0 (0%)	0 (0%)	2 (7%)	0.588	0.382
Skin rash	1 (33%)	4 (40%)	0 (0%)	1 (4%)	0.006	0.002
Average duration of prodrome to neuro-symptoms	2.33 (2-3)	5.80 (0-15)	8.5 (0-84)	10.93 (0-224)		
Median duration of prodrome to neuro-symptoms	2	5	0	1	0.455	0.286
Average duration of neuro-symptoms onset to peak	4.67 (3-6)	14.01 (0.08-60)	151.09 (0.08- 1095)	16.96 (1-120)		
Median duration of neuro symptoms onset to peak	5	6.5	23	5	0.315	0.172
Presenting symptoms						
Worsening headache/ neck stiffness	3 (33%)	5 (50%)	2 (17%)	9 (33%)	0.043	0.059
Psychomotor retardation	2 (67%)	5 (50%)	3 (25%)	14 (52%)	0.379	0.244
Behavioral change	1 (33%)	3 (30%)	8 (67%)	13 (35%)	0.357	0.200
Hallucination	0 (0%)	0 (0%)	2 (17%)	2 (7%)	0.485	0.294
Seizure	0 (0%)	2 (20%)	5 (42%)	8 (30%)	0.463	0.347
Dysphasia	1 (33%)	1 (10%)	0 (0%)	0 (0%)	0.022	0.044
Motor weakness	0 (0%)	5 (50%)	0 (0%)	11 (47%)	0.022	0.031
Sensory symptoms	1 (33%)	0 (0%)	1 (8%)	2 (7%)	0.305	0.995
Automonic symptoms	1 (33%)	1 (10%)	1 (8%)	0 (0%)	0.097	0.135
Abnormal movement	0 (0%)	2 (20%)	3 (25%)	0 (0%)	0.053	0.036

Table 3. Neurologic physical examination of patients with encephalitis with an infectious, noninfectious, or unknown etiology.

Physical exam	Etiology group				4-group analysis p	3-group analysis p
	Infectious (13)		Noninfectious (12)	Unknown (27)		
	Bacteria (3)	Virus (10)				
Meningeal irritation	2	3	1#	3ψ	0.076	0.090
signs	1	7	4#	10†	0.351	0.364
Hyperreflexia and/or						
long tract signs	1	2#	2#	8#	0.802	0.677
positive	1	5#	1	12ψ	0.131	0.068
Cranial nerve palsy	1#	0†	1‡	2β	0.305	0.995
Motor weakness						
Sensory deficit						

#, one missing data; ψ , two missing data;†, three missing data; ‡, 5 missing data; β, 13 missing data

Baseline investigations (table 4) of complete blood count (1 missing data) showed an average hemoglobin of 12.1 (8-16.1) g/dL, platelets of 246,784 (58,000-513,000) /μL and a median white blood cell count of 8,130 (1,000-32,030) cells/μL. Chemistry panel showed median creatinine of 0.78 (0.34-2.24, 1 missing data) mg/dL, and alanine aminotransferase of 27 (8-1509, 2 missing data) IU/L. Cerebrospinal fluid analysis showed median white blood cell count of 12 (0-641) cells/μL with average percentage of lymphocyte of 93.0 (6.2-100), protein of 50.5 (11-270, 2 missing data) mg/dL, and glucose of 63.5 (19-205, 2 missing data) mg/dL.

Table 4. Investigations of patients with encephalitis with an infectious, noninfectious, or unknown etiology

Investigations Median (range) except otherwise specified	Etiology group				4-group analysis p	3-group analysis p
	Infectious (13)		Noninfectious (12)	Unknown (27)		
	Bacteria (3)	Virus (10)				
Hb, g/dL (mean)	10.5 (8.4-13.6)	12.3 (8.6-15.9)	12.2 (9.1-14.4)	12.2 (8-16.1)	0.096	0.244
WBC, cells/μL	21520 (9210-32320)	7300 (1000-16650)	8160 (1870-20380)	8575 (2940-18390)	0.119	0.733
%N (mean)	77.8 (63.6-89.8)	62.7 (44.9-89.8)	70.7 (54.7-81.0)	75.0 (36.5-95.7)	0.274	0.328
Plt, /μL (mean)	227333 (125000-305000)	24020 (60000-386000)	235750 (121000-352000)	256654 (58000-513000)	0.533	0.592
Creatinine, mg/dL	0.84 (0.62-0.90)	0.82 (0.45-2.09)	0.84 (0.5-1.75)	0.71 (0.34-2.24)	0.798	0.604
ALT, IU/L	44 (42-81)	31 (10-63)	18 (11-58)	24 (0-1509)	0.196	0.248
CSF WBC, cells/ μL	278 (121-641)	6.5 (1-189)	3.5 (0-30)	17 (8-241)	0.009	0.027
CSF %Lymphocytes	45.0 (43.0-98.4)	96.5 (25.0-100.0)	100.0 (73.0-100.0)	84.0 (6.2-100.0)	0.094	0.080
Protein, mg/dL	80.0 (51.0-92.4)	69.6 (31.0-127.4)	28.8 (11-70.4)	50.0 (12.9-270.0)	0.020	0.007
Glucose, mg/dL	45 (19-69)	63.5 (40-107)	67 (51-131)	60.5 (19-205)	0.380	0.429

Of empiric antimicrobial treatments given during hospitalization (figure 6), antiviral drug was given to 27(51.9%) patients; antibacterial was given to 19(36.5%) patients (ceftriaxone to 6(11.5%), piperacillin-tazobactam to 5(9..6%), doxycycline to 5(9.6%) patients, isoniazid/rifampicin/pyrazinamide/ethambutol to 3 (5.8%) patients); anti-parasite was given to 1(1.9%) patient. At least one antimicrobial was given to 35 (67.3%) patients. Steroids was given to 22(42.3%) patients. Other treatment for encephalitis was given in 18(34.6%) patients (13 of IVIG, 8 of azathioprine, 1 of plasmapheresis, 2 of thiamine, 1 vigabatrin/biotin, 1 gabapentin/thamadol) . One case in unknown etiology group received antimicrobial (both antibacterial and antiviral drugs) drugs, steroids and other treatment for the treatment of encephalitis.

