(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 November 2016 (24.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/185003 A1

(51) **International Patent Classification**:
G01N 33/92 (2006.01) ***G01N 33/82*** (2006.01)
G01N 30/00 (2006.01)

(21) **International Application Number**:
PCT/EP2016/061373

(22) **International Filing Date**:
20 May 2016 (20.05.2016)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
15305766.6 21 May 2015 (21.05.2015) EP

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2016/185003 A1

(54) **Title**: METHOD FOR DIAGNOSING HEART FAILURE OR DYSPNEA

(57) **Abstract**: The present invention relates to a method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in a sample obtained from said patient.

METHOD FOR DIAGNOSING HEART FAILURE OR DYSPNEA

FIELD OF THE INVENTION:

The invention relates to a method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in a sample obtained from said patient.

BACKGROUND OF THE INVENTION:

Heart failure is a common, costly, disabling, and potentially deadly condition. In developed countries, around 2% of adults suffer from heart failure, but in those over the age of 65, this increases to 6–10%. Heart failure is associated with significantly reduced physical and mental health, resulting in a markedly decreased quality of life. Although some people survive many years, progressive disease is associated with an overall annual mortality rate of 10%. So, there is a permanent need in the art for new molecules for the treatment and new diagnostic of heart failure and especially for chronic heart failure (see for example Dickstein et al., 2008).

SUMMARY OF THE INVENTION:

The inventors show in a study that the ubiquinol (CoQ10H2) and the ubiquinone (CoQ10) are biomarkers of heart failure and dyspnea.

Thus, the inventions relates to a method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in a sample obtained from said patient.

DETAILED DESCRIPTION OF THE INVENTION:

Diagnostic method

The invention relates to a method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in a sample obtained from said patient.

In one embodiment, the heart failure is a chronic or an acute heart failure.

In a particular embodiment, the chronic heart failure is a systolic chronic heart failure or a diastolic chronic heart failure.

As used herein, the term “dyspnea” denotes a feeling or feelings associated with impaired breathing. The American Thoracic Society defines it as "a subjective experience of breathing discomfort that consists of qualitatively distinct sensations that vary in intensity," and recommends evaluating dyspnea by assessing the intensity of the distinct sensations, the degree of distress involved, and its burden or impact on activities of daily living. Distinct sensations include effort/work, chest tightness, and air hunger (the feeling of not enough oxygen). Dyspnea is a normal symptom of heavy exertion but becomes pathological if it occurs in unexpected situations. In 85% of cases it is due to either asthma, pneumonia, cardiac ischemia, interstitial lung disease, congestive heart failure, chronic obstructive pulmonary disease, or psychogenic causes, such as panic disorder and anxiety.

Typically, the sample according to the invention may be a blood, plasma, serum, lymph or urine sample. In a particular embodiment, said sample is blood or plasma.

Thus, the invention relates to a method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in plasma obtained from said patient.

The term "detecting" as used above includes qualitative and/or quantitative detection (measuring the expression levels) with or without reference to a control. Typically CoQ10H2 and/or CoQ10 expression may be measured for example by HPLC or other techniques.

Particularly, the invention relates to a method for diagnosing heart failure in a patient comprising a step a) consisting of measuring CoQ10H2 and/or CoQ10 expression level in a sample obtained from said patient. Particularly, the method of the invention further comprises a step of comparing the CoQ10H2 and/or CoQ10 expression level obtained in step a) to a threshold level.

Particularly, the invention also relates to a method for diagnosing dyspnea in a patient comprising a step a) consisting of measuring CoQ10H2 and/or CoQ10 expression in a sample

obtained from said patient. Particularly, the method of the invention further comprises a step of comparing the CoQ10H2 and/or CoQ10 expression level obtained in step a) to a threshold level.

The "control" may be a healthy subject, i.e. a subject who does not suffer from any chronic heart failure. The control may also be a subject suffering from heart failure or dyspnea. Preferably, said control is a healthy subject.

