Patients and Methods

This study was reviewed and approved by IRB, ethics committee or audit department of Critical care department of the faculty of medicine, Cairo University. The study runs in concordance with international ethical standards and applicable local regulatory guidelines. The study does not have any physical, psychological, social, legal, economic, or any other anticipated risks to study's participants. The study conserves participants' privacy. Investigators are responsible for keeping the security of the data. Also, the participants' data were not used for any other purpose outside this study. Personal data (e.g. Name, Contact info) were not entered in our data entry software to conserve the participants' privacy, however, each subject got a unique identifier code.

Study Design and Setting

65 consecutive cases registered for elective off-pump coronary artery bypass grafting OPCAB were recruited from 3 cardiothoracic surgery centers in this study constrained by the following inclusion and exclusion criteria:

Inclusion criteria

- Patients undergoing elective OPCAB.
- Age group between 18 and 80 years old.

Exclusion criteria

- Patients with signicant valvular heart disease, dilated or hypertophic cardiomyopathy, NYHA III or IV, EF < 40 %, need for inotropic support or intra-aortic balloon pump before surgery
- preoperative atrial fibrillation
- creatinine clearance < 60 ml/min/1.73 m2
- hyperthyoidism and hypothyroidism (serum TSH levels above or below reference ranges respectively. It was measured only upon clinical suspicion.)
- moderate to severe COPD (Shortness of breath at own pace on the level, FEV1 < 80% of predicted, or continuous use of bronchodilators for > 2 weeks).

Study's Procedure and Data Collection

Beta-blocking agents and statins were given to all patients until the morning of surgery. Oral antiplatelets were stopped 5-7 days before surgery. Euroscore II was calculated. Venous samples for measuring NT-proBNP were collected on the day of surgery before induction. Samples were sent for analysis in at critical care department laboratories, Cairo University hospitals. No specific attempts were made to standardize the anesthetic and surgical management. After conclusion of the surgery, all patients were transferred to the intensive care unit ICU intubated and mechanically ventilated. The patients were assessed for extubation within 4-8 hours of arrival in the ICU. All patients received intravenous nitroglycerin infusions for the first 24hr unless they were hypotensive. Inotropic agents were used when the patient's mean arterial pressure was below 60 mmHg and adequate perfusion could not be achieved. Potassium deficiency was promptly treated as necessary to

maintain electrolyte balance within 4-5mEq/L. Beta-blocking agents and statins were given as soon as possible postoperatively. All samples were blindly analysed. Lab staff were blinded to the clinical conditions and dlinicians were blinded to the preoperative NTproBNP sample results.

The following data were collected :

- Full history taking and clinical examination.
- Echocardiography pre-operative.
- Labs:
    - routine pre-operative labs: CBC, coagulation profile, liver and kidney functions test
    - specific: pre-operative NTproBNP
- Calcualtion of EUROSCORE II
- Data collection to evaluate incidence of complications postoperative ICU stay and till discharge from hospital including:
    - prolonged intubation
    - ischemic stroke
    - timing, duration and dose of inotropic support
    - use of intra-aortic ballon pump
    - myocardial infarction
    - arrhythmias
    - Length of postoperative ICU and hospital stay
    - death

Lab and sample analysis methods

We used ELISA immunoassay technique that allows in vitro quantitative determination of human NTproBNP concentrations in serum, plasma and biological fluids.

Test principle

ELISA (Enzyme-Linked Imuunosorbent Assay) is based on the competitive binding enzyme immunoassay technique. The microtiter plate provided in the kit has been pre-coated with an antibody specific to NTproBNP. During the reaction, NTproBNP in the sample or standard competes with a fixed amount of biotin-labeled for sites on a precoated monoclonal antibody (Ab) specific to NTproBNP.

Excess conjugate and unbound sample or standard are washed from the plate. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme subtrate reaction is ended by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450±2 nm

Machine used for reading

ELISA SET (Tecan) comprises 3 compartments:

- ELISA plate reader (spectrophotometer)
- ELISA washer (for plate well wash)
- ELISA shaker incubator (for shaking & incubating plate wells)

Samples

EDTA samples were collected and plasma samples were stored in deep freezer till measured once.