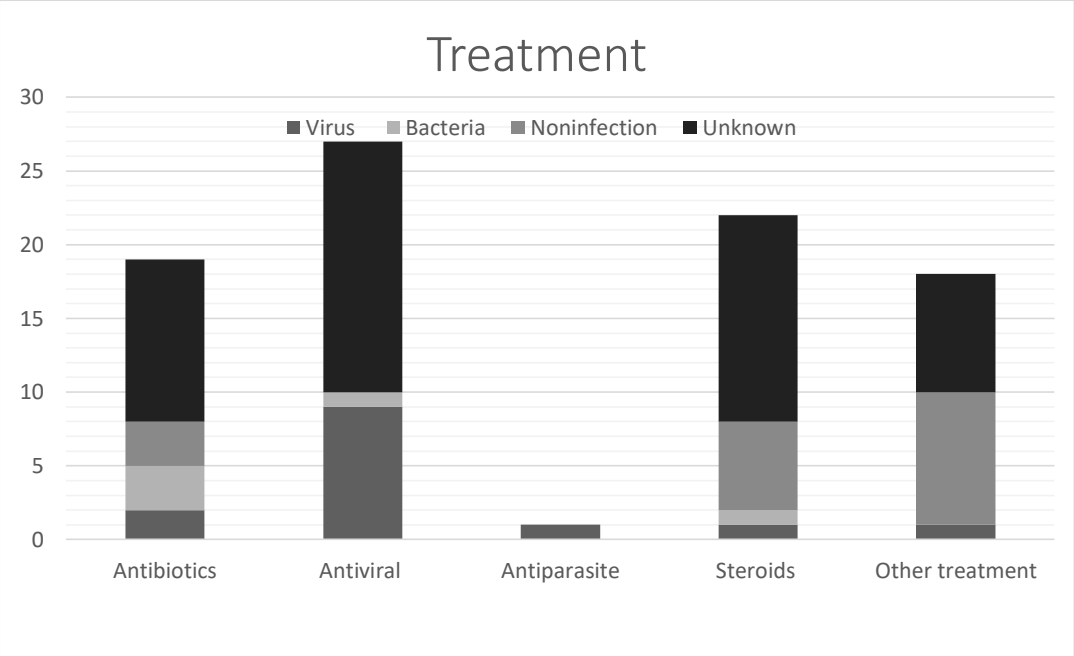

Figure 6. Treatment

All patients survived at 7 days after admission. Seven patients had full recovery within 7 days after admission. Outcome at 7 days after admission was showed (table 5 and figure 7).

Table 5. Outcome at 7 days after admission.

	Bacteria (3)	Virus (10)	Noninfection (12)	Unknown (27)
Complete recovery	1 (33.3%)	5 (50%)	0	1
Stable condition	0	0	0	1
Partial recovery	2 (66.7%)	3 (30%)	10 (83.3%)	18 (11.1%)
Clinical progression	0	2 (20%)	2 (16.7%)	7 (25.9%)

4-group analysis *p* 0.026, 3-group analysis *p* 0.008

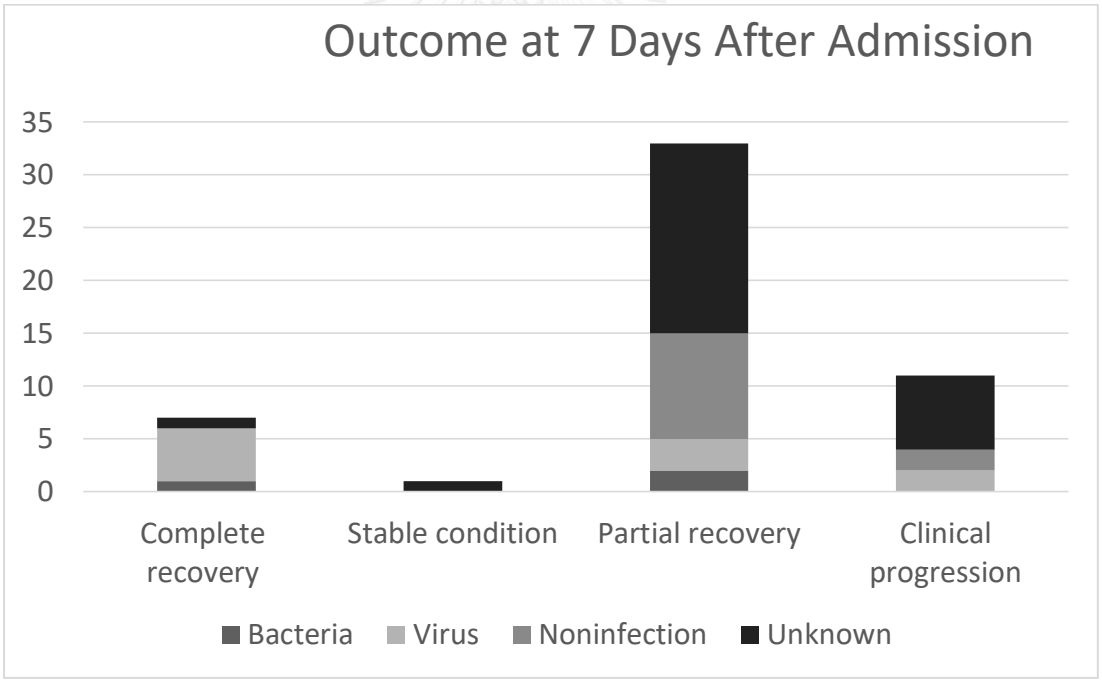

Figure 7. Outcome at 7 days after admission.

Etiology of encephalitis

1. Infectious etiology

Thirteen patients (25%) had infectious cause for encephalitis. Three patients had a bacterial etiology, and 10 patients had a viral etiology.

a) Viral agents of encephalitis (table 6)

The most frequently identified viral agent was varicella zoster virus (4 patients), followed by HSV (3 patients), CMV (2 patient) and measles (causing subacute sclerosing panencephalitis, 1 patient). All patients, except the measles case, had viral agents identified in their cerebrospinal fluid by PCR. All 10 patients had confirmed or probable viral etiology. Their ages ranged from 18 to 89 years. Four (40%) patients had skin rash (preceding varicella zoster encephalitis), 5 (50%) patients had headache, 5 (50%) patients had fever, 2 (20%) patients had nausea, 2 (20%) patients had diarrhea and one (10%) patient had myalgia. None of the patients had respiratory tract prodrome. Average duration of prodrome was 5.8 days (range 0-15) with median duration of 5 days. Five (50%) patients had worsening headache or stiffness of neck, 5 (50%) patients had psychomotor retardation, 5 (50%) had abnormal motor weakness, 3 (30%) patients had behavioral change, 2 (20%) patients had seizure, 2 (20%) patients had abnormal movement, 1 (10%) patient had dysphasia, and 1 (10%) patient had autonomic symptoms. None had hallucination nor sensory symptoms. Physical examination showed hyperreflexia and/or positive long tract signs-in 7 (70%) patients, motor weakness in 5 (50%, 1 missing data) patients, meningeal irritation in 3 (30%) patients, cranial nerve palsy in 2 (20%, 1 missing data) patients, and none showed sensory deficit (3 missing data). Average white blood cell count was 7,992 (1,000-16,650) cells/μL, hemoglobin was 12.3 (8.6-15.9) g/dL, and platelets was 240,200 (60,000-386,000) /μL. Cerebrospinal fluid analysis showed average white blood cells of 38.8 (1-189) cells/μL, percentage of lymphocytes of 80.4 (25.0-100.0), protein of 72.0 (31.0-127.4) mg/dL, and glucose of 66.6 (40-107) mg/dL.

All 4 cases of varicella encephalitis occurred in elderly patients with age range 65-89 years with 3 out of 4 cases had of diabetes mellitus. Three cases with varicella encephalitis occurred after the appearance of typical dermatomal vesicular skin lesions of herpes zoster, while one case had single vesicular lesion at buttock. One of these four cases had already completed a course of oral acyclovir treatment for herpes zoster and the dermatomal vesicular skin lesions had been crusted before neurological symptoms developed.