For example detecting CoQ10H2 and/or CoQ10 expression levels in sample may be performed by measuring the expression level of CoQ10H2 and/or CoQ10 or enzymes or mRNA levels for enzymes involved in CoQ10H2 and/or CoQ10 synthesis (detailed in Corinne Cluis, PhD Thesis manuscript, June 2014, Montreal, Quebec, CA).

Typically, the detection comprises contacting the sample with selective reagents such as probes, primers or ligands, and thereby detecting the presence, or measuring the amount, of polypeptides or nucleic acids of interest originally present in the sample. Contacting may be performed in any suitable device, such as a plate, microtiter dish, test tube, well, glass, column... In specific embodiments, the contacting is performed on a substrate coated with the reagent, such as a nucleic acid array or a specific ligand array. The substrate may be a solid or semi-solid substrate such as any suitable support comprising glass, plastic, nylon, paper, metal, polymers and the like. The substrate may be of various forms and sizes, such as a slide, a membrane, a bead, a column, a gel, etc. The contacting may be made under any condition suitable for a detectable complex, such as a nucleic acid hybrid or an antibody-antigen complex, to be formed between the reagent and the nucleic acids or polypeptides of the sample.

In a particular embodiment, the expression level of CoQ10H2 and/or CoQ10 may be determined by determining the quantity of mRNA of CoQ10H2 and/or CoQ10 of synthesis enzyme genes in blood cells.

Methods for measuring the quantity of mRNA are well known in the art. For example the nucleic acid contained in the samples (e.g., cell or tissue prepared from the patient) is first extracted according to standard methods, for example using lytic enzymes or chemical solutions or extracted by nucleic-acid-binding resins following the manufacturer's instructions. The extracted mRNA may be then detected by hybridization (e. g., Northern blot analysis).

Alternatively, the extracted mRNA may be subjected to coupled reverse transcription and amplification, such as reverse transcription and amplification by polymerase chain

reaction (RT-PCR), using specific oligonucleotide primers that enable amplification of a region in the considered gene. Preferably quantitative or semi-quantitative RT-PCR is used. Real-time quantitative or semi-quantitative RT-PCR is particularly advantageous. Extracted mRNA may be reverse-transcribed and amplified, after which amplified sequences may be detected by hybridization with a suitable probe or by direct sequencing, or any other appropriate method known in the art.

Other methods of amplification include ligase chain reaction (LCR), transcription-mediated amplification (TMA), strand displacement amplification (SDA) and nucleic acid sequence based amplification (NASBA).

Nucleic acids having at least 10 nucleotides and exhibiting sequence complementarity or homology to the mRNA of interest herein find utility as hybridization probes or amplification primers. It is understood that such nucleic acids need not be identical, but are typically at least about 80% identical to the homologous region of comparable size, more preferably at least 85% identical and even more preferably at least 90%, preferably at least 95% identical. In certain embodiments, it will be advantageous to use nucleic acids in combination with appropriate means, such as a detectable label, for detecting hybridization. A wide variety of appropriate indicators are known in the art including, fluorescent, radioactive, enzymatic or other ligands (e. g. avidin/biotin).

Probes typically comprise single-stranded nucleic acids of between 10 to 1000 nucleotides in length, for instance of between 10 and 800, more preferably of between 15 and 700, typically of between 20 and 500. Primers typically are shorter single-stranded nucleic acids, of between 10 to 25 nucleotides in length, designed to perfectly or almost perfectly match a nucleic acid of interest, to be amplified. The probes and primers are “specific” to the nucleic acids they hybridize to, i.e. they preferably hybridize under high stringency hybridization conditions (corresponding to the highest melting temperature Tm, e.g., 50 % formamide, 5x or 6x SCC. SCC is a 0.15 M NaCl, 0.015 M Na-citrate).

In a particular embodiment, the method of the invention comprises the steps of providing total RNAs obtained from the sample of the patient, and subjecting the RNAs to amplification and hybridization to specific probes, more particularly by means of a quantitative or semi-quantitative RT-PCR.

