CHAPTER 3

RESEARCH METHODOLOGY

3.1   Research Questions and Objectives

3.1.1 Research Questions

- **Primary Research Question:**

    Was flumazenil effective in the treatment of cirrhotic patients with hepatic encephalopathy measured by the improvement of clinical stage up to Grade 0 or 1 from Grade 2, 3 or 4 in 50% of cases, compared with 10% of the controls?

- **Secondary Research Questions:**

    1) What were the clinical courses of these patients in both groups in terms of the changes of mental status (improved, no change or worsened), duration of these changes and long-term outcomes (discharge or death)?

    2) Was there any difference in the long-term clinical outcomes of patients whose clinical stages of hepatic encephalopathy had been improved by flumazenil, compared with those that had not?

3) Was there any difference of the long-term clinical outcomes of the patients in the two groups?

4) What were the factors that could predict the long-term outcomes of HE patients after flumazenil administration?

5) What were the adverse reactions of flumazenil?

3.1.2 Research Objectives

• **General Objective:**

1) To assess the therapeutic effectiveness of flumazenil in terms of the fast improvement of clinical stage in the cirrhotic patients with hepatic encephalopathy.

2) To recommend the clinical position of flumazenil in the management of HE.

• **Specific Objectives:**

3) To obtain the reaction figures of flumazenil administration.

4) To identify the clinical courses of these patients.

5) To evaluate the difference of long-term clinical outcomes of the patients whose clinical stages of

hepatic encephalopathy have been improved with flumazenil, compared with those that have not.

6) To evaluate the long-term clinical outcomes of the patients with hepatic encephalopathy, regardless of their allocation to flumazenil or placebo group.

7) To determine the factors that can predict the long-term outcomes of HE patients after flumazenil administration.

8) To identify the adverse reactions of flumazenil.

3.1.3 Research Hypothesis

$$H_0 : \quad p_T \geq p_C$$

$$H_1 : \quad p_T < p_C$$

p_T : the proportion of effective responders in treatment (flumazenil) group;

p_C : the proportion of effective responders in control (placebo) group.

3.1.4 Operational Definitions

• **Clinical Stage (Grade) of Hepatic Encephalopathy:**

The **clinical stage of HE** is evaluated mainly by the Grade of Mental Status of HE[2].

Mental status is assessed using the West Haven criteria for the grading of HE (Conn et al)[2]. Details of the grading is in Appendix 1.

- Responders of Flumazenil or Placebo:

The effective responder of flumazenil or placebo is defined as that the clinical stage (mental status) should be improved at least up to Grade 0 or 1 from Grade 2, 3 or 4 during the drug administration period and the first hour after that, and the improved mental status should maintain in the following one and half hours.

The positive responder of flumazenil or placebo is defined as that the clinical stage (mental status) should be improved at least 1 grade up from the baseline and maintain in the level which should not be worse than the baseline during the whole intensive observation period.

- Long-term Clinical Outcome:

The long-term clinical outcomes of patients with hepatic encephalopathy is defined as the discharge of that patient with regained clinical stage of HE or his or her death in the hospital.

3.2   Research Design

A randomized double-blind controlled clinical trial.

It has been accepted that the randomized controlled trial[16] is the gold standard of any efficacy or effectiveness study.

The overview of this design architecture is shown in Figure 3.1.

3.3   The Sample

3.3.1 Target Population

Cirrhotic patients with hepatic encephalopathy in Shanghai area

3.3.2 Sampled Population

Consecutive HE patients admitted in the setting, who have fulfilled the eligibility criteria during the research period