Two cases of cytomegalovirus encephalitis occurred in HIV patients while on antiretroviral therapy. One patient was a 55-year-old male patient with poorly controlled diabetes mellitus diagnosed with HIV infection when he presented with *Mycobacterium simiae* septicemia 7 months prior to this admission. His CD4 at that time was 14(1.25%) cells/μL. The patient had been on antiretroviral treatment. Later he was diagnosed with cytomegalovirus retinitis and received ganciclovir injection intravitreally. He presented with left upper motor neuron facial palsy, right eye visual disturbance for one month and headache with diarrhea for one week before admission. The other case was a 39-year-old male patient diagnosed with HIV infection and diffuse large B cell lymphoma stage IV BE. His CD4 at that time was 118 (4%) cells/μL and had been on antiretroviral treatment. He was treated with 4 cycles of chemotherapy (DA-EPOCH regimen). This patient presented with nausea, vomiting and diarrhea. During hospitalization, he developed alteration of consciousness and regression of behavior without focal neurological deficit. Both cases received ganciclovir after the diagnosis of cytomegalovirus encephalitis has been made. Both cases had partial recovery of their neurologic symptoms.

Of 3 cases with HSV encephalitis, two cases had HSV-1 and the other had HSV-2. The patient with HSV-2 encephalitis was co-infected with HIV, diagnosed 2 months prior to admission. His presenting symptoms were progressive right hemichorea, left hemiparesis, cranial nerve VI palsy and post chiasmatic visual disturbance of both eyes. Magnetic resonance imaging showed ill-defined non- enhancing hyperintense T2 change at left putamen. He had been treated antiretroviral drugs and

pyrimethamine/sulfadiazine but without improvement. After the diagnosis of HSV-2 encephalitis was made with the detection of HSV-2 in the CSF, intravenous acyclovir and steroids therapy were initiated. Steroids was an adjunct therapy due to suspected vasculitis. The patient had partial improvement of neurological symptoms. Two patients with HSV-1 encephalitis did not have any comorbidity. One patient was a 59-year-old female presented with fever, myalgia, headache, behavioral change and memory deficit for 6 days. The patient received intravenous acyclovir and the neurological symptoms were fully recovered. The other patient was a 32-year-old female presented with fever, headache behavioral change and seizure 12 days prior to admission. She was referred from another hospital due to status epilepticus and aphasia. The patient received intravenous acyclovir. The neurological symptoms were partially recovered.

One case with measles encephalitis was an 18-year-old man without comorbidity presented with myoclonic seizure, aphasia and autonomic nervous system abnormality for 8 months. MRI of the brain showed non-specific white matter change of multiple areas. Electroencephalogram showed characteristic periodic activity (Rademecker complex) that was compatible with subacute sclerosing panencephalitis. His serology was positive for measles immunoglobulin G > 1:500. The patient received intravenous pulsed methylprednisolone, intravenous thiamine, oral biotin and vigabatrin without any clinical improvement. The patient finally had permanent neurological deficit.

b) Bacterial Agents of Encephalitis (table 7)

Three patients were identified to have bacteria as etiologies of encephalitis. Two patients had *Listeria monocytogenes* and one patient had *Streptococcus agalactiae*. Average duration from neurological symptoms to peak was 4.67 (3-6) days with median of 5 days. Average white blood cell count was 20,920 (9,210 – 32,320) cells/μL, hemoglobin was 10.5 (8.4-13.6) g/dL, and platelets was 227,333 (125,000-305,000) /μL. Cerebrospinal fluid analysis showed average white blood cells of 346.7 (121-641) cells/μL, percentage of lymphocytes of 62.1 (43.0-98.4)%, protein of 74.5 (51.0-92.4) mg/dL, and glucose of 44.3 (19-69) mg/dL.

The first case was a 52-year-old male without comorbidity presented with headache, vomiting, fever, left upper motor neuron facial palsy and right hemianesthesia 3 days prior to admission. Magnetic resonance imaging showed the characteristics of rhombencephalitis and microabscesses. *Listeria monocytogenes* was identified from hemoculture, but not from cerebrospinal fluid. His clinical symptoms partially improved but his left hemibody numbness persisted. Empirical treatment with ceftriaxone, acyclovir and dexamethasone had been prescribed before the pathogen was identified. Later, specific treatment with ampicillin and gentamicin was given. Neurological symptoms were partially improved at day 7 after admission. The second case was a 58-year-old male with squamous cell carcinoma of the esophagus stage IIIB. The patient was undergoing concurrent chemotherapy with cisplatin and 5FU and radiotherapy for the cancer. The patient presented fever, headache, alteration of consciousness for 3 days. The physical examination showed stiffness of neck without focal neurological deficit. *Listeria monocytogenes* was identified in both hemoculture and cerebrospinal fluid culture. Ampicillin and gentamicin were given for treatment. The neurological symptoms were fully recovered. The third patient was a 32-year-old female presented with fever, diarrhea, myalgia, behavioral change, alteration of consciousness and neurogenic bladder. On admission, she had global aphasia. The physical examination showed congestive heart failure and murmur of mitral regurgitation. Computed tomography of the brain showed bilateral temporal lobe well-defined hypodensity lesions, echocardiogram showed large vegetation at mitral valve, and hemoculture showed *Streptococcus agalactiae*. The clinical symptoms were partially improved after treatment with intravenous penicillin G sodium for 6 weeks.

2. Noninfectious Etiology of Encephalitis (table 8)

Noninfectious encephalitis in this study was defined as immune-mediated encephalitis which was subcategorized into autoimmune and paraneoplastic encephalitis.

Twelve patients in this study had noninfectious encephalitis: autoimmune encephalitis in 9 patients (anti-NMDA receptor encephalitis in 6 patients, anti-AMPA receptor encephalitis in 1 patient, Anti-LG1 encephalitis in 1 patient, Anti-GAD encephalitis in 1 patient) and paraneoplastic encephalitis in 3 patients (anti-CV2 encephalitis in one patient, anti-titin encephalitis in 1 patient, anti-myelin encephalitis in 1 patient).

Two (17%) patients had prodrome of fever, 1 (8%) patient had headache. Average duration of prodrome was 8.5 (0-84) days with median duration of 0 days. Eight (67%) patients had behavioral change, 5 (42%) patients had seizures, 3 (25%) patients had psychomotor retardation, 3 (25%) patients had orobuccal dyskinesia, 2 (17%) patients had worsening headache/ neck stiffness, 2 (17%) patients had hallucination, 1 (8%) patient had sensory symptoms, and 1 (8%) patient had autonomic symptoms. Physical examination showed hyperreflexia and/or positive long tract signs in 4 (36%, 1 missing data) patients, cranial nerve palsy in 2 (18%, 1 missing data) patients, motor weakness in 1 (83%) patient, meningeal irritation signs in 1 (90%, 1 missing data) patient, and sensory deficit in 1 (14%, 5 missing data) patient. Average white blood cell count was 8,440 (1,870-20,380) cells/μL, hemoglobin was 12.2 (9.1-14.4) g/dL, and platelets was 235,750 (121,000-352,000) /μL. Cerebrospinal fluid analysis showed average white blood cells of 7 (0-30) cels/μL, percentage of lymphocytes of 95.7 (73.0-100.0), protein of 69.5 (12.9 – 270.0) mg/dL, and glucose of 73.45 (19-205) mg/dL. Nine cases received intravenous immunoglobulin, 5 cases received azathioprine, 3 cases received pulsed methylprednisolone, 2 cases received prednisolone, and one case received gabapentin and tramadol for right ear pain which had persisted for 3 months. The outcome at 7 days after admission was full recovery only in 1 patient, 8 patients partially improved, and 3 patients were disabled and became totally dependent.

