بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

Diagnostic Accuracy of Brain Natriuretic Peptide (pro BNP) in Diagnosis of Heart Failure in Khartoum State

الدقة التشخصية للببتيد الدماغى فى تشخيص الفشل القلبي بولاية الخرطوم

A dissertation submitted in a partial fulfillment for the requirement of the master degree in Clinical Chemistry.

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قال تعالى: (قُلْ هَٰذِهِ سَبِيلِي أَدْعُو إِلَى اللَّهِ عَلَىٰ بَصِيرَةٍ أَنَا وَمَنِ اتَّبَعَنِي وَسُبْحَانَ اللَّهِ وَمَا أَنَا مِنَ الْمُشْرِكِينَ).

صدق الله العظيم

سورة يوسف (الآية: 108)

Dedication

Lovingly dedicated this thesis to

My parents

Who have always loved me

Unconditionally and whose good examples have taught me to work

Hard for

The things that I aspire to achieve.

To my lovely husband

Dr. *Yagoub Marzoug* (consultant cardiologist Alshaab teatching hospital)

Who has been a constant

Source of support and encouragement during the challenges of life.

To my lovely kids

I am truly thankful for having them in my life.

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Abstract

Heart failure is a complex , clinical syndrome of signs and symptoms that are caused by defect in cardiac structure, function or both which lead to impairment of peripheral circulation and organ oxygenation . so heart failure affecting mllions of patients world wide and lead to increasing mortality rates among patients .This study was conducted to measure serum level of pro- BNP and evaluate its role in the diagnosis of heart failure in Sudanese patients .50 blood samples were collected from patients during period from August to December 2017, chosen randomly from healthy insurance – cardiac center in Elyitona hospital in khartoum state. And 50 individuals as control group to evaluate and estimate serum pro BNP in heart failure patients . Electrochemiluminescence Immunoassay method used to s estimate serum NT –pro BNP concentration, and results were analyzed using statistical package for social science (SPSS) and Minitab and Ecxel computer programs .

The study showed that the mean of NT-pro BNP was significantly increase (P.Value 0.000) for heart failure patients .

Mean± SD for cases versus control :

(4936±7.5 versus 227± 8.6) pg\ml

The study also showed there was significant positive correlation between BNP and NYHA classes (P.Value =0.000 , r=0.618).

Also the study showed there was a negative correlation between BNP and ejection fraction and left atrium size (P.Value =0.012 r=- 0.439) and (P.Value =0.001 r= - 0 . 45) respectively .

The study showed there was no correlation between BNP and age (P,Value=0.309 r =0.145).

It is concluded that the serum NT –pro BNP was significantly increased in heart failure patient , also significant correlation between BNP and NYHA classes .

مستخلص الدراسة

الفشل القلبى او اعتلال عضلة القلب هو متلازمة معقدة من الاعراض والعلامات تحدث بسبب خلل فى وظيفة او اجزاء القلب واكثرها تاثرا العضلة القلبية او الاتنين معا مما يؤدى الى عجز او قصور فى الدورة الدموية الطرفية والامداد العضوى بالاكسجين هذه الدراسة اجريت لتقدير وتقييم فائدة قياس الببتيد الدماغى في تشخيص قصور القلب في مرضى السودان. تم جمع 50 عينة دم من المرضى خلال الفترة من أغسطس إلى ديسمبر 2017 ، تم اختيارها بشكل عشوائي من مركز القلب في مستشفى الزيتونة التخصصى في ولاية الخرطوم. و 50 فردا كمجموعة مراقبة لتقييم وتقدير الببتيدالدماغى في مرضى فشل القلب.تم قياس تركيز الببتيد المناعى بواسطة طريقة التحليل المناعي الكهربية ، وتم تحليل النتائج باستخدام حزمة إحصائية للعلوم الاجتماع وبرامج كمبيوتر مني تاب واكسل.

وأظهرت الدراسة أن متوسط الببتيدالدماغى كان يزيد بشكل ملحوظ مقارنة بمجموعة التحكم وكان الاحتمال الاحصائئ للمقارنة(0.000) لمرضى قصور القلب.

المتوسط ±الانحراف المعيارى :

(4936 ± 7.5 مقابل 227± 8.6)بيكوجرام \ديسيلتر.

كما أظهرت الدراسة وجود ارتباط إيجابي كبير بين فئتي الببتيد الدماغى ودرجات قصور القلب حسب تقسيم الجمعية الامريكية (مستوى المعنوية0.000 = ، معامل بيرسون0.618 =).

كما أظهرت الدراسة وجود ارتباط سلبي بين الببتيد الدماغى وجزء الإخراج وحجم الأذين الأيسر (مستوى المعنوية -= 0.012 -0.439= معامل بيرسون) و (مستوى المعنوية 0.001 = معامل بيرسون45 .0 - =) على التوالي.

أظهرت الدراسة أنه لا يوجد ارتباط بين الببتيد الدماغى والعمر (مستوى المعنوية 0.309 = معامل بيرسون 0.145 =).

وخلص إلى أن الببتيد الدماغى زاد بشكل كبير في مريض قصور القلب ، وكذلك ارتباط كبير بين تركيز الببتيد الدماغى ودرجات فشل القلب حسب تقسيم الجمعية الامريكية .

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LIST OF ABBREVIATIONS

BNP	Brain Natriuretic peptide
HF	Heart failure
NYHA	New York Heart Association
EF	Ejection Fraction
LA	Left Atrium
CHF	Congestive Heart Failure
ACS	Acute Coronary Syndrome

Chapter One

Introduction

Rationale

Objectives

1 INTRODUCTION and Rationale

1.1 INTRODUCTION

Heart failure is a chronic condition affecting millions of patient world wide' heart failure is a clinical syndrome that characterized by typical symptoms that may be accompanied by signs caused by a structural and or functional cardiac abnormality , resulting in reduced cardiac output and or elevated intracardiac pressures at rest or during stress .(Krumholz ,2009).

Reasons of heart failure is most commonly by damage to the heart muscle potentially as the result of a heart attack or cardiomyopathy (disease of heart)(.Heidenrich ,2013).

The heart secrete natriuretic peptide as hemostatic signal to maintain stable blood pressure and plasma volume and to prevent excess salt and water retention..(Cowie , 2013)

BNP primarily is secreted by ventricles in the heart as response to left ventricles stretching or wall tension . cardic myocyte secrete BNP precursor that is synthesized into pro BNP which consist of 108 amino acids .after its secretion pro BNP is cleaved into active terminal portion and inactive N- terminal(NT-proBNP) portion. (De bold *et al* .,1998)

There is no agreed-upon first line test for diagnose of heart failure and no simple method for New folder measuring the adequacy of cardiac out put in relation to normal level of activity, this why get BNP tested to help detect, diagnose and evaluate the severity of heart failure.BNP tested when we have symptoms of heart failure such as shortness of breath and fatigue, or when are being treated for CHF in method.

1.2 Rationale:-

Heart failure is a chronic condition affecting millions of patient worldwide, and despite the treatment available hospitalization and mortality rates remain high Heart failure is the major cause of hospitalization for patient over 65 years old. The number of patients is expected to increase by 46% between 2012-2030 and the related cost will double.

Echocardiography is the gold standard for diagnosis heart failure, but its disadvantages include high cost and ineffectiveness in patient who are obese or have concomitant chronic lung diseases with respiratory distress and may be hard to come by in some communities. Therefore biochemical markers have become useful and essential clinical tools for the easy and accurate diagnosis and prognosis of heart failure by clearly determine its incidence and stages. Also the measurement of BNP taken along with conventional clinicians in deciding treatment .recently there was no powered study performed to use BNP as diagnostic tool for heart failure in Sudan.

1.3 Objectives:-

1.3.1 General objective:-

To estimate Brain natriuretic peptides in Sudanese patients with heart failure.

1.3.2 Specific objective:-

1. To measure and compare mean concentration of BNP level in patients and control group.
2. To correlate between BNP level and age, ejection fraction, LA size and degree of breathing (NYHA classes).
3. To evaluate sensitivity and specificity of BNP in diagnosis of heart failure.
4. To calculate AUC of BNP.

Chapter Two

Literature review

2 Literature review

2-1 heart failure:-

2-1 -1Definition of Heart Failure:-

Heart failure (HF) is a complex, clinical syndrome of signs and symptoms that are caused by defects in cardiac structure, function or both Resulting in impairment of peripheral circulation and organ oxygenation. It's sometimes called congestive heart failure, although this name is not widely used nowadays. (Erica , 2016) .

Heart failure does not mean heart is stopped working it just needs some support to help it work better. It can occur at any age but is most common in older people. The heart cannot keep up with its workload .when this happens, there is not enough oxygenated blood reaching the brain and muscle and fluid begins to backup in the other tissues. The lack of oxygen causes the main symptoms of heart failure such as fatigue, shortness of breathing, and difficulty completing tasks that required exertion. (Mimi Guarne,2016)

2-1 -2 Causes of heart failure:-

The cause of heart failure is a weakened or Thickened cardiac muscle .when risk factor for heart failure are present ,there usually is inflammatory stress ,which further damages the cardiac muscle depleting cell of energy and antioxidants. (john *et al* .,2016)

Conditions that can lead to heart failure include:-

Coronary heart disease: - where the arteries that supply blood to the heart become clogged up with fatty substances (atherosclerosis), which may cause angina (chest pain) or heart attack.

High blood pressure :- this can put extra strain on the heart , which over time can lead to heart failure

Cardiomyopathy:- conditions affecting the heart muscle, where the walls of the heart chambers have become stretched ,thickened or stiff. This affects the heart ability to pump blood around the body .

Heart rhythm problems (**arrhythmias)** such as a trial fibrillation, this is the most common type , where the heart beats irregularly and faster than normal.

Congenital heart disease: - is a general term for a range of birth defect that affects the normal working of the heart

heart valves problems

Other diseases: - Chronic diseases such as diabetes, HIV, Hyperthyroidism, hypothyroidism, or build up of iron (hemochromatosis), or protein (amyloidosis), high pressure in the lungs (pulmonary hypertension) can also lead to heart failure. (vasan, 2017)

2-1 -3 Risk factors of Heart Failure:-

A single risk factor may be enough to cause heart failure , but a combination of factors also increases the risk. Risk factor include:-

High blood pressure : the heart work harder than it has to if the blood pressure is high **.**

Heart attack : is a form of coronary disease that occur suddenly .the damage occur to the heart muscle lead to defect in pumping of heart .