3.3.3 Setting

Digestive Disease Ward and Emergency Ward, Zhong Shan Hospital, Shanghai Medical University, Shanghai, China
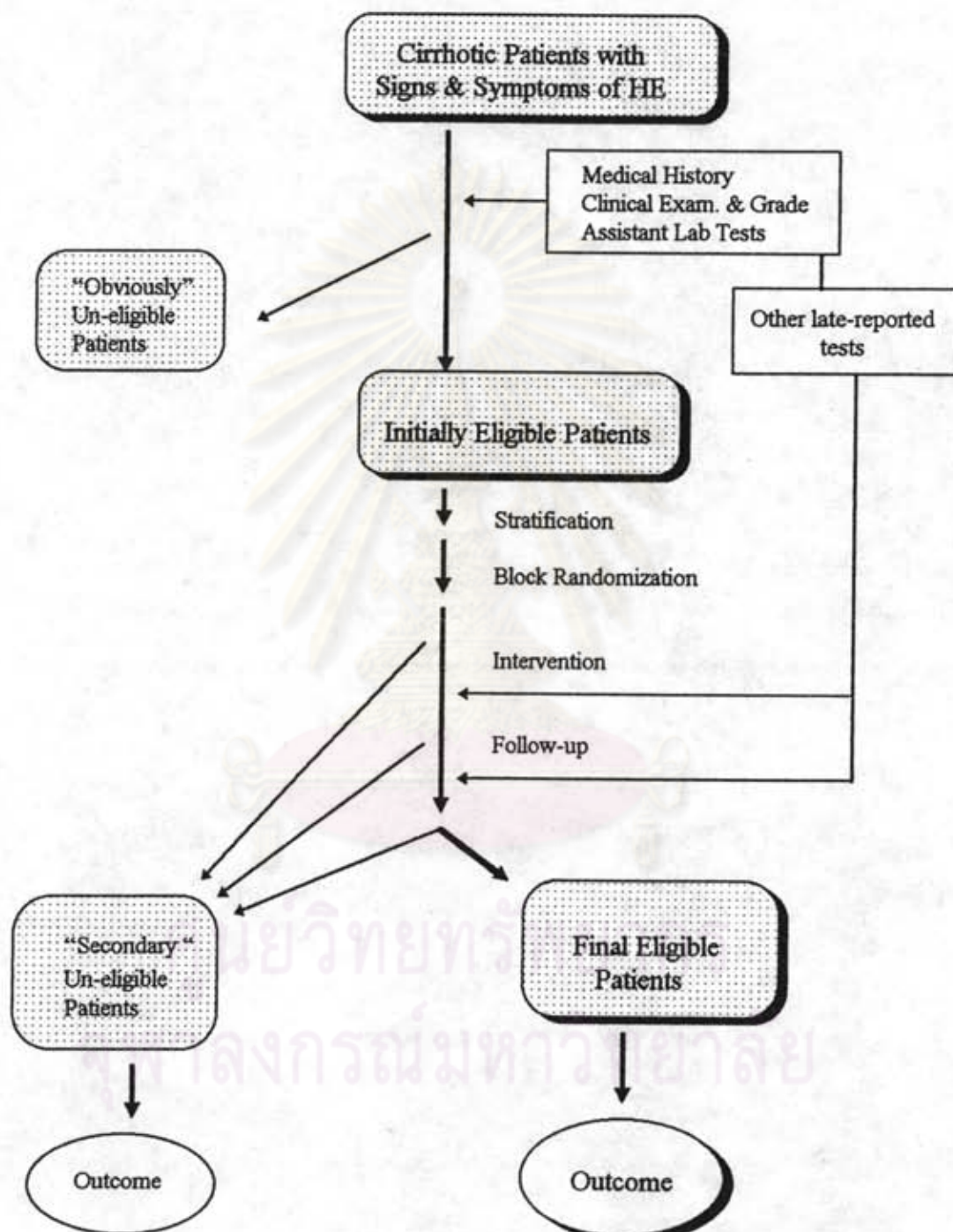
Figure 3.1   Research Design Architecture

3.3.4 Eligibility Criteria

● Inclusion criteria:

Table 3.1 Inclusion criteria

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* Patients with evidence of hepatic cirrhotic disease as shown by clinical, biochemical, image or histological abnormalities; and

* Patients who have developed the current episode of hepatic encephalopathy (clinical stage of grade 2 to 4) with clinical, biochemical, psychometric or electroencephalographic evidences for less than 3 days; and

* Patients with the age 18-65 years old.