One of 4 patients with anti-NMDA encephalitis was suspected to have benign teratoma by abdominal computed tomography.

Comparisons between Infectious, Noninfectious, and an Unknown Etiology Groups

Data was compared using two methods. Three-way or 3-group analysis compared among infectious, noninfectious and unknown etiology and four-way or 4-group analysis compared among bacterial, viral, noninfectious and unknown etiology. Baseline characteristics across the etiology groups were similar (table 1) except HIV infection which was significantly related with viral etiology (4-group analysis *p* 0.031, 3-group analysis *p* 0.051), diabetes mellitus which was related with viral etiology (4-group analysis *p* 0.046, 3-group analysis *p* 0.117), and history of long-term immunosuppressive therapy which was related to noninfectious etiology (4-group analysis *p* 0.074, 3-group analysis *p* 0.031). The clinical prodrome and neurological presenting symptoms (table 3) showed significant difference among groups. The prodromal symptoms of fever which was significantly less common in noninfectious etiology (4-group analysis *p* 0.044, 3-group analysis *p* 0.055), skin rash which was more common in infectious etiology especially viral etiology (4-group analysis *p* 0.006, 3-group analysis *p* 0.002), worsening headache/neck stiffness which was found less in noninfectious etiology (4-group analysis *p* 0.043, 3-group analysis *p* 0.059), dysphasia which was found only in infectious etiology (4-group analysis *p* 0.022, 3-group analysis *p* 0.044) motor weakness which was found more in viral and unknown etiology (4-group analysis *p* 0.022, 3-group analysis *p* 0.031), and abnormal movement was found in viral etiology (hemichorea) and anti-NMDA encephalitis (orobuccal dyskinesia) (4-group analysis *p* 0.053, 3-group analysis *p* 0.036). Physical examinations for the presence of meningeal irritation rigns, hyperreflexia, long tract signs, cranial nerve palsy, motor power weakness, sensory deficit did not differ among groups. Basic laboratory profiles (table 4) that differed among groups were white blood cell count from the cerebrospinal fluid which was high in bacterial etiology and low in noninfectious etiology (4-group analysis *p* 0.009, 3-group analysis *p* 0.027) and protein from the cerebrospinal fluid

which was less in noninfectious etiology (4-group analysis *p* 0.020, 3-group analysis *p* 0.007).

All patients survived at 7 days after admission, however details (table 5) such as full recovery, stable condition, partial improvement, and disabled outcomes were significantly different in both 4-group and 3-group analysis (*p* 0.026, *p* 0.008, respectively)

Table 6. Clinical and laboratory data regarding confirmed viral pathogens in case patients.

Infecting agent, patient	Age in years, sex	Clinical history				Clinical findings				CSF values			Sample with positive microbiologic results	Abnormal scan findings	Length of stay in days	Outcome at 7 days
		Duration of prodrome before neurologic symptoms in days	Fever	Neurologic symptoms	Other organ specific symptoms	Focal neurodeficit	Impaired cognitive function	Decreased level of consciousness and/or confusion	Other abnormal physical exam	WBC cells/mm^3	Protein mg/dL	Glucose mg/dL				
VZV																
1	73, F	14	-	Behavioral change, confusion	Dermotomal vesicles at Lt T4-9	-	+	+	Group of crusted ulcers at T4-9	40	75.7	107	CSF (PCR)	+	13	Fully recovered
2	80, F	7	-	Decreased level of consciousness, increased weakness of limbs from baseline	Dermtomal vesicles at Lt T3-5	+	+	+	Group of vesicles at T3-5	20	127.4	63	CSF (PCR)	+	67	Fully recovered
3	65, M	2	+	Photophobia, headache	NV, single vesicle at left buttock	-	-	-		113	46.2	64	CSF (PCR)	ND	15	Fully recovered
4	89, F	10	+	Decreased level of consciousness, confusion, increased weakness of left hemiparesis	-	+	+	+	Crusted vesicles at CN V3 dermatome	4	63.4	99	CSF (PCR)	-	6	Fully recovered

HSV																
5	59, F	4	+	Headache, behavioral change, memory decline	Myalgia	-	+	-	-	4	54.7	57	CSF (HSV-1 PCR)	+	10	Fully recovered
6	29, M	-	-	Lt hemiparesis, progressive Rt hemichorea, CN VI palsy, post chiasmatic VF defect	-	+	+	-	-	6	31	56	CSF (HSV-2 PCR)	+	18	Partially recovered
7	32, F	15		Headache, behavioral change, global aphasia, seizure	-	+	-	+	-	189	104	64	CSF (HSV-1 PCR)	+	26	Partially recovered
CMV																
8†	55, M	-	-	Headache, Left facial palsy, Rt visual disturbance	Diarrhea	+	-	+	OC, PPE	7	86	40	CSF (PCR)	+	65	Partially recovered
9	39, M	6	+	Decreased level of consciousness, regression behavior	Diarrhea, N/V	-	+	+	-	1	80.5	51	CSF (PCR)	+	69	Partially recovered
Measles§																
10	18, M	-	-	Myoclonic seizure, aphasia, autonomic involvement	-	+	+	N/A	-	4	51	65	Serum (Measles IgG > 1:5,000)	+	61	Disabled

NOTE. M, male; F, female; Lt, left; Rt, right; VZV, varicella zoster virus; HSV, herpes simplex virus; CMV, cytomegalovirus; CN, cranial nerve; VF, visual field; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; ND, not done; N/A, not applicable; OC, oral candidiasis; PPE, pruritic papular eruption; † HIV comorbidity; § probable diagnosis

Table 7. Clinical and laboratory data regarding confirmed bacterial pathogens in case patients.

Infecting agent, patient	Age in years, sex	Clinical history				Clinical findings				CSF values			Sample with positive microbiologic results	Abnormal scan findings	Length of stay in days	Outcome at 7 days
		Duration of prodrome before neurologic symptoms in days	Fever	Neurologic symptoms	Other organ specific symptoms	Focal neurodeficit	Impaired cognitive function	Decreased level of consciousness and/or confusion	Other abnormal physical exam	WBC cells/mm^3	Protein mg/dL	Glucose mg/dL				
Listeria monocytogenes																
11	52, M	2	+	Headache, facial weakness, Lt hemobody numbness	N/V	Lt UMN facial palsy, decreased PPS Lt hemibody	-	-	-	278	51	69	Blood	+	27	Partially improved
12	58, M	3	+	Headache, decreased level of consciousness	-	-	-	+	-	641	92.4	19	Blood, CSF	ND	26	Full recovery
Streptococcus agalactiae																
13	32, F	2	+	Behavioral change, decreased level of consciousness	diarrhea	-	+	+	Mitral regurgitation murmur	121	80	45	Blood	+	46	Partially improved

NOTE. M, male; F, female; Lt, left; PPS, pinprick sensation; CSF, cerebrospinal fluid; ND, not done

Table 8. Clinical and laboratory data regarding confirmed autoimmune and paraneoplastic etiology in case patients.

