NAGY RESEARCH MEACRO
Middle East & Africa Contract Research Organization

Statistical Analysis Plan

Single center, Open Label, controlled Study to assess the safety & efficacy of Oral Ciprodiazole® Tablets (Ciprofolxacin/ Metronidazole) versus currently used Ciprofloxacin® Tablets & Metronidazole® tablets in pelvi-abdominal infections and following IV antibiotics in post-operative period, for pelviabdominal surgeries or acute conditions

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1 Study Objective:

1.1 Primary Objectives:

1.1.1 Primary Safety:

To compare safety of oral Ciprodiazole® tablets (Ciprofolxacin/Metronidazole) versus currently used Ciprofloxacin® tablets & Metronidazole® tablets in pelvi-abdominal infections, either non-operative or post- operative following IV antibiotics.

1.1.2 Primary efficacy:

To compare efficacy of oral Ciprodiazole® tablets (Ciprofolxacin/Metronidazole) versus currently used Ciprofloxacin® Tablets & Metronidazole® tablets in pelvi-abdominal infections, either non-operative or post- operative following IV antibiotics

1.2 Secondary Objectives:

1.2.1 Secondary Safety:

- Presence of any signs/symptoms of post-operative wound infection such as redness, fever or wound discharge.
- Presence of undesirable effects on total leukocyte count and liver enzymes (SGOT & SGPT)

1.2.2 Secondary efficacy:

- To compare the complete resolution or improvement of Pelvi-abdominal infection between ciprodiazole® versus combined treatment, based on pelvi-abdominal ultrasound and others
- To compare the days for complete healing of post-operative wounds between ciprodiazole® versus combined treatment

2 Study Design

This is a single center, open Label, controlled, interventional phase IV Study conducted in Menoufia University Hospital in Egypt to compare the safety and efficacy of oral Ciprodiazole® tablets (Ciprofloxacin/Metronidazole) versus currently used Ciprofloxacin® Tablets & Metronidazole® tablets in pelvi-abdominal infection and following IV antibiotics in post-operative period, for pelvi-abdominal surgeries or acute conditions

This study is a clinical trial that will enroll 312 patients, who had pelvi-abdominal infection and/or started IV antibiotics for 48 hours, post-operative or till be able to tolerate oral intake in post-operative period, for pelvi-abdominal surgeries. They will be divided into two

treatment groups; group A: patients are treated with Ciprodiazole® tablets (Ciprofloxacin/Metronidazole) and group B (control group): patients are treated with the currently used Ciprofloxacin® Tablets & Metronidazole® tablets, using computer-generated randomization list, generated by the Data Management department (DM) of the CRO (Nagy Research) for the 2 groups who will be enrolled in this study will agree to the release of information and sign an informed consent. Subjects included in this study are enrolled for the collection of data that reflect the care they receive under routine clinical circumstances. All provided care should be according to protocol and the local standard of medical care.

The screening and enrollment period is planned to be 12 months. Subject will start receiving the treatment for a duration not exceeding 15 days. And with post-operative cases, subject will start receiving the treatment after IV antibiotics for 48 hours or till be able to tolerate oral intake. Follow up will be for 15 days from enrolment till End of treatment.

3 Study Setting

Subjects will consent to the collection of study related information while on the study. Subjects will be informed that data will be collected about their health while they are enrolled in this study. They may choose to withdraw at any time. Subjects will be advised that data collected in this study will be provided to the Sponsor, and may be reported to regulatory authorities or published in scientific journals. In any of these events, the subjects' identities will not be revealed.

3.1 Inclusion Criteria:

Subjects meeting all of the following criteria will be considered for enrolment in the study:

1. Egyptian male and female patients aged between 18-65 years' old
2. Subjects having pelvi-abdominal infections such as and not limited to: Ulcerative colitis, Diverticulitis, Cholecystitis, and PID (e.g oophoritis and salpingo-oophoritis).
3. Subjects during post-operative period for pelvi-abdominal surgery and following IV medication with Metronidazole injection plus third generation cephalosporin.
4. Subjects who are willing to sign Informed Consent Form (ICF) and ready to comply with the protocol for the duration of the study

3.2 Exclusion Criteria:

Subjects presenting with any of the following will not be included in the study:

1. Subjects with a history of hypersensitivity to any of the active ingredients of the treatments used

2. Subjects who are receiving or received any other antibiotics during the previous two weeks, rather than IV treatment in the first post-operative 48 hours, mentioned in the protocol (Metronidazole injection plus third generation cephalosporin)
3. Subjects with Pelvi-abdominal infection and performed surgery after failure of oral antibiotics.
4. Subjects having surgeries such as colorectal surgeries, appendectomy and ovarian cystectomy
5. Subjects with any medical condition requiring the usage of the following medications:

    a. Drugs that induce microsomal liver enzymes, such as Phenytoin or Phenobarbital, may accelerate the elimination of metronidazole
    b. Drugs that decrease microsomal liver enzymes activity, such as cetrimide, may prolong the half-life and decrease plasma clearance of metronidazole.
    c. Administration of theophylline in combination with Ciprofloxacin® may lead to cardiac arrest, seizures, status epilepticus, and respiratory failure
    d. Administration of corticosteroids in combination with Ciprofloxacin® may lead to tendinitis
    e. Antacids containing magnesium and aluminum, supplements and other products containing calcium, iron or zinc as they reduce the effect of Ciprofloxacin®.
    f. Tizanidine because this may cause low blood pressure and sleepiness

6. Subjects with uncontrolled diabetes mellitus; FBG > 200 mg/ml
7. Subjects with renal impairment (S. Creatinine > 1.5 mg/dL)
8. Subjects with hepatic impairment (Child-Pugh Score B-C)
9. Subjects with liver enzymes (SGOT, SGPT) > 2 times Normal range.
10. Pregnant or breast-feeding women

4 Medicinal Products:

- Ciprodiazole ® Tablets (Ciprofloxacin hydrochloride 500 mg & Metronidazole 500 mg)

5 Endpoints:

5.1 Primary Endpoints:

5.1.1 Primary Safety:

Incidence of serious/non-serious adverse events

5.1.2 Primary Efficacy:

- Complete resolution for pelvi-abdominal infection based on pelvi-abdominal ultrasound and others and/or clinical response
- Complete healing of post-operative wounds

5.2 Secondary Endpoints:

5.2.1 Secondary Safety:

- Presence of any signs/symptoms of post-operative wound infection such as redness, fever or wound discharge.
- Change in Total leukocytes count and Liver enzymes (SGOT, SGPT), between baseline (visit 1) to End of study visit (Follow up 2 visit)

5.2.2 Secondary Efficacy:

- Describe complete resolution, improvement, failure or relapse of pelvi-abdominal infection based on pelvi-abdominal ultrasound and others and or clinical response.
- Days for complete healing of post-operative wounds after 8 days of treatment (Follow-up 1 V) & 15 days of treatment (End of study visit) between the 2 groups

6 Assessment Schedule

Subjects will be enrolled for 12 months including screening visit

- Visit 1: Screening and treatment initiation visit, Day 0
- Follow-up 1 visit: Day 8 (+/-) 3 days
- Follow-up 2 visit & End of study visit: Day 15 (+/-) 3 days

7 Study Size:

Subjects having pelvi-abdominal infection, in post-operative period of pelvi-abdominal surgeries or acute conditions will be selected from the surgical department in Menoufia University Hospital and some subjects might be referred from other departments in Menoufia University Hospital such as and not limited to; the department of Gynecology and Obstetrics. Patients will be asked to participate in the study and sign the informed consent, then screening for inclusion and exclusion criteria will be done and recorded in the patient's medical records (either enrolled or screening failure).

7.1 Determination of Sample Size:

As the primary objective is to assess the safety of oral Ciprodiazole® tablets (Ciprofolxacin /Metronidazole) versus currently used Ciprofloxacin® tablets & Metronidazole® tablets following IV antibiotics in post-operative period, for pelvi-abdominal surgeries, and based on the previous study "Comparison of Intravenous/Oral Ciprofloxacin Plus Metronidazole Versus Piperacillin / Tazobactam in the Treatment of Complicated Intra-abdominal Infections".[1] Overall clinical resolution rates were statistically superior for CIP+MET (74%) compared with PIP/TAZO (63%). Mean length of stay was 14 days for CIP1MET and 17 days for PIP/TAZO patients.