Coronary artery disease :- narrowed arteries limit the heart supply of oxygen-rich blood ,resulting in weakened heart muscle.

Diabetes *:* having diabetes increases risk of high pressure and coronary artery disease.

Some diabetes medications :- some drugs like Avandia and Actos have been found to increase the risk of heart failure in some people , should discuss with doctors whether needing to make any changes.

Sleep apnea: - the inability to breathe properly while sleep at night results in low blood oxygen levels and increased risk of abnormal heart rhythms. both of these problems can weaken the heart .

Congenital heart defect: - some people who develop heart failure were born with structural heart defects.

Alcohol use:-drinking too much alcohol can weaken heart muscle and lead to heart failure.

Obesity:- people who are obese have higher risk of developing heart failure.

Irregular heartbeats: - these abnormal rhythms, especially if are very frequent and fast, can weaken the heart muscle and cause heart failure.

There are other less recognized risk factors such as nutrient deficiencies ,unhealthy diet(low in antioxidant vegetables and high in animal fats), stress ,lack of exercise.

(all of previous contribute to coronary artery disease which is major risk factor for heart failure. (Mimi Guarneri, 2016)

2- 1-4 Type of Heart Failure:-

Heart failure has two types:

systolic and Diastolic heart failure(These classification depend mainly on pathology of heart or contraction of it) Normally heart have four chambers through which blood pumps, newly oxygenated blood is pumped from the lung to the left atrium and left ventricle and out through the aorta to circulate through the rest of the body. After the oxygen has been used, the blood returns through the veins to the right atrium and right ventricle in to lungs to be re-oxygenated.(Yancy, 2013)

2-1-4-1: Systolic heart failure (left- sided heart failure) when the heart loses strength on the left side and cannot pump the blood into circulation ,it is called systolic heart failure when this occur ,the heart becomes dilated and weak. The strength of the heart muscle can be measured with an echocardiogram that measures the ejection fraction(refers to the amount, or percentage, of blood that is pumped or ejected out of the ventricles with each contraction. a normal EF is 50-70%.The term congestive heart failure refer to the accumulation of fluid in the tissue when this fluid accumulate in the legs causing pulmonary edema or into to the abdomen called ascites, a type of heart failure termed acute decompensate heart failure is emergency case.

2-1-4-2: Diastolic heart failure (right -sided **heart failure)** this type characterized by the heart becoming thicker and stiffer. When this happened the left ventricle cannot fill with sufficient blood , and not enough blood is pumped in to circulation, even if the pumping action is strong .this is why diastolic heart failure/ is sometimes referred to as heart failure with preserved ejection fraction (PEF) or right -sided. If the signs and symptoms of heart failure are present and the ejection fraction is greater than 50% diastolic heart failure maybe considered , especially if an echocardiogram shows the heart muscle is thickening.(yancy, 2013)

2-1-5 Symptoms of Heart Failure:-

Heart failure can be ongoing chronic or may start suddenly (acute). The signs and symptoms include:-

. Shortness of breath (dyspnea) when lie down

. Fatigue and weakness.

.Swelling (edema) in your legs ,ankles and feet .

.Rapid or irregular heartbeat.

.Reduced ability to exercise.

.persistent cough or wheezing with white or pink blood -tinged phlegm.

.increased need to urinate at night .

.Swelling of abdomen (ascites)

.very rapid weight gain from fluid retention.

.lack of apetite and nausea.

.difficulty concentration or decreased alertness.

.sudden , severe shortness of breath and coughing up pink , foamy mucus.

.chest pain if your heart failure is caused by a heart attack. (Allen, 2017)

2-1-6 Classifications of HF:-

classification of heart failure into classes or stages mainly dependent on progression of heart muscle weakness.

2-1-6-1: The New York heart Association (NYHA) puts the stages of heart failure into four classifications:

Class I:-

There are no restrictions of physical activity. Patients generally don't complain of being tired or experiencing shortness of breath. A patient is still able to control the disease. Regular exercise, limiting alcohol consumption, and eating healthy with moderate sodium intake, are all actions that can be taken quite easily. High blood pressure will need to be treated. Quitting smoking is crucial.

Class II:-

The patients will feel slight restrictions with everyday physical actions like bending over or walking and will be tired and shortness of breath may occur.

Class III:-

patients experience limitations during physical activity and may remain comfortable at rest, but most all physical activity will cause fatigue .under physician care ,their diet and exercise may be monitored. Diuretics ,to combat water retention , may be prescribed.

Class IV:-

Patients are virtually unable to do any physical activity without comfort and may be significant signs of cardiac problems even while resting .

The **National Heart** , **Lung and Blood Institute** estimates that 35% of patients with heart failure are in functional NYHA Class I, 35% are in Class II, 25% are in class III and 5% are in class IV .it has been estimated that between 5 and 15 % of patients have persisting sever symptoms.

2-1-6-2:- American College of Cardiology grades heart failure in four stages, and takes into account that heart can be present even before symptoms appear.

Stage A: No heart failure but a high risk due to another medical condition that can lead to heart failure significant signs of cardiac problems such as high blood pressure, diabetes, obesity or coronary artery disease.

Stage B: The heart has been damaged by the patients other medical conditions or other factor such as heart attack, valve disease, cardiomyopathy, but no symptoms are present yet but you are diagnosed with systolic left ventricular dysfunction, which means the left chamber does not pump well .most people with this stage have an echocardiogram (echo) that shows an ejection fraction (EF) of 40% or less.

Stage C: the heart is damaged and the patient is experiencing heart failure symptoms.

Stage D: the patient has severe heart failure that requires specialized care, despite receiving treatment (end- stage). (Erica Oberg, 2016).

2-1-7 Heart Failure Complications:-

When heart failure happened the heart may not be strong enough to pump out as much blood as the body needs .as it tries to move more blood, so the heart get larger, it also pumps faster, and the blood vessels narrow to get more blood out to the body. The body tries to keep the blood it has to supply the heart and brain ,this leaves less for organs like kidneys and liver .this complications include:-

2-1-7-1 Abnormal Heart Rhythm:-

In a normal heart, the upper chambers called (atria) and lower chambers (ventricles) squeeze and relax in turn to move blood through the body. Atrial fibrillation (A Fib) is one type of abnormal heart rhythm that heart failure can cause. it causes your heart to quiver and skip instead of beating .

2-1-7-2 Heart Valve Problems :-

Heart has four valves that open and close to keep blood flowing in and out of your heart . as the damage gets worse and your heart has to work harder to pump out blood ,it gets bigger . the change in size can damage the valves.

2-1-7-3 Kidney failure:-

When heart failure occur that amount of blood that supply kidney not reach to it to do normal renal function ,so it will not be able to remove enough wastes from the blood which lead to renal failure .

2-1-7-4 Anemia:-

Heart failure when cause renal failure this lead to kidney damage which lead to defect in synthesis of erythropoietin which responsible to make new red blood cells.

2-1-7-5 Liver damage:-

Heart failure can rob the liver . the fluid buildup that comes with it puts extra pressure on the portal vein , which brings blood to liver .this can scar the organ to the point where it doesn't work as well as it should.

2-1-7-6 Lung Problems:-

A damaged heart cant pump blood as effectively from the lung out to the body . blood backs up , raising pressure in the veins inside the lungs. This pushes fluid into the air sacs. As liquid build up , it gets harder to breath which cause pulmonary edema .

2-1-7-7Weight loss and Muscle loss :-

Heart failure can affect muscle and fat metabolism. In the late stages , you might lose a lot of weight and muscle mass . the muscle can get smaller and weaker.(James, 2017).

How to prevent these complications:-

Heart failure will get worse over time if don't treat it .severe heart failure can be life threatening. Treatment like weight loss , a healthy diet, exercise and medicines can protect the heart and keep healthy .follow your doctor advice and stick with your treatment plan.

2-1-8 Diagnosis of Heart Failure:-

To diagnose heart failure , will take a careful medical history, review the symptoms and perform a physical examination. will also check for the presence of risk factors such as high blood pressure or coronary artery disease or diabetes . using a stethoscope , can listen to lung for signs of congestion . the stethoscope also picks up abnormal heart sounds that may suggest heart failure . also may examine the veins in neck and check for fluid buildup in abdomen and legs (ponikowski , 2016).

After the physical exam, may also order some of these tests:-

Blood Tests:-

The doctor take a blood sample to look for signs of diseases that can affect the heart .also check for a chemical called N-terminal pro-B-type natriuretic peptide (NT-pro BNP) if diagnosis isn't certain after other tests.

Blood test can make difference in heart failure management (the accuracy of diagnosis based on history and clinical assessment only, is challenenging as symptoms are often difficult to interpret, and up to 50% of patient can be misdiagnosed with all related adverse impact , such as inappropriate care or treatment adding burden for patients and costs to healthcare systems.)

Heart failure can be diagnosed also by examination of patient and medical history are assessed. Then further tests and procedures should be done :-

Chest X-Ray:-

x-ray images help doctor see the condition of lung and heart . also it use to diagnose conditions other than heart failure that may explain signs and symptoms .

Electrocardiogram (ECG):-

This test records the electrical activity of heart through electrode attached to the skin .it helps doctor diagnose heart rhythm problems and damage to the heart .

Echocardiogram:-

An echocardiogram uses sound waves to produce a video image of the heart . this test can help doctors see the size and shape of heart along with any abnormalities .the test measures the ejection fraction , an important measurement of how well heart is pumping , and which is used to help classify heart failure and guide treatment.

Stress Test:-

Stress test measure the health of heart by how it responds to exertion . may be asked to walk on treadmill while attached to an ECG machine ,or may receive a drug intravenously that stimulates the heart similar to exercise . sometimes the stress test can be done while wearing a mask that measures the ability of heart and lungs to take in oxygen and breathe out carbon dioxide . if want to see images of heart while in exercising , may use imaging technique to visualize the heart during the test .