Condition, patient	Age in years, sex	Clinical history				Clinical findings				CSF values			Sample with positive results	Abnormal scan findings	Length of stay in days	Outcome at 7 days
		Duration of prodrome before neurologic symptoms in days	Fever	Neurologic symptoms	Other organ specific symptoms	Focal neurodeficit	Impaired cognitive function	Decreased level of consciousness and/or confusion	Other abnormal physical exam	WBC cells/mm^3	Protein mg/dL	Glucose mg/dL				
AntiNMDA receptor encephalitis																
14	30, F	-	-	Behavioral change, seizures, confusion (Benign teratoma suspected from CT whole abdomen)	-	-	+	+	-	10	20	51	CSF	ND	6	Partially improved
15	34, M	3	-	Dizziness, memory decline, decreased level of consciousness, seizures, orobuccal dyskinesia	-	-	+	+	-	14	35	70	Serum	-	74	Disabled
16	22, F	-	-	Behavioral change, decreased level of consciousness, seizures, orobuccall dyskinesia	-	+ (frontal lobe sign)	+	+	-	5	12	80	CSF	-	34	Partially improved
17	18, F	-	-	Behavioral change, confusion, orobuccal dyskinesia	-	-	+	+	-	1	26.4	64	CSF & serum	-	41	Partially improved
18	16, F	7	+	Behavioral change, confusion, visual hallucination, echolalia	Ear pain	-	+	+	Mastoiditis	1	21	57	CSF	-	9	Partially improved
19	62, F	30	+	Visual hallucination, aggressive behavior, echolalia	Ear pain	-	+	+	-	19	25	56	-	N/A	7	Disabled

Anti-AMPA receptor encephalitis 20	72, M	-	-	Behavioral change, confusion, memory decline, autonomic involvement	-	+	+	+	-	-	-	-	CSF	N/A	43	Fully recovered
Anti-LGI1 encephalitis 21	75, F	-	-	Behavioral change, seizures	-	-	-	-	-	2	11	67	CSF	ND	9	Partially improved
Anti-GAD encephalitis 22	51, F	-	-	Behavioral change	-	+ (acalculia, agraphia, apraxia)	+	-	-	-	-	-	CSF	ND	5	Disabled
Paraneoplastic encephalitis 23	59, F	-	-	Behavioral change visual hallucination, psychotic feature	-	+ (apraxia, ataxia)	+	+	-	-	-	-	Serum (antiCV2)	+	38	Partially improved
24	86, F	1	+	Decreased level of consciousness, stiff neck	-	+	-	+	-	30	70.4	61	CSF (anti-titin)	+	32	Partially improved
25	62, M	7	+	Seizures	-	-	-	+	-	-	-	-	CSF (anti-myelin)	+	5	Partially improved

CHAPTER 5
DISCUSSION AND CONCLUSION

Discussion

The results from this study corroborate the previous reports that infectious etiology of encephalitis accounts around one-fourth of all encephalitis (1). The largest study of encephalitis was done in California during 1998-2000. A total of 334 patients were enrolled, confirmed or probable viral agents of encephalitis were found in 9%, bacteria agents in 3% and parasitic agents in 1%, with possible etiology identified in 12%. In that study immune-mediated etiology was diagnosed in 10% (1). Immune-mediated encephalitis has become prevalent recently and search for autoimmune encephalitis should be performed in patients with encephalitis. In a review of 25 cross-sectional studies published between 2000-2015 immune-mediated encephalitis was accounted for 21% of all encephalitis (27). In the past, immune-mediated encephalitis had previously been assumed infectious encephalitis due to lack of appropriate investigation. In addition, the etiology of encephalitis was underdiagnosed by outdated method to identify the organism, cost and specimen handling. In most studies, infectious etiology was not extensively investigated.

The most frequently reported infectious agents of encephalitis were herpes simplex virus, varicella-zoster virus and enteroviruses. In addition, the etiology of encephalitis depends on geographical distribution; Japanese encephalitis virus was most commonly reported in Asia, tick-borne encephalitis virus in Eastern and Northern Europe/Eastern Russia, *Flavivirus* or *Alphavirus* in Northern America (27).

In Thailand, data on etiology of encephalitis was scant. The data before 2000s was derived from series or case reports. One study of pediatric patients in Bangkok between 1996 and 1998 identified viral agents in 26 patients from 40 patients (19).

Dengue virus was identified in 8 patients, Japanese encephalitis virus in 6 patients, herpes simplex virus in 4 patients, human herpes virus type 6 in 3 patients, mumps in 2 patients, enterovirus in 1 patient, varicella-zoster virus in 1 patient, and Rabies virus in 1 patient. Between 1970s and 1980s there were 1,500 to 2,500 cases of Japanese encephalitis virus reported annually(20). Routine infant vaccination for Japanese encephalitis has been introduced since 2001. During 2002 and 2008 reported cases of Japanese encephalitis to the national registry decreased four- to eight-fold from earlier decades (21). The largest study of encephalitis in Thailand was done in 2003-2005 in 5 hospitals in Bangkok and 2 hospitals in Hat-Yai (21, 22). Among 149 patients with acute encephalitis 60% were under 18 years of age and almost half met the definition of meningoencephalitis. The three most common confirmed or probable infectious agents were Japanese encephalitis virus (21 patients, 14%), enteroviruses (6 patients, 4%), and *Orientia tsutsugamushi* (6 patients, 4%).

The infectious etiology of encephalitis data in Thailand differed from other studies in Asia. A study of 127 encephalitis patients from Taiwan in 2000 and 2001 showed herpesviruses, BK virus and arboviruses as most common infectious agents(23). A study of 152 patients from India in 2007 showed enteroviruses and *Flavivirus* were more common than herpesviruses (24). While a study of 99 patients in Cambodia between 1999 and 2000 showed *Streptococcus*, BK virus, Ebstein-Barr virus and *Cryptococcus* were the common cause of encephalitis in that area (25).

A recent study in Thailand, including 103 patients with encephalitis and/or myelitis between 2010 and 2012 from a tertiary hospital in Bangkok and referral centers from 17 hospital in Thailand, identified 25 (24.3%) patients in infectious etiology (26). Among infectious agents HSV-1 was the most common, found in 6 (5.8%) patients, followed by varicella-zoster virus in 4(3.9%) patients, Japanese encephalitis virus in 3 (2.9%) patients. Immune-mediated encephalitis was identified in 25 (24%) patients.

The results from our study were similar to the last study mentioned. With an additional extensive search for pathogens by using family-wide PCR that included common viral pathogens for human encephalitis . The most common pathogens

identified were herpesviruses; varicella-zoster virus, herpes simplex virus and cytomegalovirus respectively. Enteroviruses was not found in this study. This is the evidence confirming the benefit of national routine immunization for Japanese encephalitis virus in children.

In many parts of the world, there is an increase in arbovirus infection as a leading cause of encephalitis (28), however, we did not detect acute arboviral infections in our study. The common arboviruses which are under surveillance in Thailand; Dengue 1-4, Zika, West Nile, and tick-borne encephalitis were not detected in this study. However, there is limitation for the interpretation of the results because routine serological tests for *Flavivirus* and rickettsial diseases were not included this study for all case. For patients with late presentation, PCR might have a lower yield than serology and might not detect the recent infection. We also tested for some emerging zoonotic diseases (i.e., Nipah, Chandipura, and Thogoto viruses) but the results were negative. Eventhough Nipah virus was detected in bats in Thailand (29-34) and potential vectors of Chandipura virus (35, 36) are available in Thailand, there was no report for causing a human disease yet.