So, the following parameters are used to calculate the expected sample of the study:

- Design: non-inferiority
- Sample Size = 312 Subjects (1:1)
- Ciprodiazole® Sample Size (Experimental group) = 156 subjects
- Current used regimen Sample Size (Control group) = 156 subjects
- Confidence level 95% (α error = 5%)
- Power = 80%
- Non-Inferiority margin = 2%

7.2 Sample Size & Treatment Groups:

Total of 312 Egyptian patients who have pelvi-abdominal infection or started IV antibiotics in post-operative period, for pelvi-abdominal surgeries will be enrolled in the study and randomized, using a computer system, with a ratio of 1:1, into two categories as follows:

1. Group A will receive Ciprodiazole® tablets; 156 patients
2. Group B will receive Ciprofloxacin® Tablets & Metronidazole® tablets; 156 patients

7.3 Randomization:

Subjects will be randomized into the two treatment groups with a balanced ratio of 1:1. Randomization by block Computer-generated randomization lists will be established by Data Management department (DM) of CRO (Nagy Research). The number of patients of the two treatment groups will be balanced (ratio 1:1). (156 Ciprodiazole®: 156 Ciprofloxacin® Tablets & Metronidazole® tablets).

A document describing the procedure of constitution of the randomization lists will be stored confidentially at Nagy Research CRO

Block size: 2, 4

The Randomization list will be done using website:
(https://www.sealedenvelope.com/simple-randomiser/v1/lists)

8 Data Analysis:

8.1 Analysis populations

Analysis of all efficacy variables will be performed only for patients who completed the study without protocol violation (as per protocol).

Analysis of all safety variables will be performed for all patients (intent-to-treat population), who received even 1 dose of the treatment; either Ciprodiazole® tablets or currently used Ciprofloxacin® Tablets & Metronidazole® tablets.

8.2 Statistical methods

This section provides specifications for preparation of final Statistical Analysis Plan (SAP), which will be issued prior to database lock. Any differences compared to this statistical section should be identified and documented in final SAP. Analysis will be done using SPSS version 21.

8.3 Analyses variables

8.3.1 Descriptive Analysis:

- Descriptive analysis of the collected data. All enrolled patients will be included in the safety analysis.
- All quantitative primary and secondary end point variables will be described
- Data from primary and secondary endpoints will be summarized using appropriate summary statistics i.e. n (number of subjects), mean, Standard Deviation (SD), median and range.

8.3.2 Comparative Analysis:

- All tests will be performed on the 5% level of significance.
- Chi² test for unpaired categorical variables.
- Paired t-test to estimate the change in numerical variables throughout the study visits.
- Student t-test to estimate the comparison between the subgroups for the numerical variables.
- P-values less than 0.05 will be considered statistically significant

8.4 Main criteria

- To compare safety of oral Ciprodiazole® tablets (Ciprofolxacin/Metronidazole) versus currently used Ciprofloxacin® Tablets & Metronidazole® tablets in pelvi-abdominal infections, either non-operative or post-operative following IV antibiotics.
- To compare efficacy of oral Ciprodiazole® tablets (Ciprofolxacin/Metronidazole) versus currently used Ciprofloxacin® Tablets & Metronidazole® tablets in pelvi-abdominal infections, either non-operative or post-operative following IV antibiotics.

8.5 Other criteria

- To describe presence of any signs/symptoms of post-operative wound infection such as redness, fever or wound discharge.
- To describe presence of undesirable effects on total leukocyte count and liver enzymes (SGOT & SGPT) between baseline (visit 1) to End of study visit (Follow up 2 V)
- To compare the complete resolution, improvement, failure or relapse of Pelvi-abdominal infection between ciprodiazole® versus combined treatment, based on pelvi-abdominal ultrasound and others
- To compare the number of days for complete healing of post-operative wounds between ciprodiazole® versus combined treatment

8.6 Primary Safety analysis

A. Number of participants experiencing Serious Adverse Events (SAEs). [Time Frame: visit 1 to End of study visit].

- Frequency distribution (number & percent) of occurrence of SAEs necessitating a change or discontinuation of treatment. Chi² test will be used to determine p-value and significance between the 2 groups.

- Frequency distribution (number & percent) of type and severity of SAEs. Chi² test will be used to determine *p*-value and significance between the 2 groups.

B. Number of participants experiencing Adverse Events (AEs) leading to permanent discontinuation of the study drug. [Time Frame: visit 1 to End of study visit].