Cardiac computerized tomography (CT) scan:-

In a cardiac CT scan , lie on a table inside a doughnut – shaped machine. An X-ray tube inside the machine rotates around the body and collects images of the heart and chest . **.**

Magnetic resonance imaging (MRI):-

in a cardiac MRI , lie on a table inside a long tube like machine that produces a magnetic field ,which aligns atomic particles in some of the cell. Radio waves are broadcast toward these aligned particles , producing signals that create images of heart

Coronary angiogram: - In this test, a thin flexible tube (catheter) is inserted into a blood vessel at groin or in arm and guided through the aorta into coronary arteries. a dye injected through the catheter makes the arteries supplying the heart visible on an x-ray, helping doctors spot blockages.

Myocardial biopsy:-

In this test, the doctor insert a small**,** flexible biopsy cord into a vein in neck or groin, and small pieces of heart muscle are taken. This test may be performed to diagnose certain types of heart muscle diseases that cause heart failure (Hobbs, 2002.)

2-1-9 Medication of HF:

This medications help reduce the symptoms of congestive heart and can actually improve heart muscle function . there are several main classes of medications that are used for treating weak heart muscle .your physician will choose medication based on your symptoms and whether your heart needs to be strengthened or relaxed. This type include :-

1-beta blockers:-

This class of drugs not only slows your heart rate and reduces blood pressure but also limits or reverses some of the damage to heart if the patient have systolic heart failure . examples include carvedilol, bisoprolol (Zebeta).

These medicines reduce the risk of some abnormal heart rhythms and lessen chance of dying unexpectedly . also reduce signs and symptoms of heart failure , improve heart function and help live longer.

2-Angiotensin II receptor blockers:-

These drugs have the same benefits as ACE inhibitors . they may be an alternative for people who can't tolerate ACE inhibitors. Include Cozaar and Diovan .

3- Diuretics:-

Often called water pills , it makes urinate more frequently and keep fluid from collecting in the body . diuretics, such as furosemide (Lasix) decrease fluid in lungs so patient can breathe more easily . because diuretics make the body lose potassium and magnesium , the doctor also prescribe supplements of these minerals and monitor level of them in blood through regular blood tests.

4- ACE (Angiotensin Converting Enzyme) Inhibitors :-

These drugs help people with systolic heart failure live longer and feel better . ACE inhibitors are type of vasodilator , a drug that widens blood vessels to lower blood pressure , improve blood flow and decrease the workload on the heart . Examples lisinopril (Zestril) and captopril (Capoten).

Aldosterone antagonists :-

These drugs include spironolactone and eplerenone (Inspra) . these are potassium - spring diuretics , which also have additional properties that help people with severe

systolic heart failure live longer . unlike some other diuretics , spironolactone can raise the level of potassium in the blood to dangerous level ,so talk doctor if increased potassium is a concern , and learn if need to modify intake of food that high in potassium .

Inotropes :-

These are intravenous medication used in people with severe heart failure in the hospital to improve heart pumping function and maintain blood pressure.

Digoxin :-

This drug , also referred to digitalis , increases the strength of heart muscle contraction . it also tends to slow the heart beat. Digoxin reduces heat failure symptoms in systolic 1qheart failure . It may be more likely to be given to someone with heart rhythm problem, such as atrial fibrillation. (Rakel,2017)

2-2 BNP (Brain Natriuretic Peptide):-

BNP: An important new cardiac test

It's not common for a new diagnostic test to have an immediate impact on clinical practice, but BNP is just such a test. Best of all, it's a simple, safe blood test that can help doctors evaluate complex cardiac functions.

2-2-1 Definition of BNP:-

BNP belongs to a family of protein hormones called natriuretic peptides .each member of this group is produced by a different part of circulatory system. ANP is produced by the muscle cell in the upper pumping chambers of the heart (the atria)., BNP is produced in the larger and more powerful lower chambers (the ventricles) ., CNP is produced mainly in blood vessels ., and DNP is found in the blood plasma but probably originates in the heart itself. These natriuretic peptides have an important role in regulating the circulation. Scientists have learned the most about ANP and BNP. Both act on blood vessels, causing them to dilate or widen .they also work on the kidneys, causing them to excrete more salt and water. In addition, the natriuretic peptides reduce the production of various hormones that narrow blood vessels, boost the heart rate, or affect fluid retention, example include adrenaline, angiotensin, and aldosterone. The net effect of natriuretic peptides is to promote urine excretion, relax blood vessels, lower blood pressure, and reduce the hearts workload. they are part of the body s natural defense mechanisms designed to protect the heart from stress .and they surge into action when they are needed most , when the heart itself is under siege .(Yasue, 1994)

BNP subsequently was in porcine brains. These peptides have several actions such as down -regulating sympathetic nervous system and the renin- angiotensin aldosterone system. (Sudoh, 1988)

2-2-2 Biosynthesis of BNP: BNP is synthesized as a 134 – amino acids preprohormone (preproBNP), encoded by the human gene NPPB . Removal of the 25- residue N- terminal signal peptide generates the prohormone , pro BNP , which is stored intracellularly as an o-linked glycoprotein , pro BNP is subsequently cleaved between arginine -102 and serine 103 by a specific convertase (probably fur in or corin) into NT-pro BNP and biologically active 32 -amino acid polypeptide BNP -32 ,

which are secreted into the blood in equimolar amounts. Processing of proBNP may be regulated by O- glycosylation of residues near the cleavage sites. (Schellenberger, 2006)

B-type natriuretic peptide and N-terminal pro b-type are substance that are produced in the heart and released when the heart is stretched and working hard to pump blood. BNP test may be ordered when a person has signs and symptoms that could be due to heart failure such as difficulty breathing, fatigue, swelling in the feet, ankles, legs and abdomen. BNP is primarily used to help detect, diagnose and evaluate the severity of heart failure; it can be used along with other cardiac biomarker tests, to detect heart stress and damage and or along with lung function test to distinguish between causes of shortness of breath. (Levin and Gardener 1998).

2-2-3 BNP in Heart Failure:-it sounds like alphabet soup, but it's actually modern cardiology at its best .BNP helps the body compensate for congestive heart failure (CHF) ., measurements of BNP help doctors diagnose and treat this serious condition. In CHF , the heart chambers are dilated , or enlarged . heart muscle cells are stretched as the chambers swell with extra blood that cant be pumped out efficiently . The stretched muscle cells produce extra BNP, which pours into the bloodstream .and doctors now have simple, accurate, inexpensive tests to measure BNP in blood samples. A bedside test can even yield diagnostic information right in the emergency department. BNP is very helpful in diagnosis CHF. A normal BNP level is about 98% accurate in ruling out the diagnosis, freeing doctors to hunt for other conditions that may be causing shortness of breath or fluid retention. In most labs, high levels are less conclusive, but in patient with suspected CHF, levels of about 900 pg/ml in 50-75 year old or above 1200 pg/ml in older patients support the diagnosis up to 90% of the time, most of patient who do not have true CHF have elevated BNPs due to severe lung or renal disease. The table below lists some of the conditions that can raise or lower BNP level. (Tsutamaoto, 1997)

Non cardiac conditions that change BNP levels:-

Increase BNP:-

1. Increase age
2. Female gender
3. Lung , liver and kidney disease
4. High blood pressure
5. Overactive thyroid
6. Excessive cortisol level
7. Certain rare tumor
8. Brain hemorrhages

Decrease BNP:-

1. Obesity
2. Medications
3. Ace inhibitors
4. Spironolactone diuretics
5. Beta blockers

Heart failure can be confused with other conditions and may co-exist with them. Level can help doctors differentiate b/w heart failure and other problems such as lung disease. Although BNP and NT-BNP are usually used to recognized heart failure , an increased level in people with acute coronary syndrome (ACS) indicates an increased risk of recurrent events. Thus a health practitioner may use either BNP or N-T pro BNP to evaluate risk of future cardiac event risk of future cardiac event in some one with ACS. (Rutten 2016)

Result of test mean higher -than normal results suggest that aperson has some degree of heart failure, and the level of BNP in the blood is related to its severity. Higher level of BNP is often associated with aworse outlook (prognosis) for the person. Anormal level of NT- pro BNP based on Cleveland clinic's reference range is: less than 125pg/ml for patients aged 0-74 years. Less than 450 pg /ml for patients aged 75-99 years. (Januzzi, 2006)

Chapter Three

Materials and Methods

3. Material and method:

3.1 Materials:-

3.1.1 Study approach:-

A quantitative method was used to estimate BNP concentration in AL Khartoum state during the period from August to December 2017.

3.1.2 Study design:-

Descriptive case – control study.

3.1.3 Study area and period:-

This study was conducted in Khartoum state, zytona hospital from August to December 2017.

3.1.4 Study population:-

The study included 50 patients with heart failure their age from 20-90 and people without HF as control, their age was matched.

3.1.5: Sample size:-

The study included one hundred volunteered to participate in the study , 50 patient with heart failure, and 50 individual serve as control.

3.1.6: inclusion criteria:-

Sudanese patients with heart failure with different classes, and volunteer were included in this study.

3.1.7: exclusion criteria:-

The criteria of exclusion based on excluding any patient with renal diseases**.**

3.1.8: Ethical consideration:

Verbal informed consent obtained from each participant in the study regarding acceptance to participate in the study and reassurance of confidentiality before the specimen was collected for research purpose

3.1.9 Data collection:-

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concerning each case examined.

3.1.10: sample collection and processing:-

About 5ml of venous blood were collected from each participant (both case and control). The sample collected under aseptic conditions and placed in sterile heparin container, then centrifuged for 5 minutes at 3000 RPM to obtain sample for BNP, then the obtained sample were kept at 2-8 c until the time of analysis.

3.2 Method:-

Estimation of BNP by using ESL technology (sandwish assay)

Principle of method: 1st incubation: antigen pro BNP specific antibody, and a monoclonal NT-pro BNP Specific antibody labeled with ruthenium complex from a sandwich complex, 2nd incubation: after addition of streptavidin – coated micro particle, the complex becomes bound to the solid phase via interaction of biotin and streptavidin . The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured on to the surface of the electrode. UN bound substances are then removed with pro cell and pro cell M. Application of avoltage to the electrode then induces chemiluminescent emission which is measured by photomultiplier. (Appendix 11).