For bacterial etiology, two of our patients had *Listeria monocytogenes* and both had the pathogen detected from blood culture which suggests that hemoculture should be routinely performed in acute encephalitis patient.

Immune-mediated encephalitis accounted for one-fourth of cases in this study. The clinical presentations of immune-mediated encephalitis were similar to infectious encephalitis excep fever, worsening headache/neck stiffness, rash, dysphasia, motor weakness which were presented less in immune-mediated encephalitis than other causes while orobuccal dyskinesia was found only in anti-NMDA encephalitis. This differential diagnosis should be kept in mind because the treatment is different from infectious encephalitis. In the western countries, approximately half of the anti-NMDA encephalitis patients had teratomas (37), but in the study from China, approximately 10% of patients had teratomas (38). Our study is similar to the study from China because only 1 of 6 (17%) patients with anti-NMDA encephalitis had teratoma. This may

suggest the difference in associated condition of anti-NMDA encephalitis in the western and eastern countries.

Cerebrospinal fluid profile might suggest bacterial encephalitis which had significantly higher number white blood cell than other causes while the noninfectious encephalitis had the lowest number of white blood cells and protein.

Patients with VZV encephalitis might not have active skin lesion at the onset of neurological symptoms. Baseline characteristic of HIV infection and the presence of skin rash were associated with viral infection. Dysphasia was associated with infectious etiology, abnormal movement was associated with viral etiology and anti-NMDA encephalitis, motor weakness was associated with viral and unknown etiology.

There are some limitations in our study. Firstly, the number of patients enrolled in the study did not meet the number expected. Secondly, approximately three-fourths of our cases were retrospective. Twenty-eight patients had unknown etiology encephalitis. We were able to test archived samples of cerebrospinal fluid in 17 patients in unknown etiology group and did full panel family PCR for viruses, but none were detected. For the remaining 10 cases with unknown etiology, we could not process additional tests due to unavailability of archived sample. Thirdly, serological tests for *Flavivirus* and rickettsial diseases were not included routinely in our study, as mentioned above. It is possible that some cases with rickettsial or viral etiology might be undetected. Fourthly, our institution is in the metropolitan area of Thailand. The encephalitis etiology differs by the regions so the results of this study might not be applied to other regions of Thailand.

Conclusion

Infection caused by herpesviruses was the most prevalent viral etiology, similar to studies from most developed countries. Emerging viral pathogens were not detected to cause encephalitis in this study. A quarter of patients presenting with acute encephalitis in this study had immune-mediated encephalitis. Fewer ratio of anti-NMDA encephalitis patients with teratomas than in western case series. Autoimmune and

paraneoplastic encephalitis should be kept in the differential diagnosis in patients with acute encephalitis.

REFERENCES

1. Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. Clin Infect Dis. 2003;36(6):731-42.

2. Cizman M, Jazbec J. Etiology of acute encephalitis in childhood in Slovenia. Pediatr Infect Dis J. 1993;12(11):903-8.

3. Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988-1997. Clin Infect Dis. 2002;35(2):175-82.

4. Sivertsen B, Christensen PB. Acute encephalitis. Acta Neurol Scand. 1996;93(2-3):156-9.

5. Vora NM, Holman RC, Mehal JM, Steiner CA, Blanton J, Sejvar J. Burden of encephalitis-associated hospitalizations in the United States, 1998-2010. Neurology. 2014;82(5):443-51.

6. Stahl JP, Mailles A, Dacheux L, Morand P. Epidemiology of viral encephalitis in 2011. Med Mal Infect. 2011;41(9):453-64.

7. Olival KJ, Daszak P. The ecology of emerging neurotropic viruses. J Neurovirol. 2005;11(5):441-6.

8. Arbo MJ, Fine MJ, Hanusa BH, Sefcik T, Kapoor WN. Fever of nosocomial origin: etiology, risk factors, and outcomes. Am J Med. 1993;95(5):505-12.

9. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644-55.

10. Hughes WT, Armstrong D, Bodey GP, Feld R, Mandell GL, Meyers JD, et al. From the Infectious Diseases Society of America. Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. J Infect Dis. 1990;161(3):381-96.

11. Guillaume V, Lefeuvre A, Faure C, Marianneau P, Buckland R, Lam SK, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods. 2004;120(2):229-37.

12. Johnson N, Wakeley PR, Mansfield KL, McCracken F, Haxton B, Phipps LP, et al. Assessment of a novel real-time pan-flavivirus RT-polymerase chain reaction. Vector Borne Zoonotic Dis. 2010;10(7):665-71.

13. Kumar S, Jadi RS, Anakkathil SB, Tandale BV, Mishra AC, Arankalle VA. Development and evaluation of a real-time one step reverse-transcriptase PCR for quantitation of Chandipura virus. BMC Infect Dis. 2008;8:168.

14. Lwande OW, Lutomiah J, Obanda V, Gakuya F, Mutisya J, Mulwa F, et al. Isolation of tick and mosquito-borne arboviruses from ticks sampled from livestock and wild animal hosts in Ijara District, Kenya. Vector Borne Zoonotic Dis. 2013;13(9):637-42.

15. Anthony SJ GT, Rejmanek D, et al. Laboratory Protocols for PREDICT Surveillance. Version 2. USAID; 2013.

16. Granerod J, Crowcroft NS. The epidemiology of acute encephalitis. Neuropsychol Rehabil. 2007;17(4-5):406-28.

17. Stahl JP, Mailles A. What is new about epidemiology of acute infectious encephalitis? Curr Opin Neurol. 2014;27(3):337-41.

18. Venkatesan A. Epidemiology and outcomes of acute encephalitis. Curr Opin Neurol. 2015;28(3):277-82.

19. Chokephaibulkit K, Kankirawatana P, Apintanapong S, Pongthapisit V, Yoksan S, Kositanont U, et al. Viral etiologies of encephalitis in Thai children. Pediatr Infect Dis J. 2001;20(2):216-8.

20. Chunsuttiwat S. Japanese encephalitis in Thailand. Southeast Asian J Trop Med Public Health. 1989;20(4):593-7.

21. Olsen SJ, Supawat K, Campbell AP, Anantapreecha S, Liamsuwan S, Tunlayadechanont S, et al. Japanese encephalitis virus remains an important cause of encephalitis in Thailand. Int J Infect Dis. 2010;14(10):e888-92.

22. Olsen SJ, Campbell AP, Supawat K, Liamsuwan S, Chotpitayasunondh T, Laptikulthum S, et al. Infectious causes of encephalitis and meningoencephalitis in Thailand, 2003-2005. Emerg Infect Dis. 2015;21(2):280-9.

23. Lee TC, Tsai CP, Yuan CL, Wei CY, Tsao WL, Lee RJ, et al. Encephalitis in Taiwan: a prospective hospital-based study. Jpn J Infect Dis. 2003;56(5-6):193-9.

24. Joshi R, Mishra PK, Joshi D, Santhosh SR, Parida MM, Desikan P, et al. Clinical presentation, etiology, and survival in adult acute encephalitis syndrome in rural Central India. Clin Neurol Neurosurg. 2013;115(9):1753-61.