Total RNAs can be easily extracted from the sample. For instance, the sample may be treated prior to its use, e.g. in order to render nucleic acids available. Techniques of cell or protein lysis, concentration or dilution of nucleic acids, are known by the skilled person.

In another embodiment, the expression level of CoQ10H2 and/or CoQ10 genes may be measured by DNA microarray analysis. Such DNA microarray or nucleic acid microarray consists of different nucleic acid probes that are chemically attached to a substrate, which can be a microchip, a glass slide or a microsphere-sized bead. A microchip may be constituted of polymers, plastics, resins, polysaccharides, silica or silica-based materials, carbon, metals, inorganic glasses, or nitrocellulose. Probes comprise nucleic acids such as cDNAs or oligonucleotides that may be about 10 to about 60 base pairs. To measure the expression level of CoQ10H2 and/or CoQ10 genes, a sample from a test subject, optionally first subjected to a reverse transcription, is labelled and contacted with the microarray in hybridization conditions, leading to the formation of complexes between target nucleic acids that are complementary to probe sequences attached to the microarray surface. The labelled hybridized complexes are then detected and can be quantified or semi-quantified. Labelling may be achieved by various methods, e.g. by using radioactive or fluorescent labelling. Many variants of the microarray hybridization technology are available to the man skilled in the art (see e.g. the review by Hoheisel, Nature Reviews, Genetics, 2006, 7:200-210).

Detection of CoQ10H2 and/or CoQ10 expression level in the sample may also be performed by measuring the level of CoQ10H2 and/or CoQ10 synthesis enzymes. In the present application, the "level of CoQ10H2 and/or CoQ10 synthesis enzymes" means the quantity or concentration of said CoQ10H2 and/or CoQ10 enzymes or their functional activities.

Such methods comprise contacting a sample with a binding partner capable of selectively interacting with CoQ10H2 and/or CoQ10 enzymes present in the sample. The binding partner is generally an antibody that may be polyclonal or monoclonal, preferably monoclonal.

The presence of the molecule can be detected using standard electrophoretic and immunodiagnostic techniques, including immunoassays such as competition, direct reaction, or sandwich type assays. Such assays include, but are not limited to, Western blots; agglutination tests; enzyme-labeled and mediated immunoassays, such as ELISAs; biotin/avidin type assays; radioimmunoassays; immunoelectrophoresis; immunoprecipitation, etc. The reactions generally include revealing labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, or other methods for detecting the formation of a complex between the antigen and the antibody or antibodies reacted therewith. Said

fluorescence-activated cell sorter is a machine that can rapidly separate the cells in a suspension on the basis of size and the color of their fluorescence.

The aforementioned assays generally involve separation of unbound molecule in a liquid phase from a solid phase support to which antigen-antibody complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e. g., in membrane or microtiter well form); polyvinylchloride (e. g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

More particularly, an ELISA method can be used, wherein the wells of a microtiter plate are coated with a set of antibodies against the proteins to be tested. A sample containing or suspected of containing the marker is then added to the coated wells. After a period of incubation sufficient to allow the formation of antibody-antigen complexes, the plate(s) can be washed to remove unbound moieties and a detectably labeled secondary binding molecule is added. The secondary binding molecule is allowed to react with any captured sample marker protein, the plate is washed and the presence of the secondary binding molecule is detected using methods well known in the art.

One preferred method utilizes immunohistochemistry, a staining method based on immunoenzymatic reactions using monoclonal or polyclonal antibodies to detect cells or specific proteins such as tissue antigens. Typically, immunohistochemistry protocols involve at least some of the following steps:

1) antigen retrieval (eg., by pressure cooking, protease treatment, microwaving, heating in appropriate buffers, etc.);

2) application of primary antibody (i.e. anti- CoQ10H2 or anti-CoQ10 enzymes antibody) and washing;

3) application of a labeled secondary antibody that binds to primary antibody (often a second antibody conjugate that enables the detection in step 5) and wash;

4) an amplification step may be included;

5) application of a detection reagent (e.g. chromagen, fluorescently tagged molecule or any molecule having an appropriate dynamic range to achieve the level of or sensitivity required for the assay);

6) counterstaining may be used and

7) detection using a detection system that makes the presence of the proteins visible (to either the human eye or an automated analysis system), for qualitative or quantitative analyses.