Standard curve preparation for calculation of results

Standard was reconstituted with 1 ml of sample diluent. This produces a stock standard of 20ng/mL. The standard is allowed to rest for 15 min with gentle agitation prior to serial dilutions. The undiluted standard serves as high

standard concentration (20ng/mL) and the sample diluent serves as zero standard concentration. (Fig.11)

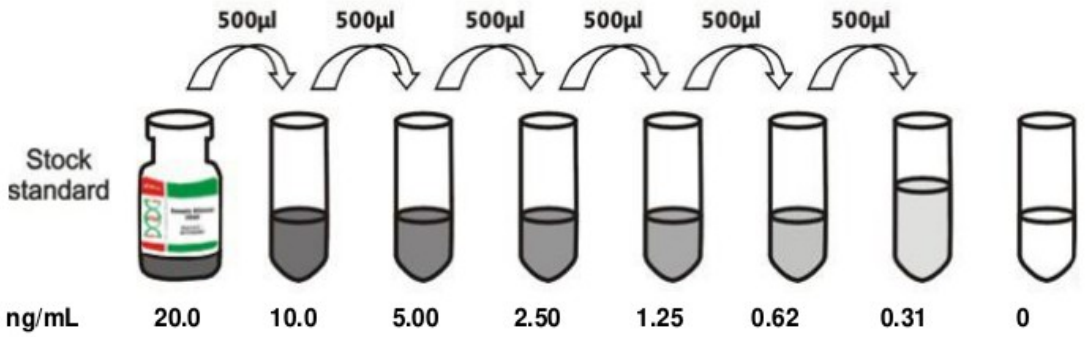

Figure 11:

A curve is plotted with serial standard dilutions log graph, plotting the mean absorbance for each standard on the X-axis against the concentration on the Y-axis and draw a best fit curve through the points on the graph. (Table5 & Fig.12)

Table 5: optical density fo r standard dilutions

Concentration ng/mL	20	10	5	2.5	1.25	0.625	0.312	0
OD(absorbance)	2.622	2.36	1.63	1.048	0.658	0.4	0.258	0.06

Calculation of results

The concentration of NTproBNP in the samples is then determined by plotting the OD (optical density) of the samples on the standard curve.

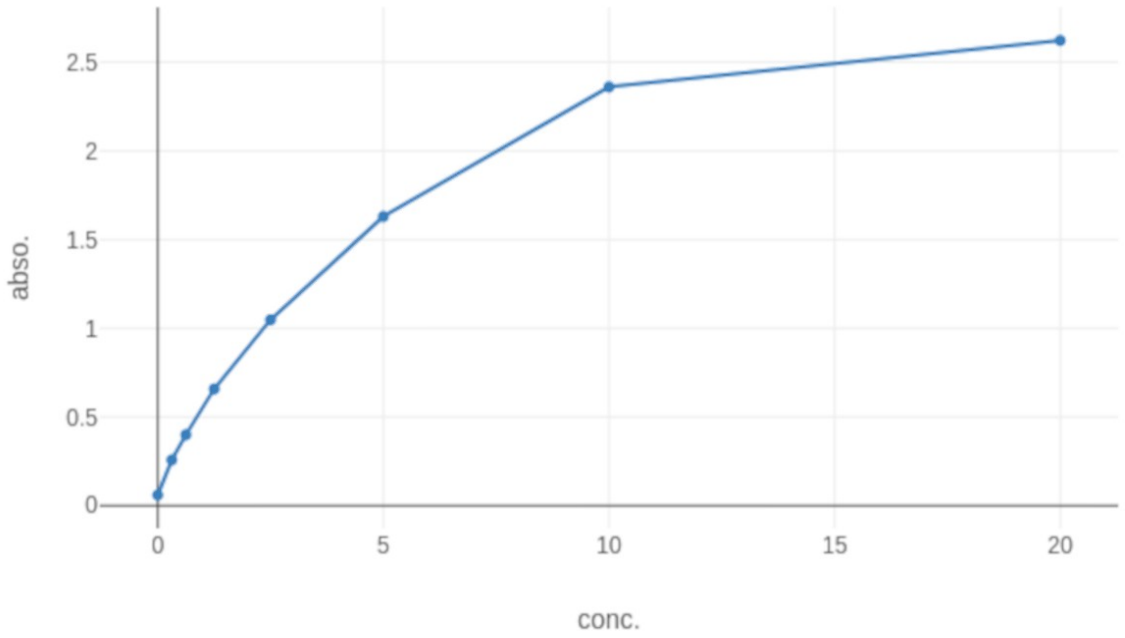

Figure 12: standard curve for calculation of NTproBNP results,.

range 0312-20 ng/mL

Study's Outcomes

Primary outcomes:

- low output heart failure (inotropic support at second post-operative day, adrenaline > 50ng/kg/min or dobutamine > 10mcg/kg/minat any time and/or need for intra-aortic balloon pump)

Secondary outcome parameters:

- mortality
- arrhythmias
- perioperative myocardial Infarction
- length of ICU
- length of postoperative hospital stay
- prolonged intubation (Intubation more than 24 hours postoperatively and/or reintubation following planned extubation).

Data Analysis and Statistical Methods

An Excel spreadsheet was established for the entry of data. We used validation checks on numerical variables and option-based data entry method for categorical variables to reduce potential errors. Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum, maximum and interquartile ranage in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test . Correlations between quantitative variables were done using Spearman correlation coefficient . ROC curve was constructed with area under curve analysis performed to detect best cutoff value of NTproBNP for detection of outcomes. P-values less than 0.05 were considered as statistically significant.