25. Srey VH, Sadones H, Ong S, Mam M, Yim C, Sor S, et al. Etiology of encephalitis syndrome among hospitalized children and adults in Takeo, Cambodia, 1999-2000. Am J Trop Med Hyg. 2002;66(2):200-7.

26. Saraya A, Mahavihakanont A, Shuangshoti S, Sittidetboripat N, Deesudchit T, Callahan M, et al. Autoimmune causes of encephalitis syndrome in Thailand: prospective study of 103 patients. BMC Neurol. 2013;13:150.

27. Boucher A, Herrmann JL, Morand P, Buzele R, Crabol Y, Stahl JP, et al. Epidemiology of infectious encephalitis causes in 2016. Med Mal Infect. 2017;47(3):221-35.

28. Johnson RT. Acute encephalitis. Clin Infect Dis. 1996;23(2):219-24; quiz 25-6.

29. Hotez PJ, Bottazzi ME, Strych U, Chang LY, Lim YA, Goodenow MM, et al. Neglected tropical diseases among the Association of Southeast Asian Nations (ASEAN): overview and update. PLoS Negl Trop Dis. 2015;9(4):e0003575.

30. Thanapongtharm W, Linard C, Wiriyarat W, Chinsorn P, Kanchanasaka B, Xiao X, et al. Spatial characterization of colonies of the flying fox bat, a carrier of Nipah virus in Thailand. BMC Vet Res. 2015;11:81.

31. Wacharapluesadee S, Boongird K, Wanghongsa S, Ratanasetyuth N, Supavonwong P, Saengsen D, et al. A longitudinal study of the prevalence of Nipah virus in Pteropus lylei bats in Thailand: evidence for seasonal preference in disease transmission. Vector Borne Zoonotic Dis. 2010;10(2):183-90.

32. Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. J Virol Methods. 2007;141(1):97-101.

33. Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, et al. Bat Nipah virus, Thailand. Emerg Infect Dis. 2005;11(12):1949-51.

34. Wacharapluesadee S, Samseeneam P, Phermpool M, Kaewpom T, Rodpan A, Maneeorn P, et al. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. Virol J. 2016;13:53.

35. Geevarghese G, Arankalle VA, Jadi R, Kanojia PC, Joshi MV, Mishra AC. Detection of chandipura virus from sand flies in the genus Sergentomyia (Diptera: Phlebotomidae) at Karimnagar District, Andhra Pradesh, India. J Med Entomol. 2005;42(3):495-6.

36. Sudeep AB, Bondre VP, Gurav YK, Gokhale MD, Sapkal GN, Mavale MS, et al. Isolation of Chandipura virus (Vesiculovirus: Rhabdoviridae) from Sergentomyia species of sandflies from Nagpur, Maharashtra, India. Indian J Med Res. 2014;139(5):769-72.

37. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, et al. Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol. 2008;7(12):1091-8.

38. Huang X, Fan C, Wu J, Ye J, Zhan S, Song H, et al. Clinical analysis on anti-N-methyl-D-aspartate receptor encephalitis cases: Chinese experience. Int J Clin Exp Med. 2015;8(10):18927-35.

APPENDIX

Case Record Form

Case Number…….…….. Contact person ……..

Date of admission..... Length of stay .....days

1. Demographic data

Souce (1) KCMH Hospital (2) other hospital…….

Sex (1) Male (2) Female

Age (year) …….yrs……..month

Marital Status (1) single (2) married (3) divorced

Hometown (province) .....

Present address (province) .....

Occupation (1) medical/paramedical personnel(2) farmer/agriculture

(3) government officer (4) employee

(5) officer (6) business owner

(7) other.....

2. Risk Factors

Contact with animals / sick person

(1) Yes, please specify (2) No

1.1 dog 1.2 cat

1.3 cattle 1.4 pig

1.5 mosquito 1.6 sick patient (specify relationship to the patient....., type of illness……..)

1.7 others (specify.....)

Eating/contact raw food

(1) Yes, please specify (2) No

1.1 pork 1.2 beef

1.3 poultry 1.4 seafood

1.5 freshwater aquatics 1.6 other (specify)……

Travel history within 1 months

(1) Yes Place……..Duration before the event……. (2) No

3. Host status

(1) Normal (2) Immunocompromised

If Immunocompromised host

(1) DM : duration of illness ……yrs

Last HbA1C within 1 year (if available)……..%

End organ damage (specify)……..

(2) liver disease : (specify)…….. duration of illness ……yrs

(3) Chronic kidney disease (Cr ≥ 3 mg/dL) : duration of illness ……yrs

(specify cause if possible)……

Last creatinine within 1 year ……

(4) HIV : duration of illness ……yrs

Last CD4 count within 1 year……

Last viral load within 1 year……

previous opportunistic infection (specify)……

active opportunictic infection (specify)……

(5) Hematologic malignancy : (specify)…… duration of illness ……..……yrs

(6) Autoimmune disease : (specify)…….. duration of illness ……..…yrs

(7) On long term steroid/immunosuppressive : total duration……yrs

Medications / dose of last prescription……

(8) Organ transplant : organ …… duration prior to the event……yrs

(9) Solid organ malignancy : organ / stage…… Time of Dx prior to the event…….yrs

Current treatment for the malignancy

9.1 Yes, detail……

9.2 No

(10) Alcohol abuse : amount ……gram of alcohol drink/day duration …….. yrs

Direct head trauma within 1 year (1) Yes : duration before the event……..months……..days

(2) No

Head Surgery (1) Yes : duration before the event…….. months……..days

(2) No

4. Current medication

(1) Yes (2) No

please specify (drugs/dose per day)……

5. Present illness

Prodromal symptom	(1) Yes (detail below)	(2) No	
Fever	(1) Yes	(2) No	duration ..... Days
URI symtoms	(1) Yes	(2) No	duration ..... Days
Diarrhea	(1) Yes	(2) No	duration ..... Days
Nausea/vomitting	(1) Yes	(2) No	duration ..... Days
Myalgia	(1) Yes	(2) No	duration ..... Days
UTI symptom	(1) Yes	(2) No	duration ..... Days
Headache	(1) Yes	(2) No	duration ..... Days
Skin rash	(1) Yes	(2) No	duration ..... Days

Other please specify……..

Prodrome to neuro onset please specify.....day

Neurologic symptoms			
Behavioral/personality changes	(1) Yes	(2) No	
Decreased level of consciousness	(1) Yes	(2) No	
Stiff neck	(1) Yes	(2) No	
Seizures	(1) Yes	(2) No	
confusion	(1) Yes	(2) No	
dysphasia	(1) Yes	(2) No	
memory decline	(1) Yes	(2) No	
hemiparesis	(1) Yes (please specify.....)		(2) No
monoparesis	(1) Yes (please specify.....)		(2) No
paraparesis	(1) Yes (please specify.....)		(2) No
quadriparesis	(1) Yes	(2) No	
sensory symptom	(1) Yes (please specify.....)		(2) No
bowel & bladder dysfunction	(1) Yes	(2) No	
Neuro-onset to peak	please specify.....day		

Past history		
CNS infection in the past	(1) Yes	(2) No
If yes, time of illness	(1) within 1 year	(2) >1 years

6. Physical examination

vital signs BT …… degree Celcius RR …… / min

PR ……../ min BP……..mmHg

HEENT	(1) normal	(2) abnormal……..
Cardiovascular system	(1) normal	(2) abnormal……..
Respiratory system	(1) normal	(2) abnormal……..
Gastrointestinal sysmtem	(1) normal	(2) abnormal…….
Musculoskeletal system	(1) normal	(2) abnormal……..
Mucocutaneous system	(1) normal	(2) abnormal……..