- Frequency distribution (number & percent) of causes of treatment discontinuations. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of occurrence of AEs necessitating a change or discontinuation of treatment. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of type and severity of AEs. Chi² test will be used to determine p-value and significance between the 2 groups.

8.7 Primary Efficacy analysis:

A. Complete resolution for Pelvi- abdominal infection based on pelvi-abdominal Ultrasound

- Frequency distribution (number & percent) of complete resolution for pelvi-abdominal infection. Chi² test will be used to determine p-value and significance between the 2 groups.

B. Complete healing of the post-operative wounds [Time Frame: Follow-up 1 V to End of study visit].

- Frequency distribution (number & percent) of complete healing of the post-operative wounds. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of incomplete healing of the post-operative wounds. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of no healing of the post-operative wounds. Chi² test will be used to determine p-value and significance between the 2 groups.

8.8 Secondary Safety analysis:

A. Presence of any signs/symptoms of post-operative wound infection such as redness, fever or wound discharge [Time Frame: visit 1 to End of study visit].

- Frequency distribution (number & percent) of presence of any signs/symptoms of post-operative wound infection such as redness, fever or wound discharge. Chi² test will be used to determine p-value and significance between the 2 groups.

B. Change in Total leukocytes count between baseline (visit 1) to End of study visit

- Descriptive analysis (mean, Standard Deviation, Median, Interquartile Range, Minimum and Maximum) of total leucocyte will be from baseline (visit 1) to End of study visit. Percent change will be calculated between baseline to End of study. Paired t-test will be used to determine p-value and significance between baseline and at the end of the study. Unpaired t-test will be used to determine the p-value and significance between Changes in total leucocyte between 2 groups.

C. Change in Liver enzymes (SGOT) between baseline (visit 1) to End of study visit

- Descriptive analysis (mean, Standard Deviation, Median, Interquartile Range, Minimum and Maximum) of Liver enzymes (SGOT) will be from baseline (visit 1) to End of study visit. Percent change will be calculated between baseline to End of study. Paired t-test will be used to determine p-value and significance between baseline and at the end of the study. Unpaired t-test will be used to determine the p-value and significance between Changes in Liver enzymes (SGOT) between 2 groups.

D. Change in Liver enzymes (SGPT) between baseline (visit 1) to End of study visit

- Descriptive analysis (mean, Standard Deviation, Median, Interquartile Range, Minimum and Maximum) of Liver enzymes (SGPT) will be from baseline (visit 1) to End of study visit. Percent change will be calculated between baselines to End of study. Paired t-test will be used to determine p-value and significance between baseline and at the end of the study. Unpaired t-test will be used to determine the p-value and significance between Changes in Liver enzymes (SGPT) between 2 groups.

8.9 Secondary Efficacy analysis:

A. Complete resolution, improvement, failure or relapse of Pelvi- abdominal infection

- Frequency distribution (number & percent) of complete resolution, improvement, failure or relapse for Pelvi- abdominal infection. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of improvement for Pelvi- abdominal infection. Chi² test will be used to determine p-value and significance between the 2 groups.
- Frequency distribution (number & percent) of failure for Pelvi- abdominal infection. Chi² test will be used to determine p-value and significance between the 2 groups.

- Frequency distribution (number & percent) of relapse for Pelvi- abdominal infection. Chi² test will be used to determine p-value and significance between the 2 groups.

B. Days for complete healing of post-operative wounds after 8 days of treatment (Follow up 1 visit) & 15 days of treatment (Follow up 2 visit or End of study visit)

- Descriptive analysis (mean, Standard Deviation, Median, Interquartile Range, Minimum and Maximum) of days for complete healing versus combined treatment will be after 8 days of treatment (Follow-up 1 V) & 15 days of treatment (End of study visit). Percent change will be calculated between baseline to End of study. Paired t-test will be used to determine p-value and significance between baseline and at the end of the study. Unpaired t-test will be used to determine the p-value and significance between Changes in complete healing between 2 groups.