Procedure:-

Plasma BNP measurement: blood samples were collected using lithium heparin tubes centrifuged at 3,500 g for 15 min at 4 c. Immunoassay that uses biotinylated anti BNP monoclonal antibody and streptavidin -coated magnetic solid- phase particles that were used to attract the immune binding of latex particles coupled with horseradish peroxidase and a monoclonal antibody fragment .this generated an electrochemical detection signal proportional to the level of BNP in the patient sample , this method was valid and considered as a reference method . it was based on a two-step sandwich immunoassay ,detection was done by chemiluminescence .the electrochemiluminescence immunoassay (ELICA) is intended for use on Elecsys and **cobase** immunoassay analyzers. Serum NT -pro BNP levels were measured with an Elecsys pro BNP reagent kit (Roche diagnostic, USA) the limits of detection were 5-35000 pg /ml or 0.6 – 4130 pgmol/l. The result was provided within 18 min. (Appendix 11).

3.3 Quality control: -

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3. 4 Statistical analysis:-

Data obtained from this study was performed using statistical package for the social science (SPSS). T-test was used to compare the mean subgroup values. Chi-

square testing was used to compare the categorical variables. Pearson's correlation was used to estimate the correlation between two variables. For the analysis of the receiver operating characteristic (ROC) curve is used for determing specifity and sensitivity of test. for drawing Grafes use excel version 2010, for ploting scatter used minitab version 16 .

Differences were considered significant when the p values were less than 0.05.

ROC curve:-

The term ROC stands for Receiver Operating Characteristic. ROC curves are frequently used to show in a graphical way the connection trade off between clinical sensitivity (the fraction of people with the disease that the test correctly identifies as positive) and specificity (the fraction of people without the disease that the test correctly identifies as negative) for every possible cut off for a test or a combination of tests. In addition the area under the ROC curve gives an idea about the benefit of using the test (s) in question which used to show ability of test to discriminate between patients and control. Accuracy of test is measured by the area under curve or AUC. an area of 1 represents a perfect test. This curve it used in clinical biochemistry to choose the most appropriate cut -off for a test. The best cut-off has the highest true positive rate together with the lowest false positive rate .as the area under an ROC curve is a measure of the usefulness of a test. (Suzanne, 2008)

Chapter Four

Results

4. Result: -

The result of biochemical determinant of serum pro BNP in patient with heart failure are given in tables and figures:-

Table (4-1) illustrates the comparison of mean of BNP concentration in patient with heart failure compared to control group.

The mean of pro BNP was significantly increase in heart failure patients compared to control group (P.value = 0.000).

Mean ±SD for cases versus controls:

(4936 ± 7.5 versus 227±8.6)

Table (4-2): show the frequency and percentage of BNP in case group. 56.5% of patients the BNP ranged between 1000-5000.

Figure (4-1):-show age distribution among case group. 60% of patient over 50 year.

Figure (4-2):-show the gender distribution among case group. 60% of patients were males.

Figure (4-3): shows the frequency and percentage of BNP among case group.

Figure (4-4): shows the frequency and percentage of NYHA classes. 62.7% of patients classified as NYHA (II), 31.4% classified as NYHA (III), 5.9% classified as NYHA (IV).

Figure (4-5): show correlation between BNP concentration and age.

The scatter showed there was no correlation between BNP and age (r= - 0.145 P. Value =0.309).

Figure (4-6): show correlation between BNP and ejection fraction.

The scatter showed there was a significant negative correlation between BNP and ejection fraction (r= -0.349 P .Value = .012)

Figure (4-7): show correlation between BNP and Left atrium size.

The scatter showed there was significant negative correlation between BNP and LA size. (P. Value =0.001 r=-.45)

Figure (4-8) show correlation between BNP and NYHA classes. (P. Value = 0.000 r= .618)

The scatter showed there was significant moderate positive correlation between BNP and NYHA classes.

Figure (4-9) show the ROC curve which determine the specificity and sensitivity of BNP test and AUC of BNP test.

The curve show BNP is perfect test for heart failure diagnosis (AUC =1).

Tale (4-1) comparison of mean of BNP level among study group

variable	Case N=50 (mean/SD)	Control N=50 (mean/SD)	p-value
BNP (pg/ml)	4936.45± 7.5	227±8.6	0.000

Result given in mean ±SD p.value ≤ 0.05 consider significant. Independent sample T.TEST was used for comparison

Table (4-2) Frequency and percentage of the BNP range in case group

BNP (pg/ml)	Frequency	Percent
500-1000	9	17.6%
1000-5000	29	56.5%
5000-10000	7	13.7%
Above 10000	6	11.8%

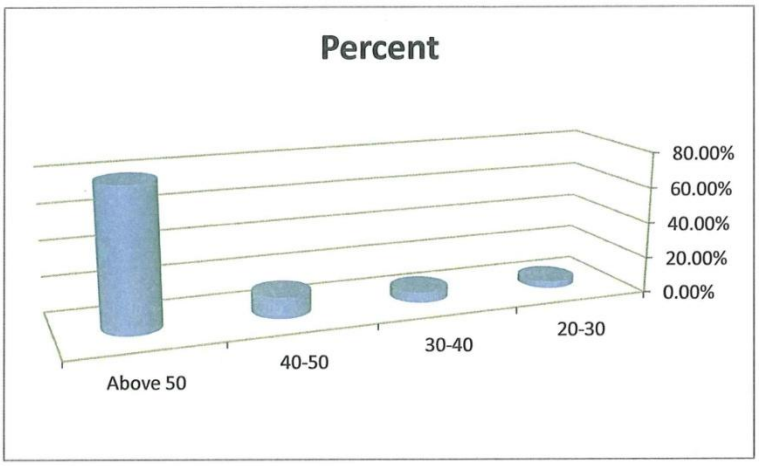


Figure (4-1) Age distribution among case group

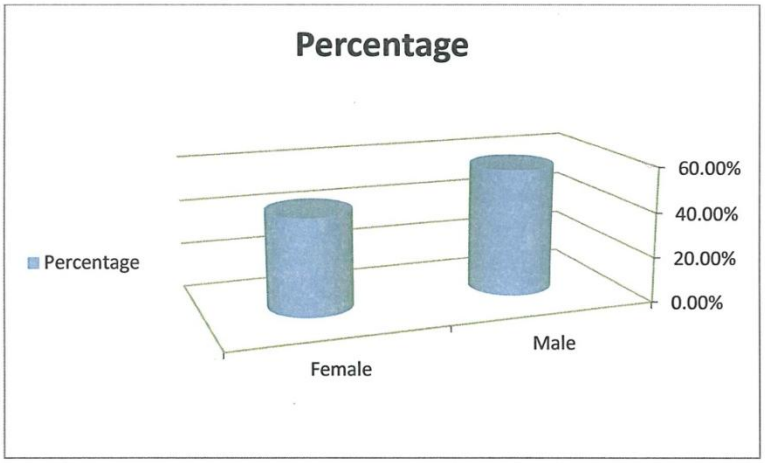
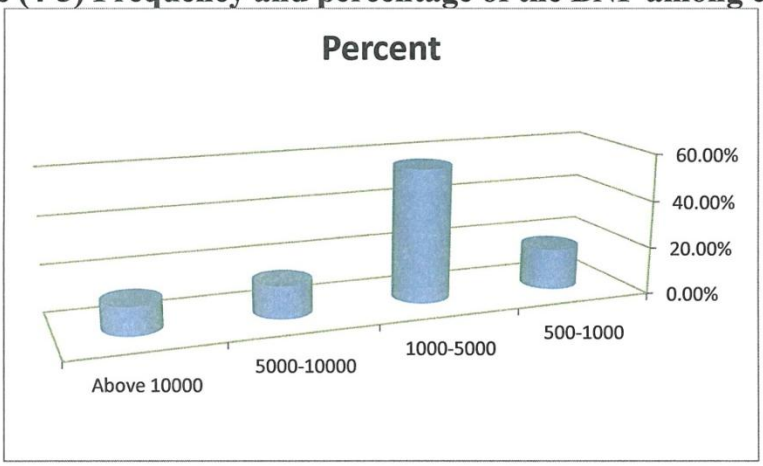