Neurologic system **if abnormality presents at baseline before this illness please remark

coma status/consciousness	(1) fully alert, coherent, oriented	(2) confused, inattention
	(3) rousable	(4) aphasia
	(5) coma	
Kernig's	(1) Positive (Rt., Lt.)	(2) Negative
Brudzinski's	(1) Positive	(2) Negative
Neck stiffness	(1) Positive	(2) Negative
Deep tendon reflex	(1) normal	(2) hyperreflexia
	(3) areflexia	(4) asymmetric
		(detail.....)
Clonus	(1) Positive (Rt., Lt.)	(2) Negative
Babinski's sign	(1) extensor response (Rt., Lt., both)	
	(2) plantar response(Rt., Lt., both)	
	(3) withdraw (Rt., Lt, both)	(4) equivocal (Rt., Lt., both)
		others.....

Cranial nerve

CN II	(1) normal	(2) abnormal (Rt., Lt., both)
CN III	(1) normal	(2) abnormal (Rt., Lt., both)
CN IV	(1) normal	(2) abnormal (Rt., Lt., both)
CN VI	(1) normal	(2) abnormal (Rt., Lt., both)
CN VII	(1) normal	(2) abnormal (Rt., Lt., both)
CN VIII	(1) normal	(2) abnormal (Rt., Lt., both)

CN XII (1) normal (2) abnormal (Rt., Lt., both)

Nystagmus (1) yes (specify)…… (2) no

Muscle weakness (1) hemiparesis (2) quadriparesis

(3) paraparesis (4) radiculopathy

(5) Equivocal (6) none

Motor power grade	right	left
	upper grade……	upper grade……
	lower grade……..	lower grade……..

Muscle tone (1) normal (2) Spastic

(3) hypotonia (4) Equivocal

(5) rigidity

Sensory (1) normal (2) Abnormal (specify below)

2.1 cord level .....

2.2 hemisensory pattern…….

2.3 segment pattern……

7. LAB and Imaging

Imaging

Imaging modality (1) MRI (2) CT

(3) MRI and CT (4) None

Imaging compatible with symptoms (1) Yes (3) No

Imaging

findings/patterns……

……..

Time of imaging after neuro onset ……..hour……..days

Chest x-ray (1) Normal (2) Abnormal ……

CBC

Hemoglobin (1) normal…….. (2) Anemia (M<13 g/dL,F< 12 g/dL)……

(3) polycythemia (M > 18.5 g/dL, F > 18 g/dL)……..

WBC (1) normal…….. (2) leukopenia (<4000)……

(3) leukocytosis (>11000)……..

Type of WBC Predominate / %

(1) neutrophil ……(2) lymphocyte…….

(3) Eosinophil……(4) Monocyte……

Platelet (1) normal…… (2) thrombocytopenia (<150,000)……..

(3) thrombocytosis(>500,000)……

Blood chemistry

BUN (1) ≤20 (2) >20

Cr (1) 0-3 (2) >3

LFT (1) Normal (2) Abnormal (specify)……..

Urine

Urinary examination (1) Normal (2) Abnormal (specify) ……

CSF profiles (1) Normal (2) Abnormal

Time between neuro-onset and LP please specify……..day

Antibiotics/antifungal/antiviral before LP ……duration……days

Open pressure ……cmH2O

CSF RBC count……

CSF WBC count……..

WBC Neu%..... Lym% …… Eos%.....

other% …….

CSF protien……..

CSF glucose…….. / Serum glucose……

Pathogen iden from CSF specimen

Direct stain : Gram's stain…… AFB……..mAFB……Wright…….

India Ink……..

Serology : Cryptococcal Ag……..

Molecular : Multiplex PCR for bacterial meningitis ……..

PCR for TB ……

PCR for viruses (specify by circle, detail)……..

HSV-1, HSV-2, Adenovirus type 3/7/21, Pan-enterovirus, JEV

CMV, VZV, EBV, WNV, Dengue virus 1-4, Rabies virus, JC virus

Nipah virus, HHV6 virus, Tick-borne encephalitis Virus, Chandipura virus

HTLV-1 virus, Others……

Culture : Bacteria ……..

Fungus……..

Mycobacterium……

Encephalitis autoantibody panel: (specify)……..

Pathogen iden from Blood

Culture : Bacteria ……

Serology : Rickettsia IFA First serum…….....Second serum ……

Leptospirosis IgM First serum……Second serum ……..

Dengue IgG/IgM First Serum…… Second Serum……..

Molecular : PCR Leptispira & Rickettsia……

PCR for Dengue……

Pathogen from Respiratory specimen

Molecular : PCR for influenza……

8.Treatment

Antibiotic	(1) Yes (specify)……	(2) No
Antiviral	(1) Yes (specify)……	(2) No
Antifungal	(1) Yes (specify)……	(2) No
corticosteroid	(1) Yes (specify)……	(2) No

Other.....

9. Outcome at 7 days after admission

(1) complete recovery

(2) partial recovery

(3) disabled

(4) death

if dead 4.1 from encephalitis 4.2 from other infectious diseases

4.3 from non-infectious conditions

10. Outcome at 30 days after admission

(1) complete recovery

(2) partial recovery

(3) disabled

(4) death

if dead 4.1 from encephalitis 4.2 from other infectious diseases

4.3 from non-infectious conditions

VITA

Benjawan Skulsujirapa, M.D.

Born on Jan 5th, 1982 in Suratthani province

Education:

2006 - Doctor of Medicine (First honor), Chulalongkorn University, Bangkok

2012 - Diploma of the Thai Board of Internal Medicine

Work:

- General Practitioner

May 2006 – April 2007 Suratthani Provincial Hospital, Suratthani, Thailand

May 2007 – April 2008 Wieng-Sra Hospital, Suratthani, Thailand

May 2008 – April 2009 Kirirat Nikom Hospital, Suratthani, Thailand

- Resident in Internal Medicine

June 2009 – May 2012 King Chulalongkorn Memorial Hospital, Bangkok, Thailand

- Internist

July 2012 – April 2015 Bangkok Huahin Private Hospital, Prachuap Khirikhan, Thailand

Current:

2015 – present: Fellow in training, Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok

Publications:

1. Skulsujirapa B, Suankratay C. Vector-borne diseases. In: Vanichanan J, Putcharoen O. Infectious Diseases Across the Regions. 1st ed. Bangkok: Trithep Book Process; 2017:179-270

2. Skulsujirapa B, Paitoonpong L. Post-exposure Prophylaxis. In: Suwanpimolkul G, Jutivorakool K, Paitoonpong L, Putcharoen O, Suankratay C. Infectious Disease Emergencies. 1st ed. Bangkok: Trithep Book Process; 2016:119-171

3. Skulsujirapa B, Jongwutiwes S, Pattanawong U, Suankratay C. Cystoisospora belli infection: the first case series in Thailand. Poster session presented at: European Congress of Clinical Microbiology and Infectious Diseases, 26th Annual Conference of the European Society of Clinical Microbiology and Infectious Diseases; 2016 April 9-12; Amsterdam, Netherlands.