8.10 Interim analysis

Optional

9 Results:

9.1 Demographic Data and Vital Signs

9.2 Medical history

9.3 Concomitant Medication

9.4 Primary End Point:

9.4.1 Primary Safety

9.4.1 Primary Efficacy

9.5 Secondary End Point:

9.5.1 Secondary Safety

9.5.1 Secondary Efficacy

9.1 Demographic Data and vital signs

Table 1 Demographic Data and vital signs among treatment Groups

	Group A (N=156)	Group B (N=156)	*p*-value
Gender (male), N (%)			
Age (Yrs.), mean (SD)			
BMI, mean (SD)			
Pulse, mean (SD)			
Systolic, mean (SD)			
Diastolic, mean (SD)			

9.2 Medical History

Table 2 Number and percent of medical history among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Med history 1, N (%)			
Med history 2, N (%)			
Med history 3, N (%)			

9.3 Concomitant Medication

Table 3 Number and percent of Concomitant medication among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
N			
ConMed1, N (%)			
ConMed2, N (%)			
ConMed3, N (%)			

9.4 Primary End Point

9.4.1 Primary Safety

Table 4 Number and percent of Adverse Event necessitating a change or discontinuation of treatment among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Total No of SAE, N (%)			
SAE1, N (%)			
SAE2, N (%)			
SAE3, N (%)			

Table 5 Number and percent of Serious Adverse Event among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Total No of SAE, N (%)			
SAE1, N (%)			
SAE2, N (%)			
SAE3, N (%)			

Table 6 Number and percent of causes of treatment discontinuations among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Total No of treatment discontinuations, N (%)			
Cause1, N (%)			
Cause2, N (%)			
Cause3, N (%)			

Table 7 Number and percent of Adverse Event necessitating a change or discontinuation of treatment among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Total No of AE, N (%)			
AE1, N (%)			
AE2, N (%)			
AE3, N (%)			

Table 8 Number and percent of Adverse Event among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Total No of AE, N (%)			
AE1, N (%)			
AE2, N (%)			
AE3, N (%)			

9.4.2 Primary Efficacy

Table 9 Number and percent of complete resolution for pelvi- abdominal infection among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
No of complete resolution for pelvi-abdominal infection, N (%)			
No of incomplete resolution for pelvi-abdominal infection, N (%)			

Table 10 Number and percent of complete healing of the post-operative wounds among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Complete healing, N (%)			
Incomplete healing, N (%)			
No healing, N (%)			

9.5 Secondary End Point

9.5.1 Secondary Safety:

Table 11 Number and percent of signs/symptoms of post-operative wound infection among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
No of signs/symptoms of post-operative wound infection, N (%)			
Redness, N (%)			
Fever, N (%)			
Wound discharge, N (%)			

Table 12 Percent Change in mean Total leukocytes among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Visit 1, mean ± SD			
End of study visit, mean ± SD			
% Change			
***p*-value between V1 & End of study visit**			
***p*-value between 2 groups**			

Table 13 Percent Change in mean SGOT among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Visit 1, mean ± SD			
End of study visit, mean ± SD			
% Change			
p-value between V1 & End of study visit			
p-value between 2 groups			

Table 14 Percent Change in mean SGPT among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Visit 1, mean ± SD			
End of study visit, mean ± SD			
% Change			
p-value between V1 & End of study visit			
p-value between 2 groups			

9.5.2 Secondary Efficacy:

Table 15 Number and percent of complete resolution, improvement, failure or relapse for Pelvi- abdominal infection among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Complete resolution, N (%)			
Improvement, N (%)			
Failure or relapse, N (%)			

Table 16 Percent Change in mean days for complete healing of post-operative wounds after 8 days of treatment (Follow up 1 visit) among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Visit 1, mean ± SD			
Follow up 1 visit, mean ± SD			
% Change			
p-value between V1 & Follow up 1 visit			
p-value between 2 groups			

Table 17 Percent Change in mean days for complete healing of post-operative wounds after 15 days of treatment (Follow up 2 visit or End of study visit) among treatment groups

	Group A (N=156)	Group B (N=156)	*p*-value
Visit 1, mean ± SD			
Follow up 2 visit, mean ± SD			
% Change			
p-value between V1 & Follow up 2 visit			
p-value between 2 groups			

10 Reference:

1- Cohn S, Lipsett P, Buchman T, Cheadle W, Milsom J, O'Marro S et al. Comparison of Intravenous/Oral Ciprofloxacin Plus Metronidazole Versus Piperacillin/Tazobactam in the Treatment of Complicated Intra-abdominal Infections. Annals of Surgery. 2000;232(2):254-262.