● Exclusion criteria:

Table 3.2 Exclusion criteria

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* Ingestion of any drug for the specific treatment of HE earlier than the preceding 3 days; or

* Intake of synthetic Bzs (e.g., diazepam) in the preceding week; or

* Preexisting neurological diseases; or

* End-stage of primary or secondary hepatic cancer; or

* End-stage renal failure, as defined by serum $Cr > 5$ mg/dl and BUN > 75 mg/dl; or

* Respiratory failure, as defined by $PO_2 < 60$ mmHg, $PCO_2 > 50$ mmHg or both; or

* Hemodynamic instability, as defined by heart rate > 120/min, blood pressure $< 90/60$ mmHg or both; or

* Pregnancy; or

* Refusal of permission for patients' participation from relatives.

Justification of eligibility criteria: Cirrhotic patients who have moderate to severe HE (Grade 2 to 4), which obviously disturb their own and their family members' common life, are the eligible subjects, who are expected to acquire the benefit from the proposed treatment. Mild HE cases will not be enrolled. Because the aging problem could generally cause mental slow-action, the eligible age range is selected as less than 65. Previous specific treatment for HE and previous ingestion of Bz-like agents, which might interfere the purity of the effect of the proposed treatment should also be examined and excluded. However, since HE is a severe complication of liver diseases, practically and ethically, it is very unusual not to administer the therapies for HE after the diagnosis. Routine management is accepted. Other diseases that have clinical manifestations mimic HE should be excluded. Obviously, we can not perform this new treatment to the pregnant women and to those who or whose close family members don't agree to participate in this project.

3.3.5 Sample Size Estimation

Sample size formula[52] for comparing two proportions of two independent groups will be used, since the primary outcome is the proportion of the patients who have been improved by the studied intervention.

$$n_{grp} = \frac{\{ Z_\alpha \times \sqrt{[2 \times p \times (1-p)]} + z_\beta \times \sqrt{[p_T \times (1-p_T) + p_C \times (1-p_C)]} \}^2}{\{ p_C - p_T \}^2}$$

where, α = .05 z_α = 1.645 (one-tail)

β = .10   power=.90 Z_β = 1.28 (one-tail)

p_C = 10%   Proportion of patients whose clinical stages of HE have been effectively improved in the control (placebo) group

p_T = 50%   Proportion of patients whose clinical stages of HE have been effectively improved in the treatment (flumazenil) group

p = (p_C + p_T) / 2 = 30%

thus, n = 20.53

21 patients / each group were supposed to be recruited.

Justifications for selecting these two proportions: The proportions of patients whose clinical stages are supposed to be improved by the studied

intervention in treatment and control group and their clinical significance should be considered. In this research, the expected proportion of patients responding to flumazenil, which could only be estimated from the previous uncontrolled or controlled studies, say 40% to 80%. The regimen of the control group is not only a placebo agent but also a combination of conventional therapies for HE -- glutamates and BCAA, a part reason of which is for ethics and also the routine management of HE patients in wards. Because the glutamates and BCAA are both equally used in the two groups and the effectiveness is very slowly, the different response rates in the treatment and control group will be considered mostly as the effect of flumazenil or possibly the combination of flumazenil and glutamates and/or BCAA. To be more conservative in this study, 10% of patients in the control group are expected to be improved because of the effect of the conventional therapies (glutamates and BCAA) or the possibly spontaneous remission, though previous studies showed almost no patient responded to the placebo.

3.4   Experimental Maneuver

3.4.1 Sample Collection

The eligible patients were all patients who met the eligibility criteria from all the HE patients during the study period in the research setting, and were allocated to the treatment or control group by randomization. The baseline data were obtained immediately before the randomization and intervention. Because some laboratory data which could demonstrate the un-eligibility of certain patients were not available at the first enrollment, or some unpredictable but fatal accidence (severe GI bleeding, etc.) happened during the study period, these patients had to be excluded even intervention had been given. They were to be analyzed separately.