Various immunoenzymatic staining methods are known in the art for detecting a protein of interest. For example, immunoenzymatic interactions can be visualized using different enzymes such as peroxidase, alkaline phosphatase, or different chromogens such as DAB, AEC, or Fast Red; or fluorescent labels such as FITC, Cy3, Cy5, Cy7, Alexafluors, etc. Counterstains may include H&E, DAPI, Hoechst, so long as such stains are compatable with other detection reagents and the visualization strategy used. As known in the art, amplification reagents may be used to intensify staining signal. For example, tyramide reagents may be used. The staining methods of the present invention may be accomplished using any suitable method or system as would be apparent to one of skill in the art, including automated, semi-automated or manual systems.

The method of the invention may comprise a further step consisting of comparing CoQ10H2 and/or CoQ10 expression levels with a control reference.

The invention thus relates to a method for diagnosis heart failure in a patient comprising determining the expression level of CoQ10H2 and/or CoQ10 in a sample obtained from said patient and comparing said expression level to a threshold value. As used herein, "expression level of CoQ10H2 or CoQ10" synthesis enzymes refers to an amount or a concentration of a transcription product, for instance mRNA coding for CoQ10H2 or CoQ10 synthesis related enzymes or controlling the quinone synthesis for instance the mRNA level for the CLK1 gene, or of a translation product (Aguilaniu et al., Gene Dev. 2005). Typically, a level of mRNA expression can be expressed in units such as transcripts per cell or nanograms per microgram of tissue. A level of a polypeptide can be expressed as nanograms per microgram of tissue or nanograms per milliliter of a culture medium, for example. Alternatively, relative units can be employed to describe an expression level.

In a preferred embodiment, when the measure of CoQ10H2 or CoQ10 enzymes synthesis expression is performed by rt qPCR, the expression level of the gene in a patient suffering of chronic heart failure is increased by at least 35%, preferably by at least 40%, preferably by at least 50%; preferably by at least 60 %, preferably by at least 70%, preferably by at least 80%, more preferably by at least 90%, even more at least 100% compared to a control reference. In other words, preferably, when the gene expression is measured by rt qPCR, the quantity of mRNA encoding the enzyme in a patient suffering of chronic heart

failure is increased by at least 35%, preferably by at least 40%, preferably by at least 50%; preferably by at least 60 %, preferably by at least 70%, preferably by at least 80%, more preferably by at least 90%, even more at least 100% compared to a control reference.

Typically, a "threshold value", "threshold level" or "cut-off value" can be determined experimentally, empirically, or theoretically. A threshold value can also be arbitrarily selected based upon the existing experimental and/or clinical conditions, as would be recognized by a person of ordinary skilled in the art. Preferably, the person skilled in the art may compare the expression levels of CoQ10H2 or CoQ10 synthesis enzymes related genes obtained according to the method of the invention with a defined threshold value.

Preferably, said threshold value is the mean expression level of CoQ10H2 or CoQ10 synthesis related enzymes of a population of healthy individuals. As used herein, the term "healthy individual" denotes a human which is known to be healthy, i.e. which does not suffer from chronic heart failure, has never been subjected to such chronic heart failure, and does not need any medical care.

Typically, the skilled person in the art may determine the expression level of CoQ10H2 or CoQ10 in a biological sample, preferably blood, of 100 individuals known to be healthy. The mean value of the obtained expression levels is then determined, according to well known statistical analysis, so as to obtain the mean expression level of CoQ10H2 or CoQ10. Said value is then considered as being normal and thus constitute a threshold value. By comparing the expression levels of CoQ10H2 or CoQ10 to this threshold value, the physician is then able to diagnose chronic or acute heart failure. Indeed, by comparing the level of CoQ10H2 or CoQ10 obtained in a biological sample, preferably blood or urine, of a given subject to a threshold value, one can easily determine whether said subject suffers from chronic or acute heart failure or not.