Figure (4-2) Gender distribution among case group

Figure (4-3) Frequency and percentage of the BNP among case group



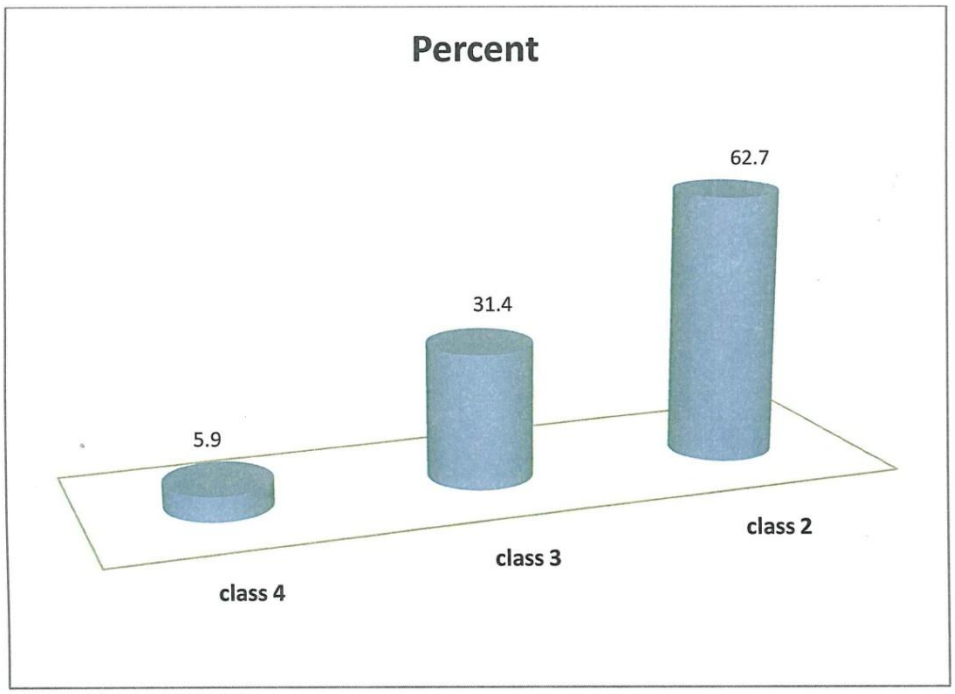


Figure (4-4) frequency and percent of NYHA classes

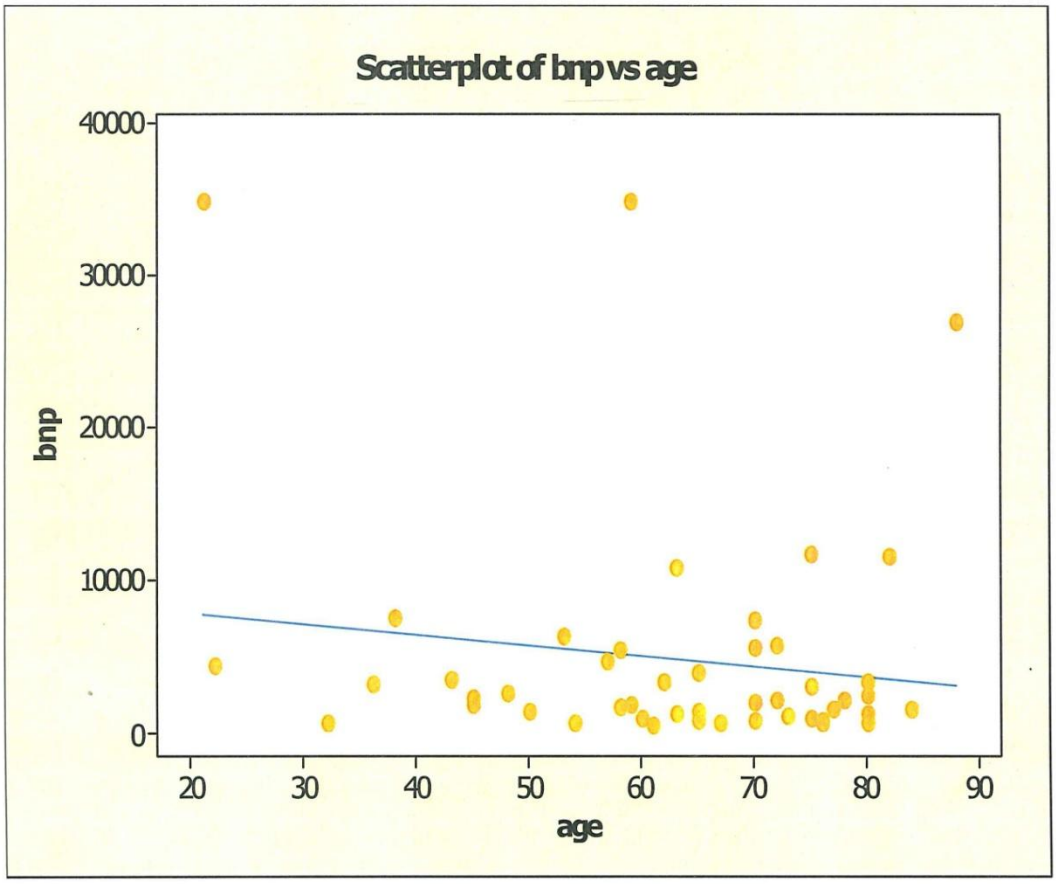


Figure (4-5) correlation between BNP concentration and age (r =-0.145 P.Value =0.309)

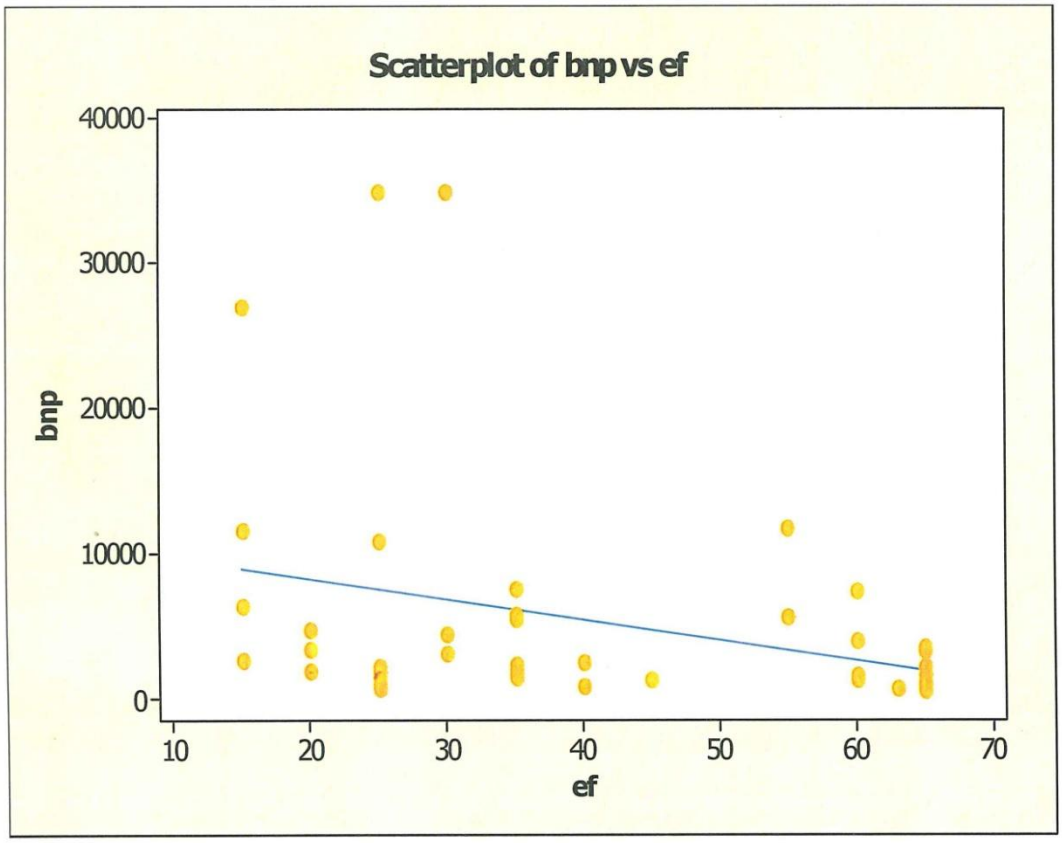


Figure (4-6) correlation between BNP and ejection fraction (r=-0.349 P.Value 0.012)

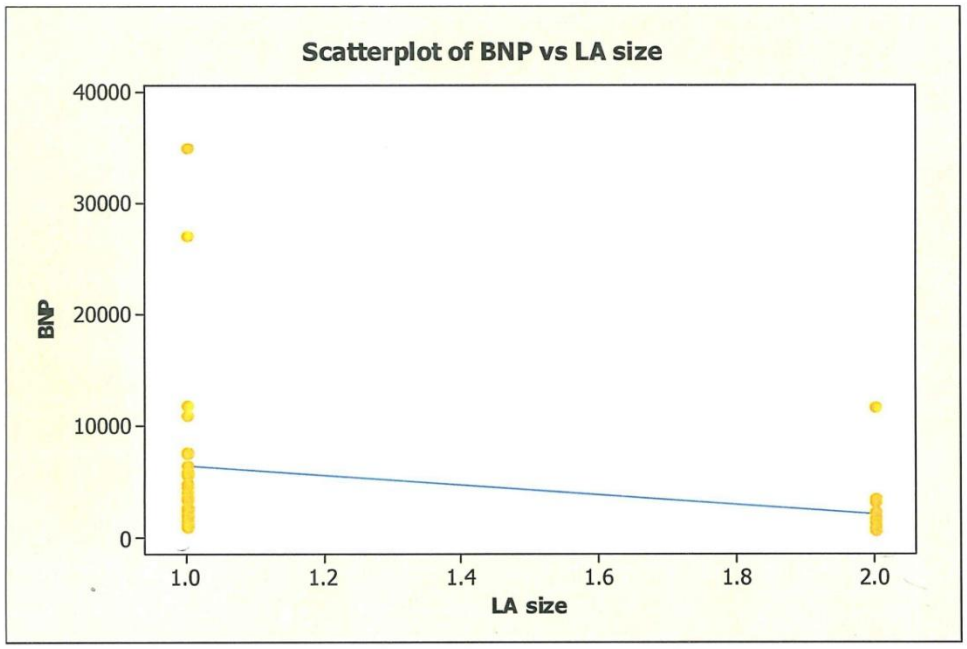
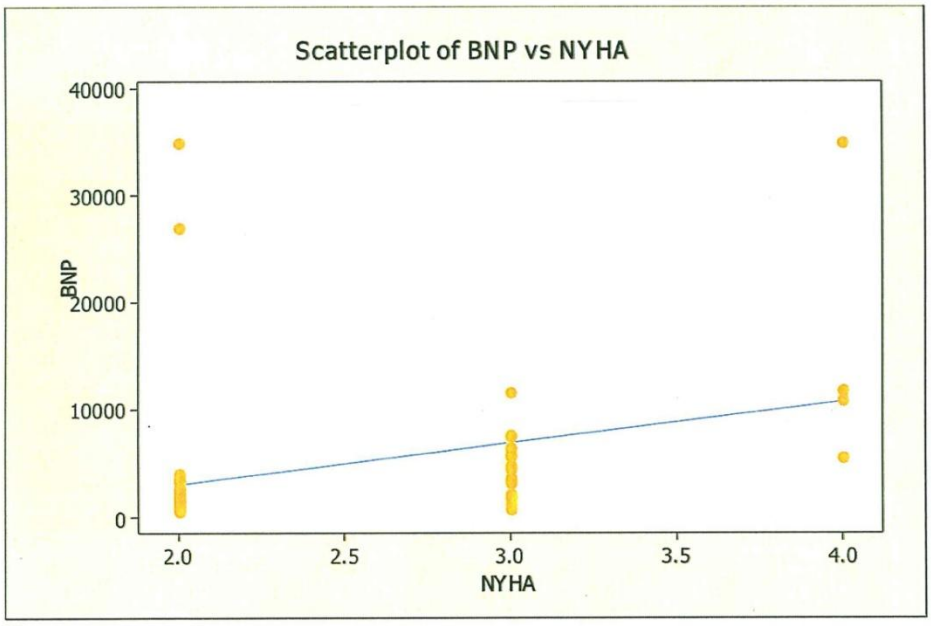


Figure (4-7) correlation between BNP and left atrium size (LA size)

(r =-0.45 p,value = 0.001)



Figure(4-8) correlation between BNP and NYHA classes

(r =.618 p.value=0.000)