3.4.2 Randomization

Block randomization after stratification[53] has been introduced into this research.

Three strata were obtained by dividing the study subjects at baseline according to the clinical stage 2, 3 and 4 of HE. Each stratum contained several blocks with the size number of 2. The preparation of block

randomization was performed by the supervisor of this research and the pharmacist. The pharmacist prepared the interested drug -- flumazenil (0.5 mg/ml/ampoule × 2 ampoules) and the placebo -- normal saline (1 ml/ampoule × 2 ampoules) in the numbered sealed envelope, arranged the block, and gave the label on each ampoule according to consecutive sequence.

After the subject had been allocated into respective stratum according to their clinical stage of HE at the initial point, randomization was processed immediately before the proposed intervention.

Justification of this allocation - randomization, stratification and block[53]:

1)   Randomization is the must for any efficacious or effective trials; and block allocation is suitable for the trials with relative small sample size.

2)   HE is a complex syndrome which is influenced by many factors (such as underlying liver disease, liver function, precipitating factors, etc.). Its main clinical manifestation is the neuropsychiatric abnormalities, which are the final result of those various factors and best represent the severity of the encephalopathy.

The clinical stage of HE is not only a prognostic variable, but also a primary outcome variable, which defines whether the intervention shows effectiveness or positiveness.

3.4.3 Blindness

Double-blindness[53] was introduced.

In a trial with relative subjective assessments, double-blindness (mask to the patient and the evaluator) is also a must.

After written informed consent had been obtained from the eligible patient's relative, a nurse opened the envelope with the label number corresponding to the subject and processed the drug administration according to the already-determined procedure. The investigators was not informed about the grouping of the patient until the end of the trial.

Official breaking of the blindness in an emergency was prepared, when allergy, hemodynamic unstableness, GI bleeding or anxiety or other fatal occasions occurred.

3.4.4 Intervention and Follow-Up

The intervention was processed immediately after the randomization.

A) During the period before the proposed intervention, routine work was processed, such as to set up intravenous route, try to remove or correct any precipitating factors, to maintain the vital signs stable and to give the conventional treatment with glutamate (sodium and potassium, 20 ml × 2% in 500 ml × 5% Glucose solution, respectively) and BCAA (500 ml × 7%) regularly to both groups.

B) In the following intervention period, each patient in treatment group received 1.0 mg flumazenil in 18 ml saline solution (total 20 ml) intravenously within 5 minutes. The patient in control group was injected only 20 ml saline solution.

C) All the patients (both groups) were then continuously given BCAA (still that 500 ml × 7%) for the following 3 hours.

D) All the patients were intensively observed and evaluated (see Measurement part) on clinical

stage during the period of B) and C), which was totally 2.5 hours.

E) After that intensive observation period (2.5 hours), other therapies except flumazenil according to the clinical situations were administered optionally according to routine ward work. All the patients were proposed to be daily followed-up until discharge or death in the hospital.

F) Notes: During the trial period, the generally accepted therapeutic method for HE, lactulose, was not available, another effectiveness-proved agent, neomycin, was not applied either, because of its ototoxicity and nephrotoxicity.

3.5 Measurements

3.5.1 Variables For Selecting Eligible Subjects

Table 3.3   Variables for selecting eligible patients

- Evidence of cirrhosis:
    * clinical,
    * biochemical and/or
    * histological abnormalities
- Renal function:
    * serum BUN (<75 mg/dl)
    * serum Cr (<5 mg/dl)
- Respiratory function:
    * PO_2 (>60 mmHg)
    * PCO_2 (<50 mmHg)

- Evidence and clinical stage of HE:
    * clinical,
    * biochemical,
    * neuropsychometric and/or
    * electroencephalographic abnormalities
- Cardiovascular function:
    * BP (>90/60mmHg)
    * heart rate (<120/mins)
- History for Exclusion Criteria items A, B, C, D and H.
- Consent

3.5.2 Variables For Predicting Long-Term Outcomes

Evaluated at entry of randomization.