Accordingly, the physician would be able to adapt and optimize appropriate medical care of a subject in a critical and life-threatening condition suffering from chronic heart failure. The determination of said prognosis is highly appropriate for follow-up care and clinical decision making.

Therefore, the invention is drawn to a method for diagnosis of chronic heart failure in a patient comprising the following steps:

a) determining the expression level of CoQ10H2 or CoQ10 in a sample obtained from said patient;

b) determining the mean expression level of CoQ10H2 or CoQ10 in a biological sample of a population of healthy individuals, preferably 100 healthy individuals; and

c) a step of comparing the expression level of CoQ10H2 or CoQ10 obtained of a) to the mean expression level of CoQ10H2 or CoQ10 obtained in b).

The present invention also relates to kits for the diagnosis of chronic heart failure, comprising means for detecting CoQ10H2 or CoQ10 expression level.

According to the invention, the kits of the invention may comprise an anti- CoQ10H2 or anti-CoQ10 synthesis enzymes antibody; and another molecule coupled with a signalling system which binds to said CoQ10H2 or CoQ10 synthesis enzymes antibody.

Typically, the antibodies or combination of antibodies are in the form of solutions ready for use. In one embodiment, the kit comprises containers with the solutions ready for use. Any other forms are encompassed by the present invention and the man skilled in the art can routinely adapt the form to the use in immunohistochemistry.

The present invention also relates to CoQ10H2 or CoQ10 synthesis related enzymes as a biomarker for the diagnosis of chronic heart failure.

In another embodiment, the invention relates to an in vitro method for monitoring a patient's response to chronic heart failure or dyspnea treatment which comprises a step of measuring the expression level of CoQ10H2 or CoQ10 synthesis related enzymes, or a step of measuring the activity of CoQ10H2 or CoQ10 synthesis related enzymes, in a sample from a patient.

Thus, the present invention provides the use of CoQ10H2 or CoQ10 as a biomarker for the monitoring of anti chronic heart failure therapies.

According to the invention, the expression level of CoQ10H2 or CoQ10 synthesis related enzymes may be determined to monitor a patient's response to chronic heart failure treatment.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1: ROC curve analysis for Q10 levels (n=116), Control patients (n=53) vs heart failure patients (n=63) patients. AUCs were

0.8137 (CI 95%: 0.7356 to 0.8919) - P value < 0.0001.

Figure 2: CoQ10H2 and CoQ10 plasma concentration levels in patients.

Left column: concentration of CoQ10H2; Right column: concentration of CoQ10. T= control patients; D= dyspnea patients ; IC= heart failure patients.

Table 1: Statistical data of the ROC curve (figure 1)

Area under the ROC curve	
Area	0.8137
Std. Error	0.03987
95% confidence interval	0.7356 to 0.8919
P value	< 0.0001
Data	
Control	53
IC Patient	63
Missing Controls	0
Missing Patients	0

EXAMPLE:

Material & Methods

Patient Inclusions

All the 116 subjects underwent transthoracic echocardiography for left ventricular ejection fraction (LVEF) assessment (Fig. 1). Control patients (C) without cardiovascular risk or echocardiographic abnormalities were recruited from the general population. Individuals with dyspnea and normal left ventricular ejection fraction (D) and patients with stable systolic chronic heart failure (IC) were recruited from the cardiology department at Rangueil Hospital, Toulouse. The inventors used a threshold value of LVEF <45% to sort individuals into 3 groups:

C, healthy control

D, patients with LVEF ≥45%,

IC, chronic heart failure patients with LVEF <45%.

They monitored 63 IC cases out of the 116 subjects tested. The inventors defined two comparative groups:

1- (C) individuals with cardiovascular risk factors and normal left ventricular ejection fraction (62 <LVEF <81%);

2- (D) individuals with cardiovascular risk factors and dyspnea and normal left ventricular ejection (62 <LVEF <81%); <44%).