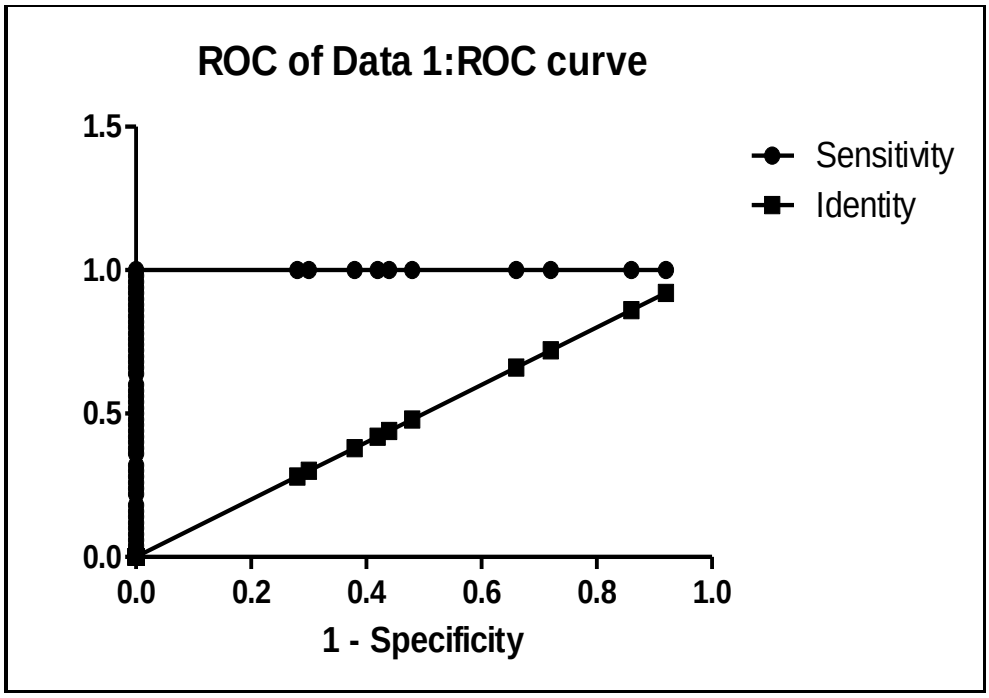


Figure (4-9) Show ROC curve

Result of Area

Area under the ROC curve	
Area	1.000
Std. Error	0.0
95% confidence interval	1.000 to 1.000
P value	< 0.0001
Data	
Control	50
Patient	50
Missing Controls	0
Missing Patients	0

Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio
< 17.50	8.000	2.223% to 19.23%	100.0	92.89% to 100.0%	
< 22.50	14.00	5.819% to 26.74%	100.0	92.89% to 100.0%	
< 27.50	28.00	16.23% to 42.49%	100.0	92.89% to 100.0%	
< 32.50	34.00	21.21% to 48.77%	100.0	92.89% to 100.0%	
< 37.50	52.00	37.42% to 66.34%	100.0	92.89% to 100.0%	
< 42.50	56.00	41.25% to 70.01%	100.0	92.89% to 100.0%	
< 50.00	58.00	43.21% to 71.81%	100.0	92.89% to 100.0%	
< 57.50	62.00	47.18% to 75.35%	100.0	92.89% to 100.0%	
< 61.50	70.00	55.39% to 82.14%	100.0	92.89% to 100.0%	
< 64.00	72.00	57.51% to 83.77%	100.0	92.89% to 100.0%	
< 385.0	100.0	92.89% to 100.0%	100.0	92.89% to 100.0%	
< 749.0	100.0	92.89% to 100.0%	98.00	89.35% to 99.95%	50.00
< 793.5	100.0	92.89% to 100.0%	96.00	86.29% to 99.51%	25.00
< 799.5	100.0	92.89% to 100.0%	94.00	83.45% to 98.75%	16.67
< 807.5	100.0	92.89% to 100.0%	92.00	80.77% to 97.78%	12.50
< 836.0	100.0	92.89% to 100.0%	90.00	78.19% to 96.67%	10.00
< 867.0	100.0	92.89% to 100.0%	88.00	75.69% to 95.47%	8.33
< 899.0	100.0	92.89% to 100.0%	86.00	73.26% to 94.18%	7.14
< 965.5	100.0	92.89% to 100.0%	84.00	70.89% to 92.83%	6.25
< 1012	100.0	92.89% to 100.0%	82.00	68.56% to 91.42%	5.56
< 1047	100.0	92.89% to 100.0%	80.00	66.28% to 89.97%	5.00
< 1105	100.0	92.89% to 100.0%	78.00	64.04% to 88.47%	4.55
< 1267	100.0	92.89% to 100.0%	76.00	61.83% to 86.94%	4.17
< 1408	100.0	92.89% to 100.0%	74.00	59.65% to 85.37%	3.85
< 1461	100.0	92.89% to 100.0%	72.00	57.51% to 83.77%	3.57
< 1520	100.0	92.89% to 100.0%	70.00	55.39% to 82.14%	3.33
< 1558	100.0	92.89% to 100.0%	68.00	53.30% to 80.48%	3.13
< 1608	100.0	92.89% to 100.0%	66.00	51.23% to 78.79%	2.94
< 1680	100.0	92.89% to 100.0%	64.00	49.19% to 77.08%	2.78
< 1805	100.0	92.89% to 100.0%	60.00	45.18% to 73.59%	2.50
< 1918	100.0	92.89% to 100.0%	58.00	43.21% to 71.81%	2.38
< 1989	100.0	92.89% to 100.0%	56.00	41.25% to 70.01%	2.27
< 2127	100.0	92.89% to 100.0%	54.00	39.32% to 68.19%	2.17
< 2254	100.0	92.89% to 100.0%	52.00	37.42% to 66.34%	2.08
< 2309	100.0	92.89% to 100.0%	50.00	35.53% to 64.47%	2.00
< 2436	100.0	92.89% to 100.0%	48.00	33.66% to 62.58%	1.92
< 2640	100.0	92.89% to 100.0%	46.00	31.81% to 60.68%	1.85
< 2962	100.0	92.89% to 100.0%	44.00	29.99% to 58.75%	1.79
< 3210	100.0	92.89% to 100.0%	42.00	28.19% to 56.79%	1.72
< 3350	100.0	92.89% to 100.0%	40.00	26.41% to 54.82%	1.67
< 3478	100.0	92.89% to 100.0%	38.00	24.65% to 52.82%	1.61
< 3573	100.0	92.89% to 100.0%	36.00	22.92% to 50.81%	1.56
< 3832	100.0	92.89% to 100.0%	32.00	19.52% to 46.70%	1.47
< 4270	100.0	92.89% to 100.0%	30.00	17.86% to 44.61%	1.43
< 4622	100.0	92.89% to 100.0%	28.00	16.23% to 42.49%	1.39
< 5099	100.0	92.89% to 100.0%	26.00	14.63% to 40.35%	1.35
< 5614	100.0	92.89% to 100.0%	24.00	13.06% to 38.17%	1.32
< 5783	100.0	92.89% to 100.0%	22.00	11.53% to 35.96%	1.28
< 6125	100.0	92.89% to 100.0%	18.00	8.576% to 31.44%	1.22
< 6961	100.0	92.89% to 100.0%	16.00	7.170% to 29.11%	1.19
< 7570	100.0	92.89% to 100.0%	14.00	5.819% to 26.74%	1.16
< 9272	100.0	92.89% to 100.0%	12.00	4.534% to 24.31%	1.14
< 11268	100.0	92.89% to 100.0%	10.00	3.328% to 21.81%	1.11
< 11755	100.0	92.89% to 100.0%	8.000	2.223% to 19.23%	1.09
< 19446	100.0	92.89% to 100.0%	6.000	1.255% to 16.55%	1.06
< 31015	100.0	92.89% to 100.0%	4.000	0.4881% to 13.71%	1.04

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion:-

The prevalence rate of heart failure is increasing and its mortality is still high despite many drugs and surgical device have been developed recently . since a half of the patients with heart failure have no symptoms , furthermore , the symptoms and signs of heart failure are not specific nor sensitive , the diagnosis of heart failure is often difficult. So measuring serum BNP Level used as gold standard biomarkers for the diagnosis and prognostic stratification of heart failure .From the finding of this study it appears that the serum BNP concentration were significantly increased in patient with heart failure compared to control group . this result agreed with another study carried by (Bozkurt et al ., 2013), showed significant increased in heart failure patient compared to control . this result is in excellent agreement with international guidelines which used BNP to discriminate the origin of dyspnea (cardiac versus non – cardiac e.g bronchial asthma).

The finding of this study showed there was a negative correlation between BNP and ejection fraction and left atrium size. for LA size (r=.-.45 P. Value =.001) for ejection fraction (r=-0.349 P .Value =0.012)this result is agreed with another study carried by (Karakilic et al .,2010),

The finding of this study showed there was no correlation between age and BNP ,This result is agreed with another study carried by (Korean et al ., 2005) and (levy and larson ., 2002) .

This study showed that BNP serum level was positively correlated with NYHA classes. (r= .618 P.Value =.000). this result is in excellent agreement with previous reported studies carried by (lambaS., 2002).our study showed an excellent agreement with previous reported study show that serum BNP Levels of patients with NYHA 11-IV dyspnea and having evidence of heart failure were more significantly than the BNP serum levels of patients who do not have any symptoms or they have minimal of heart failure which carried by (McCullough, P.A 2002).

In this study has many limitations which may effects in result. Firstly it was a study based on only one participating center, secondly small population compared with the real current population with heart failure, short period time about four month only.

5-2 CONCLUSION

- According to the results of this study it is concluded that:
- Serum BNP levels were increased in patients with heart failure.
- Serum BNP is negatively correlated with Ejection fraction and left atrium size and positively correlated to NYHA classes and not correlated with age.
- BNP is sensitive test for diagnosis heart failure.

5-3 RECOMMENDATIONS

1. BNP should be used as routine investigation in the laboratories of cardiac center.
2. Should be used as sensitive test for diagnosis of heart failure.
3. patients with heart failure it's important to look after health this include take medications , have a healthy diet, Exercise regularly, stop smoking ,limit alcohol consumption, regular reviews and monitoring. To manage the heart failure every patient should follow this zones handout (Appendix 111).