◊ **General subject's characteristics:**

Table 3.4   Patients' general characteristics

• Age	• Precipitating factors of HE:
• Etiology of cirrhosis:	* GI bleeding from varices,
* posthepatitic,	* increased dietary protein,
* alcoholic,	* constipation,
* etc.	* infections (peritoneal,
• Duration of HE before	pulmonary, urinary, etc.)
randomization	* abdominal paracentesis,
• Episodes of previous HE	* severe diarrhea or vomiting
• Child-Pugh scores:	* dehydration,
* ascites,	* electrolyte and/or acid-
* serum albumin,	base disturbances,
* serum bilirubin,	* hypotension,
* prothrombin time,	* hypoxia,
* encephalopathies	* surgical procedures,
• Serum transaminases	* etc.
• Hemoglobin	

◊ **Clinical stage**

Grade of mental status of HE (in Appendix 1)

◊ **The Components of PSE Sum and Index** (in Appendix 2)

◊ **Special variable for prediction**

  * Results of the interested intervention (flumazenil)

    -- Responder or Non-responder

3.5.3 Outcome Variables and Measurement

◊ **Clinical courses**

> Clinical stage (HE Grade)
>
> PSE Sum and Index
>
> Discharge
>
> Death

◊ **Adverse reaction of HE**

● **Outcome Measurement and Instruments**

◊ **For primary research question**

> * The improvement of mental status during the intensive observation period
>
> ⇒ By clinical grading, and optionally by PSE Sum and Index.

◊ **For secondary research questions**

> * The clinical courses after flumazenil administration
>
> ⇒ By clinical examinations, PSE Sum and Index.
>
> * Predictor variables for the long-term outcomes
>
> ⇒ By history-taking, laboratory tests and clinical examinations.
>
> * Adverse reactions of flumazenil
>
> ⇒ By clinical examinations.

3.6 Consideration Of Some Confounding Factors

3.6.1 Selection Bias

This is a hospital-based clinical research, which requires to recruit the eligible patients consulting to the hospital as the subjects. Therefore, those HE patients who do not come to the hospital will not be included. The subclinical cases were not included, either, for routine therapy is effective to them. However, HE is such a severe condition that almost all patients are sent by their family members or colleagues, which reflects the true severe spectrum of HE. To avoid the possible missing HE cases during the research period, all the consecutive HE patients from the out-clinic or the emergency-room were checked, among which the eligible patients were to be obtained.

3.6.2 Assessment Bias

To avoid the potential bias from the subjective assessment of the clinical stage of HE, detailed operational definitions have been prepared and the investigators have been trained.

There are some factors besides the encephalopathy itself that can influence the appearance of mental status, such as age, personality, history of intellectual

education. However it is hard to match these factors within two groups. Thereby the clinical stage of the patient had not been only evaluated according to the operational definition but also compared with his baseline stage and common daily life situation as well.

3.6.3 Contamination

Administration of flumazenil, the experimental therapy, which could only be prescribed by the investigators, had not been used for the patients in the control group during the whole research period.

- In the intervention period, only the patients in treatment group received flumazenil.

- In the follow-up period until discharge or death of the patients, the corresponding physicians of the patients had been informed not to prescribe flumazenil to any of the patients in the project.

3.6.4 Co-intervention

Co-intervention, any additional therapeutic maneuvers carried out unequally in the two groups, could occur before, during and/or after the research period.

- Before the recruitment of the subjects, some of the HE patients might had received some treatment

for HE, especially previously used Bzs, though rare, which would severely damage the therapeutic effect of flumazenil purely on HE only. Strict selection for the patients with negative special therapeutic history for HE was the main criteria.