3- (IC, positive control) patients with chronic stable systolic heart failure (18 <LVEF <44%). Moreover, all individuals were extensively phenotyped to check for clinical and biochemical parameters as shown in table 1.

Groups	T	D	IC
LVEF %	73 (65-81)	71 (62-81)*	33 (18-44)
Age (years)	55 (72-45)	55 (69-38)	55 (83-23)
Male %	78	78	67
Hypertensive %	0	44	44
Diabetes %	0	33	33
Obesity %	0	44	56
Dyslipidemia %	0	56	44
Heredity %	0	33	11
BMI	24 ± 3	28 ± 3	30 ± 5
Systolic blood pressure (mm Hg)	128 ± 16	138 ± 12	124 ± 19
Diastolic blood pressure (mm Hg)	81 ± 9	80 ± 14	76 ± 9
Etiologies			
Ischemic %	_	_	33
Hypertensive %	_	_	11
Valvular %	_	_	11
Idiopatic dilated %	_	_	44
Alcool abuse %	_	_	0
Atrial fibrillation %	_	_	33

Labs (mean ± s.d.)			
BNP (pg/ml)	11 ± 9	15 ± 10	164 ± 151=
Na+ (mM)	140 ± 1	140 ± 1	138 ± 4
creatinine (µM)	80 ± 6	83 ± 9	96 ± 23
Hb (g/dl)	14.7 ± 1.1	14.3 ± 1.1	14.1 ± 1.8
Leukocytes (cells/µl)	5735 ± 1225	6159 ± 1433	6765 ± 1533
Lymphocytes (cells/µl)	1865 ± 412	1988 ± 428	1918 ± 499
Neutrophils (cells/µl)	3498 ± 898	3630 ±1073	4107 ± 1153

Table 2: Cardiovascular risk factors, clinical and biochemical parameters of study groups

In this table 2, proportion of individuals with the indicated risk factor for each of the 4 groups of the study is indicated as percentages or averaged value:

(C) Individuals with cardiovascular risk factors and normal left ventricular ejection fraction (62 <LVEF <81%);

(D) Individuals with cardiovascular risk factors and dyspnea and normal left ventricular ejection (62 <LVEF <81%); <44%).

(IC, positive control) patients with chronic stable systolic heart failure (18 <LVEF <44%).

All individuals were extensively phenotyped to check for clinical and biochemical parameters. * indicates P <0.05 for statistical comparison between groups. = indicates P <0.05 for statistical comparison between ALVD and IC groups. BNP, B-type natriuretic peptide.

Analysis of CoQ10H2 and CoQ10 plasma levels

Blood sampling was performed on dry tubes containing EDTA.

CoQ10H2 and CoQ10 were monitored by HPLC. The chromatographic system consisted of an isocratic solvent delivery pump (Thermo Scientific SpectraSystem P4000), with a 50 mm × 2.1 mm reverse phase micro column C18 particle diameter 3.5 µm (Waters X-Terra). Methanol was used as mobile phase with a flow of 0.4 ml/min. The column temperature was set at 25°C. Ten microliter of each sample was introduced into the column

using an automatic injector (Thermo Scientific SpectraSystem AS3000). The column effluent was monitored with a dual wavelength ultraviolet detector (Thermo Scientific SpectraSystem UV2000) set at 275 nm

A standard solution was prepared solubilizing CoQ10 and CoQ10H2 in ethanol to obtain a concentration of 1 mg/ml, then an appropriate dilution was made in blank of excipients of each formulation and finally in mobile phase. The final concentration of the CoQ working standard solutions was 2 μg/ml.

Results

Control patients had a Q10H2 plasma concentration of 783 ± 68 nM, whereas plasmatic levels in dyspnea patients and in HF patients were significantly reduced with 396 ± 50 and 414 ± 33 nM, respectively. Control patients had a Q10 plasma concentration of 83 ± 13 nM, whereas plasmatic levels in dyspnea patients and in IC patients were not significantly increased with 141 ± 27 and 112 ± 16 nM, respectively.