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Appendices

SUDAN UNIVERSITY OF SCIENCE AND TECHONLOGY

Questionnaire

A- Personal information :-

Name

Age (20-40) (40-60) (60-70) (≥70)

Gender (M) (F)

Address

Tel

Height

Weight

BMI

B- Risk factor:-

DM

HTN

Known Ischemic Heart disease

Smoking

Obesity

Alcohol abuse

C- Presentation :-

*** Symptom**

Shortness of breath (Yes) (No)

NYHA (I) (II) (III)

Lower limp swelling (Yes) (No)

*** Sign**

Raised JVP (Yes) (No)

S_3 (Yes) (No)

Bibasal cruckels (Yes) (No)

Chest wheezes (Yes) (No)

Tender hepatomegally (Yes) (No)

Bilateral LLO (Yes) (No)

D- Lab test:-

Hb %

S creatnine

Estimated GFR

E- Pro-BNP:-

Reason to measure it:-

HF likely (Yes) (No)

HF unlikely (Yes) (No)

Pro-BNP result:-

F- Echo finding:-

EF %

LA size (dilated) (not dilated)

Diastolic dysfunction

G- Final clinical and /or echo finding :

DCM

RCM

HHD

ICM

PPCM

HCM

IHD

Valvular heart disease

H- HF confirm :- (Yes) (No)

ms_04842464190V12.0

Elecsys proBNP II

cobas®

REF	Σ	SYSTEM
04842464 190	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 271
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 091

Intended use

Immunoassay for the in vitro quantitative determination of N-terminal pro B-type natriuretic peptide in human serum and plasma. This assay is indicated as an aid in the diagnosis of individuals suspected of having congestive heart failure and detection of mild forms of cardiac dysfunction.[1,2,3,4,5,6,7,8]

The test also aids in the assessment of heart failure severity in patients diagnosed with congestive heart failure.[9,10]

This assay is further indicated for the risk stratification of patients with acute coronary syndrome[11,12,13,14,15] and congestive heart failure, and it can also be used for monitoring the treatment in patients with left ventricular dysfunction.[1,2,16,17,18,19,20]

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Heart failure is a clinical syndrome characterized by systemic perfusion inadequate to meet the body's metabolic demands as a result of a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/ or elevated intracardiac pressures at rest or during stress.[1,2,3] Left ventricular dysfunction can be one of the functional precursors of heart failure.[1,2]

Heart failure is a progressive disease where in both hospitalized and ambulatory patients, most deaths are due to cardiovascular causes, mainly sudden death and worsening HF.[1,2]

The typical terminology used to describe HF is based on measurement of the Left Ventricular Ejection Fraction (LVEF). According to latest ESC guidelines, HF comprises a wide range of patients, from those with normal LVEF [typically considered as ≥ 50 %; HF with preserved EF (HFpEF)] to those with reduced LVEF [typically considered as < 40 %; HF with reduced EF (HFrEF)]. Patients with an LVEF in the range of 40-49 % represent a 'grey area', which is now defined as HF with midrange EF (HFmrEF).[1,2,3] Clinical information and imaging procedures are used to confirm the diagnosis of heart failure.[1,2,3]

The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. The following natriuretic peptides have been described: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP).[21,22]

ANP and BNP, as antagonists of the renin-angiotensin-aldosterone system, influence by means of their natriuretic and diuretic properties, the electrolyte and fluid balance in an organism.[23,24,25] In subjects with left ventricular dysfunction, serum and plasma concentrations of BNP increase, as does the concentration of the putatively inactive amino-terminal fragment, NT-proBNP. ProBNP, comprising 108 amino acids, is secreted mainly by the ventricle and, in this process, is cleaved into physiologically active BNP (77-108) and the N-terminal fragment NT-proBNP (1-76).[22,23]

Several studies have demonstrated the significant role of natriuretic peptide testing, including NT-proBNP, in heart failure management from diagnosis to monitoring, leading to the recommendation to use them in clinical practice by major international guidelines with often highest level of evidence and recommendation.[1,2]

Based on the symptoms, the severity of heart failure is classified in stages (New York Heart Association classification [NYHA] I-IV). When patients are grouped according to their NYHA classification, NT-proBNP levels increase with increasing class numbers and reflect the severity of cardiac impairment.[9,10]

Heart failure symptoms are often non-specific and do not help to discriminate between heart failure and other conditions, such as (non-cardiogenic) pulmonary edema, chronic obstructive pulmonary disease (COPD), pneumonia or sepsis.[1,2]

The European Society of Cardiology Heart Failure Guidelines recommends natriuretic peptides, including NT-proBNP, as an initial diagnostic test.[1] Patients with NT-proBNP below the recommended NT-proBNP cutoffs for non-acute and acute onsets are unlikely to have HF, and therefore do not require echocardiography - and elevated NT-proBNP help to identify patients who require further cardiac investigation.[1] When used with the recommended cutoff, the Elecsys proBNP assay yields negative predictive values ranging from 97 % to 100 % depending on age and gender.[10]

The test is also useful in the early stages of heart failure, where symptoms may be transient rather than present all the time.[3] The high sensitivity of NT-proBNP allows also the detection of mild forms of cardiac dysfunction in asymptomatic patients with structural heart disease.[4,5,6,7,8]

NT-proBNP can also be used for prognostic applications in patients with acute coronary syndrome. The GUSTO IV study, with more than 6800 patients, showed that NT-proBNP was the strongest independent predictor of one year mortality in patients with acute coronary syndrome.[15]

In patients hospitalized for acute decompensated heart failure, pre-discharge measurement of natriuretic peptides is useful to categorize patient's risk at discharge.[1,16] Changes in NT-proBNP levels during hospitalization demonstrated to be a strong predictor of outcomes.[16,26,27,28,29] A decrease in NT-proBNP values of ≥ 30 % has shown to be correlated with favorable outcome, while an increase in NT-proBNP values > 30 % was correlated with 6.6 times higher risk of rehospitalization or death in 6 months.[16]

In chronic heart failure, serial measurement of NT-proBNP concentration can be used to monitor the disease progression, to predict outcomes and evaluate the success of treatment.[1,2,17,18,20,30,31]

Elevated NT-proBNP values are strongly predictive of adverse outcomes and rising values identify a risk, while significant lowering of NT-proBNP denotes improved outcomes and better prognosis.[1,2,17,32]

When NT-proBNP levels change during treatment of chronic heart failure, decrease over the course of the disease correlated with improved clinical outcomes.[1,2,18,20] This interpretation of NT-proBNP results remains unchanged when using the new drug class Angiotensin receptor–neprilysin inhibitor[1,2] (ARNI, e.g. sacubitril-valsartan): In contrast to BNP, NT-proBNP degradation is not inhibited by the drug so that NT-proBNP results are not increased by the mode of action of the drug.[19,33,34] In patients treated with sacubitril-valsartan, rapid and sustained reduction of NT-proBNP levels has been observed, reflecting reduced wall stress[33] and benefits of the drug correlating with a lower rate of cardiovascular death and heart failure hospitalization.[20]

NT-proBNP can be used before non-cardiac surgery to evaluate patients' perioperative cardiac risk.[35]

In addition NT-proBNP can be used to identify patients at higher risk of cardiotoxicity which can lead to heart failure and may be helpful in monitoring the use and dosing of cardiotoxic tumor drugs[1,36,37] or interventions causing fluid retention or volume overload (e.g. COX-2 inhibitors, nonsteroidal anti-inflammatory drugs).[38,39,40,41,42,43,44,45]

In meta-analysis including 95617 patients without history of cardiovascular disease, NT-proBNP concentration strongly predicted first-onset heart failure and augmented chronic heart disease and stroke prediction, suggesting that NT-proBNP could serve as a biomarker in new therapeutic approaches that integrate heart failure into cardiovascular disease primary prevention.[46]

The Elecsys proBNP II assay contains two monoclonal antibodies which recognize epitopes located in the N-terminal part (1-76) of proBNP (1-108).

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

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- 1st incubation: Antigen in the sample (15 µL), a biotinylated monoclonal NT-proBNP-specific antibody, and a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complex[a)] form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as PRO-BNP II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-NT-proBNP-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-NT-proBNP antibody (mouse) 1.1 µg/mL; phosphate buffer 40 mmol/L, pH 5.8; preservative.

R2 Anti-NT-proBNP-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-NT-proBNP antibody (sheep) labeled with ruthenium complex 1.1 µg/mL; phosphate buffer 40 mmol/L, pH 5.8; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, NH_4-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (lower detection limit) + coefficient of correlation > 0.95.

Stable for 3 days at 20-25 °C, 6 days at 2-8 °C, 24 months at -20 °C (± 5 °C).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04842472190, proBNP II CalSet, for 4 x 1.0 mL
- REF 04917049190, PreciControl Cardiac II, for 4 x 2.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

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Calibration

Traceability: This method has been standardized against the Elecsys proBNP assay (REF 03121640122). This in turn is traceable to pure synthetic NT-proBNP (1-76) by weight.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Cardiac II.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

Conversion factors: pmol/L x 8.457 = pg/mL

pg/mL x 0.118 = pmol/L

Limitations - interference

The assay is unaffected by icterus (bilirubin < 428 µmol/L or < 25 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 17.1 mmol/L or < 1500 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

There is no high-dose hook effect at NT-proBNP concentrations up to 35400 pmol/L (300000 pg/mL).

In vitro tests were performed on 51 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

In extremely rare cases (global incidence: < 1 in 10 million), patients may show discrepant results when tested with the assay kit (values < lower detection limit) due to a NT-proBNP genetic variant.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

5-35000 pg/mL or 0.6-4130 pmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 5 pg/mL (< 0.6 pmol/L). Values above the measuring range are reported as > 35000 pg/mL (> 4130 pmol/L) or up to 70000 pg/mL (8260 pmol/L) for 2-fold diluted samples.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 5 pg/mL (0.6 pmol/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with NT-proBNP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 1770 pmol/L or > 15000 pg/mL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Dilutions of up to 1:10 may entail maximum deviations of 25 % from the theoretical value.

Clinical data

Interpretation of NT-proBNP values

With increasing age atherosclerosis and aging processes of the heart (e.g. fibrosis) result in cardiac dysfunction. Development of cardiac dysfunction is individually different and clinically asymptomatic in its early stages.[47,48] NT-proBNP levels reflect cardiac function or dysfunction respectively. With increasing age elevated levels of NT-proBNP are more frequently found in apparently healthy individuals, thus reflecting the increasing frequency of cardiac dysfunction.

NT-proBNP values need to be interpreted in conjunction with the medical history, clinical findings and other information (e.g. imaging, laboratory findings, accompanying disorders, treatment effects).