- During the intervention period, experimental therapy combined with standard therapies, just as in the real clinical practice, was applied. Because the conventional therapies were equally distributed in both groups, the different studied primary outcome in the two groups should be contributed to the studied drug, flumazenil and to the spontaneous remission by chance.

- Co-intervention was a very likely problem in the follow-up period. Though keeping blindness could avoid the intended extra care for the patients in treatment group, it was very difficult and even impossible to make the therapies for all patients in both groups equivalently in a long period because of the different characteristics of the subjects. However, this was the daily routine work for these patients. Since it seemed the co-intervention was, in a sense, unavoidable, the long-term clinical consequences were listed only

in the secondary question and the corresponding analysis was mostly of description.

3.6.5 Compliance

In this short study period, every patient had fully complied to the "treatment" during the intensive observation period. The following daily checking for their status was one part of routine clinical work. The uncooperation of the patients was one of the aspects of HE.

3.7   Data Collection

The demographic data and clinical status at the baseline were obtained from all the eligible patients whether their relatives agreed to be enrolled or not. The data of the un-eligible HE patients were also recorded. These data would show how representative the subjects (recruited patients) were.

3.7.1 Demographic and Medical History Data

Obtained by history-taking, previous medical record and clinical examination at the baseline.

3.7.2 General Laboratory Data

By laboratory tests at baseline.

3.7.3 Clinical Stage (Mental Status)

Evaluated by one investigator (the physician) at baseline 0, 5, 10, 20, 30, 45, 60, 75, 90, 120 and 150 minutes after the commencing of the intervention, and daily at 8 am until the date of discharge or death. The investigator was blind of the drug administration (flumazenil or placebo) until the end of the research.

The clinical stage of the patient at one certain time point was evaluated according to the operational definition and compared with his baseline stage and common daily life situation as well.

3.7.4 PSE Sum and Index

The 5 components of this instrument was supposed to be performed at the following time points in the intensive observation period. In the follow-up period, mental status and asterixis have been examined daily at 8 am and NCT time, EEGs and venous ammonia concentration were supposed to be tested twice weekly until the date of discharge or death of that patient.

However, because of the unavailability of EEGs and unfeasibility to get the blood sample for ammonia tests so often, most of the data from EEGs were incomparable, some serials of ammonia concentration were

also missing. Some patients could not perform the NCT because of their clinical status (3 or 4).

Table 3.5 Timepoints of evaluation of mental status and PSE Index

Components	0	5	10	20	30	45	60	75	90	120	150 min
Mental Status	x	x	x	x	x	x	x	x	x	x	x
NCT time	x				x						x
Asterixis	x	x	x	x	x	x	x	x	x	x	x
EEGs	x	x	x	x	x	x	x	x	x	x	x
Ammonia	x				x						x

3.8 Data Analysis

Table 3.6 Primary and secondary outcome analysis

For Primary Outcome:	
⇒ The difference of proportions of patients whose clinical stages of HE have been effectively improved between the treatment and control group	*Chi-square test. If the expected frequencies are less than 5, Fisher's exact test is adopted.
For Secondary Outcomes:	
⇒ Clinical courses (changes of mental status, discharge or death)	*Descriptive and graphic statistics
⇒ Difference of the long-term outcomes of patients whose clinical stages of HE have or have not been improved by flumazenil	*Descriptive statistics
⇒ Difference of the long-term outcomes of HE patients in the treatment or control group	*Descriptive statistics
⇒ Predictor variables for long-term outcomes	*Descriptive statistics
⇒ Adverse reactions of flumazenil	*Descriptive statistics

Moreover, the timepoint of the change of the mental status has also been compared.

Because of the sample size in the whole study is quite small, let alone the size in the subgroup, it is impossible to draw any inferential conclusion from the secondary research questions. Thus, most of the analysis were descriptive. However, it has been tried to perform some analytical statistics, like Chi-square, logistic regression and etc..

3.9   Ethical Consideration

- It is ethical to perform a controlled trial because whether the drug or the treatment is of value is not well proven or the efficacy and effectiveness have not been generally accepted.

- Therapeutic safety is guaranteed because all the patients will receive standard therapies besides the placebo and the studied drug, which, in addition, has not shown any severe adverse reactions in all the previous trials.

- Written informed consent had been obtained from the relatives of the patient before commencing the study.

3.10   Limitations

- Subjective assessment of the clinical stage of HE could be a potential source of bias in this research.

- The effect of flumazenil on the long-term outcomes (survival) of HE was in the secondary question.

- Dose-reaction effect, economic view and another type of HE (fulminant hepatic failure) were not included in this study.

3.11   Expected Benefits and Applications

The treatment of HE requires attention to multiple problems and pathophysiological mechanisms. Since flumazenil can improve HE within minutes while other therapies by days, it is reasonably to suggest that given its effectiveness and the relevant clinical courses, flumazenil can be first applied to the HE patient, hoping to reverse the neuropsychiatric symptoms as quick as possible and to yield prognostic information. The potential for Bz antagonist therapy which is likely to challenge synergistically other therapies would help physicians select and plan their case management scheme, decrease the nurses' workload of the patients with bizarre and uncooperative behavior and avoid using the

sedatives to control their impaired neuropsychiatric behavior. The favorable result of the clinical management of HE is expected to be utilized for the necessary cost-effectiveness analysis to evaluate whether the benefits yielded by these available treatment resources would outweigh the cost.

3.12   Obstacles and Strategies to Solve the Problems

3.12.1 Recruitment of HE Patients

Zhong Shan Hospital is a general university-teaching hospital. The consecutive recruitment of all the HE patients in a short period of time needs the cooperation among the Department of Internal Medicine, Surgery, Emergency and Outpatient and the laboratories. It also depends on the diagnostic acumen of the corresponding doctors.

Therefore, the following strategies have been carried out before and during the research period:

- Short course or lecture on HE for the residents;
- Close contact among the Chief Residents of these departments;
- Daily preparation of the Ward of Digestive Diseases;

- Consultant group;

- Proper assistance from the administration office.

3.12.2 Standardization of Evaluation of HE

To avoid inter-observer and minimize intra-observer variability, one resident have been intensively trained to process the assessment of clinical stage of HE patients during the whole research period. However, in case of her absence, another resident of GI division who had also been trained took her work over. This happened not often. Her and his work have been examined by the consultants regularly.

The quality of lab tests for other measurement of HE have been checked routinely as the regulation.

3.12.3 Obtaining of Written Informed Consent

The Chinese patients and their relatives are not very familiar with the form of consent, especially of a written informed consent not for surgery. They might not be very willing to sign the consent immediately, which would delay or prevent the recruitment of eligible patients. Well-organized explanation of the research about the purpose, procedure, possible adverse effect (though rare) and safety guarantee have been verbally delivered to the relatives of the patients. Written

consent were obtained from most of the cases, while others gave oral consent.

3.13   Administration and Time Schedule

3.13.1 Administration

- Permission have been obtained from the Research and Ethical Committees of Zhong Shan Hospital.
- A meeting was held for the research team and the corresponding medical staff of the Departments of Internal Medicine, Surgery, Outpatient, Emergency and Laboratories to solicit their cooperation to take part in the study.
- One resident has been appropriately trained by the consultants to ascertain the accuracy and the reliability of assessment of clinical stage of HE.
- A special record form has been prepared.
- Regular monitoring the measurement has been made.

3.13.2 Time Schedule

April, 1995 to	1	2	3	4	5	6	7	8	9	10	11	12	13
April, 1996	Ap	My	Jn	Jy	Ag	Sp	Oc	Nv	Dc	Ja	Fb	Mr	Ap
Preparation	<----->												
Data collection		<-->											
Data analysis											<------------>		
Write-up												<------------>	