Cutoff values

A number of studies support a decision threshold for NT-proBNP of 125 pg/mL. NT-proBNP values < 125 pg/mL exclude cardiac dysfunction with a high level of certainty in patients with symptoms suggestive of heart failure e.g. dyspnea.[1,3,49,50] NT-proBNP values > 125 pg/mL may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications (myocardial infarction, heart failure, death).

Recommended cutoffs in patients with diagnosed stable chronic heart failure

Patients with stable heart failure (n = 721) were compared to the reference group (n = 2264).

ROC plot analysis at the cutoff value of 125 pg/mL showed a sensitivity of 88 %, a specificity of 92 %, a negative predictive value (NPV), and a positive predictive value (PPV) of 96.7 % and 80.6 %, respectively.

Expected values

NT-proBNP concentrations in the reference group are shown in the following tables. The most appropriate decision threshold apparent from these distributions is 125 pg/mL.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Reference group

The circulating NT-proBNP concentration was determined in samples from 1981 blood donors aged between 18 and 65 as well as 283 elderly patients aged between 50 and 90, both populations without known cardiac risks, symptoms or medical history.

The descriptive statistics for NT-proBNP concentrations (pg/mL) in the reference group are shown in the following table:

	All					
Age (years)	N	Mean	SD	Median	95th percentile	97.5th percentile
18-44	1323	35.6	30.2	20.4	97.3	115
45-54	408	49.3	63.3	30.7	121	172
55-64	398	72.6	84.4	47.3	198	263
65-74	102	107	85.9	85.1	285	349
≥ 75	33	211	152	174	526	738

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All						
Age (years)	N	Mean	SD	Median	95th percentile	97.5th percentile
Total	2264	50.3	62.4	27.9	149	196

Males						
Age (years)	N	Mean	SD	Median	95th percentile	97.5th percentile
18-44	815	27.7	25.5	20.0	62.9	85.8
45-54	278	39.0	63.6	21.6	83.9	121
55-64	259	57.2	74.5	37.7	161	210
65-74	61	105	87.9	83.9	241	376
≥ 75	13	163	116	151	486	486
Total	1426	39.8	55.3	20.0	113	169

Females						
Age (years)	N	Mean	SD	Median	95th percentile	97.5th percentile
18-44	508	48.2	32.8	37.1	116	130
45-54	130	71.5	56.7	55.4	169	249
55-64	139	101	94.0	79.6	247	287
65-74	41	109	83.8	85.2	285	301
≥ 75	20	243	167	191	738	738
Total	838	68.2	69.3	47.8	177	254

In the pediatric population aged between 1 and 18 the following NT-proBNP values were obtained using the Elecsys proBNP assay, REF 03121640122:[51]

Age (years)	N	NT-proBNP (ng/L)	
		75th percentile	97.5th percentile
1-3	13	231	320
4-6	21	113	190
7-9	32	94	145
10	11	73	112
11	69	93	317
12	21	95	186
13	23	114	370
14	18	68	363
15	24	74	217
16	24	85	206
17	24	71	135
18	12	53	115

Correlation of NT-proBNP with NYHA classification in patients diagnosed with CHF

NT-proBNP values (pg/mL) for patients with restricted left ventricular ejection fraction (majority under therapy).

NYHA functional class				
	NYHA I	NYHA II	NYHA III	NYHA IV
N	182	250	234	35
Mean	1016	1666	3029	3465
SD	1951	2035	4600	4453
Median	342	951	1571	1707
5th percentile	33.0	103	126	148
95th percentile	3410	6567	10449	12188
% > 125 pg/mL	78.6	94.0	95.3	97.1

Patients presenting acute dyspnea - ICON (International Collaborative of NT-proBNP) study[10]

NT-proBNP concentrations were determined in samples from 1256 patients presenting with acute shortness of breath to emergency departments at four hospitals. This population included patients with a prior history of hypertension, coronary artery disease, myocardial infarction, heart failure, or pulmonary disease. 720 subjects were found to be suffering from acute exacerbation of heart failure, while the remainder were determined to present dyspnea due to other causes. The descriptive statistics for NT-proBNP concentrations (pg/mL) for both groups are shown in the following table:

ICON Population	Acute dyspnea without acute heart failure			Acute dyspnea with acute heart failure		
Age (years)	< 50	50-75	> 75	< 50	50-75	> 75
Mean	163	500	1209	7947	7964	10519
SD	484	1239	2703	9093	12892	15961
Median	42	121	327	5044	3512	5495
5th percentile	5	10	24	393	416	658
25th percentile	16	44	139	2257	1608	2154
95th percentile	104	402	910	9825	9262	11900
97.5th percentile	778	2101	7916	36201	29089	35183
Min.	1	1	2	196	38	17
Max.	4386	10467	15725	43177	117390	117390
N	150	281	105	33	251	436

Result interpretation in patients presenting acute dyspnea

By using the optimal cutoffs established by the ICON study group and shown in the table below, physicians can increase the specificity and accuracy for diagnosing heart failure in patients presenting acute dyspnea in the emergent setting.

Category	Optimal cut-point pg/mL	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Rule in cut-point						
< 50 years (n = 184)	450	97	93	79	99	94
50-75 years (n = 537)	900	90	82	83	88	85
> 75 years (n = 535)	1800	85	73	92	55	83
Rule out cut-point						
All patients	300	99	60	77	98	83

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory

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Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

cobas e 411 analyzer					
	Repeatability				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	44.0	5.19	1.84	0.22	4.2
Human serum 2	126	14.9	3.06	0.36	2.4
Human serum 3	2410	284	31.7	3.74	1.3
Human serum 4	33606	3966	922	109	2.7
PC CARDII[b)]1	82.0	9.68	2.11	0.25	2.58
PC CARDII2	2318	274	27.3	3.22	1.18

b) PC CARDII = PreciControl Cardiac II

cobas e 411 analyzer					
	Intermediate precision				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	44.0	5.19	2.02	0.24	4.6
Human serum 2	126	14.9	3.23	0.38	2.6
Human serum 3	2410	284	44.2	5.22	1.8
Human serum 4	33606	3966	1288	152	3.8
PC CARDII1	82.0	9.68	2.27	0.27	2.8
PC CARDII2	2318	274	36.6	4.32	1.6

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	64	7.55	1.21	0.14	1.9
Human serum 2	124	14.6	1.82	0.22	1.5
Human serum 3	14142	1669	182	21.5	1.3
PC CARDII1	77.0	9.09	1.41	0.17	1.8
PC CARDII2	2105	248	24.8	2.92	1.2

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	pg/mL	pmol/L	pg/mL	pmol/L	%
Human serum 1	46	5.43	1.44	0.17	3.1
Human serum 2	125	14.75	3.43	0.40	2.7
Human serum 3	32930	3885	546	64.4	1.7
PC CARDII1	77.0	9.09	2.12	0.25	2.7
PC CARDII2	2170	256	59.4	7.01	2.7

Method comparison

A comparison of the Elecsys proBNP II assay, REF 04842464190 (y) with the Elecsys proBNP assay, REF 03121640122 (x) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 2133

Passing/Bablok[52]

$y = 0.977x + 1.89$

Linear regression

$y = 0.999x - 13.36$

$\tau = 0.946$ $r = 0.996$

The sample concentrations were between approximately 5 and 30022 pg/mL (approximately 0.6 and 3543 pmol/L).

Analytical specificity

The Elecsys proBNP II assay does not show any significant cross reactions with the following substances, tested with NT-proBNP concentrations of approximately 230 pg/mL and 2300 pg/mL (max. tested concentration):

Cross-reactant	**Concentration tested**
Adrenomedullin	1.0 ng/mL
Aldosterone	0.6 ng/mL
Angiotensin I	0.6 ng/mL
Angiotensin II	0.6 ng/mL
Angiotensin III	1.0 ng/mL
ANP_{28}	3.1 µg/mL
Arg-vasopressin	1.0 ng/mL
BNP_{32}	3.5 µg/mL
CNP_{22}	2.2 µg/mL
Endothelin	20 pg/mL
NT-proANP$_{1-30}$ (preproANP$_{26-55}$)	3.5 µg/mL
NT-proANP$_{31-67}$ (preproANP$_{56-92}$)	1.0 ng/mL
NT-proANP$_{79-98}$ (preproANP$_{104-123}$)	1.0 ng/mL
Renin	50 ng/mL
Urodilatin	3.5 µg/mL

Functional sensitivity

50 pg/mL (5.9 pmol/L)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

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HEART FAILURE ZONES

Every Day

Make sure that you do the following EVERY DAY:

- Weigh yourself before breakfast. Compare today's weight with your *dry weight*. Dry weight is your weight when you do not have extra fluid in your body. Make sure you know your dry weight. **Your dry weight is 1 pound less than your weight on the first day home from the hospital. _____ - 1 lb = _____ lbs or the weight determined by your healthcare provider.**
- Take ALL medications as prescribed.
- Check for swelling in your feet, ankles, legs and stomach.
- Limit sodium in your diet as prescribed by your healthcare provider (read food labels for sodium content); **My sodium limit is _____ mg/day**
- Be active and exercise every day.

Which zone are you in today? Green, Yellow or Red?

Green Zone

ALL CLEAR – This zone is your goal.

You do not have symptoms or they are mild. You have:

- **No new or worsening:**
  - Shortness of breath
  - Swelling of feet, ankles, legs or stomach
  - Fatigue/tiredness
- **Stable weight** (weight is within 4 pounds of your dry weight)

Yellow Zone

CAUTION – This zone is a warning zone.

Call your heart failure doctor or nurse if you:

- **Gain or lose 4 or more pounds** from your dry weight
- **Have new or worsening:**
  - Shortness of breath when active or at night when lying down
  - Swelling of feet, ankles, legs or stomach
  - Tiredness (less energy than usual)
  - Dizziness that lasts more than a minute
  - Need to urinate more often at night
  - Dry cough
- **Feel uneasy and know something is not right**
- **Have a change of appetite** (less hungry)

Doctor to call: _____

Red Zone

EMERGENCY

Go to the emergency room or call 911 if you:

- Struggle to breathe or are short of breath while sitting still
- Have chest pain that is new or gets worse
- Are confused or cannot think clearly

It is important to have an office visit in one week (7 days) after you leave the hospital, *even if you feel well.*

Please keep your scheduled appointment: Date: _____ Time: _____