ROC curve analysis for Q10H2 levels revealed that Q10H2 levels have discriminant power for HF diagnostic with and AUC of 0.8137 (CI 95%: 0.7356 to 0.8919) - P value < 0.0001 (Figure 2 and Table N).

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

Corinne Cluis, PhD Thesis manuscript, June 2014, Montreal, Quebec, CA

CLAIMS:

1. A method for diagnosing heart failure or a dyspnea in a patient comprising a step consisting of detecting Ubiquinol (CoQ10H2) and/or Ubiquinone (CoQ10) expression level in a sample obtained from said patient.

2. A method according to claim 1 wherein the heart failure is a chronic or an acute heart failure.

3. A method according to claims 1 or 2 wherein the sample is blood, plasma, serum, lymph or urine.

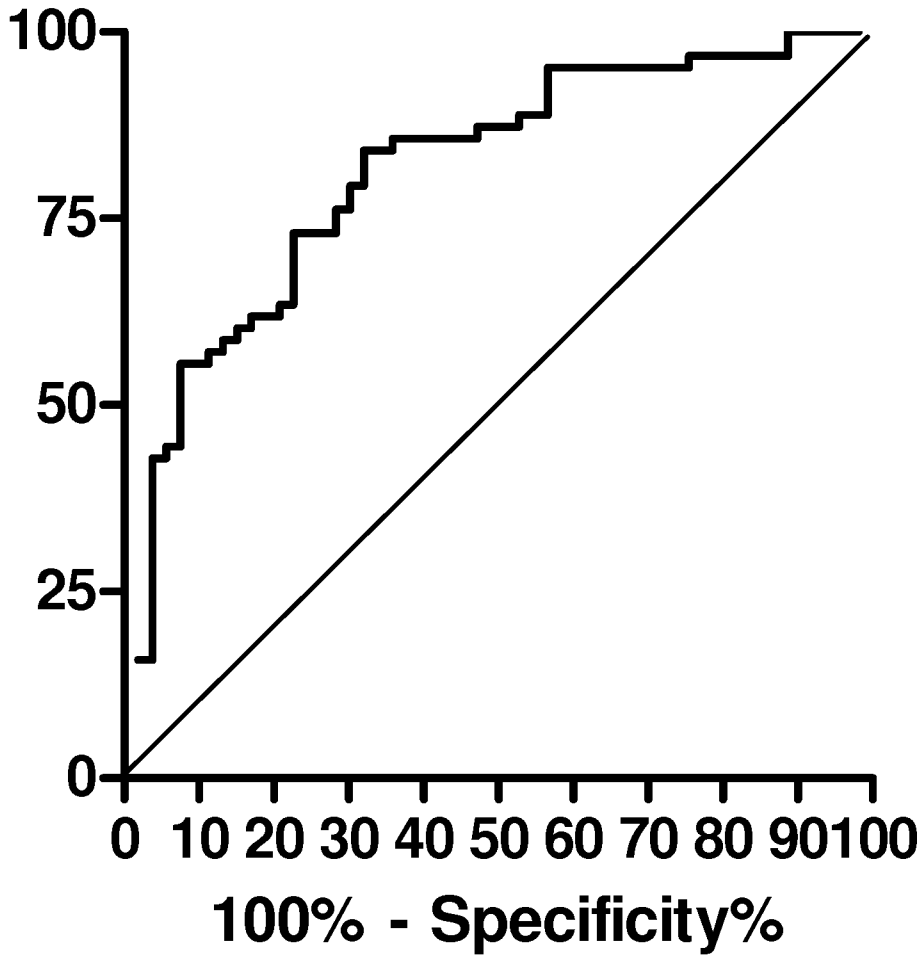


Figure 1

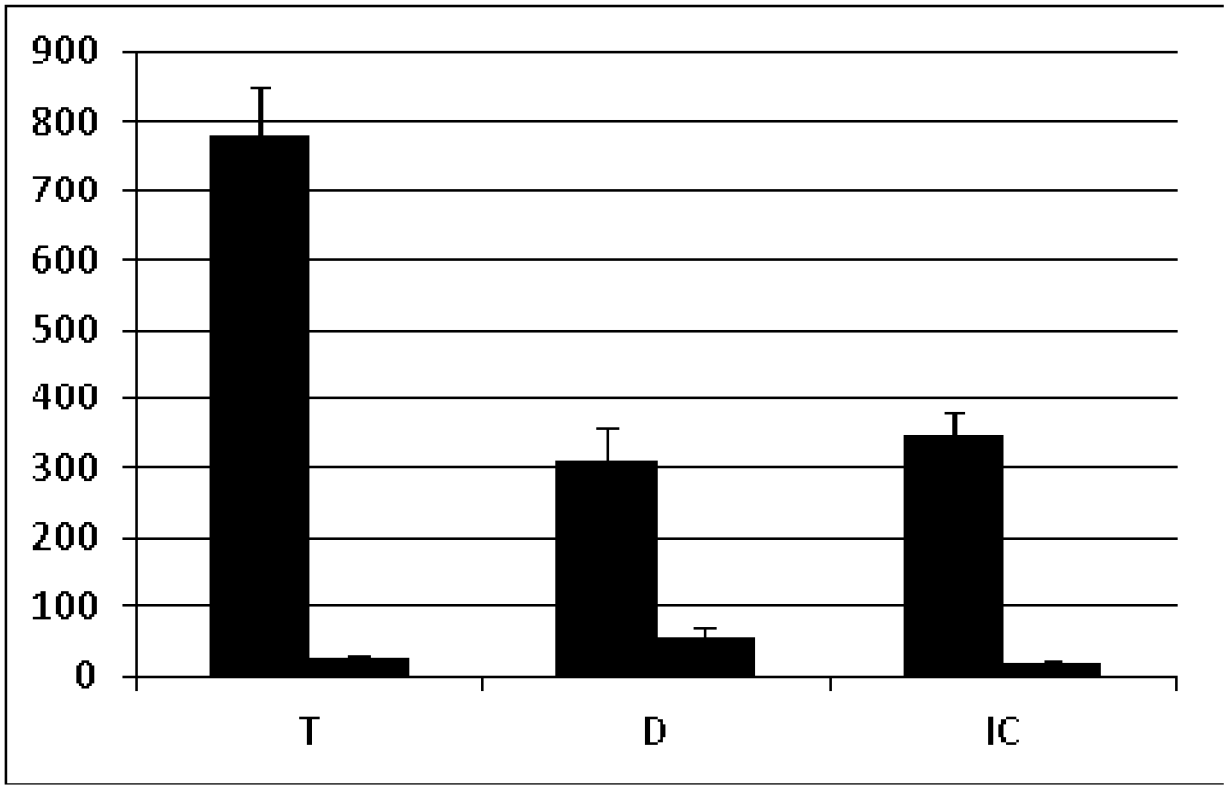


Figure 2

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/061373

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/92 G01N30/00 G01N33/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMONE ONUR ET AL: "Association between serum level of ubiquinol and NT-proBNP, a marker for chronic heart failure, in healthy elderly subjects", BIOFACTORS, vol. 41, no. 1, 2 January 2015 (2015-01-02), pages 35-43, XP055205021, ISSN: 0951-6433, DOI: 10.1002/biof.1198 the whole document ----- -/--	1-3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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Date of the actual completion of the international search	Date of mailing of the international search report
22 June 2016	30/06/2016

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLYNEUX S L ET AL: "Coenzyme Q10", JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY, vol. 52, no. 18, 28 October 2008 (2008-10-28), pages 1435-1441, XP025572005, ELSEVIER, NEW YORK, NY, US ISSN: 0735-1097 [retrieved on 2008-10-21] the whole document	1-3
X	SENES MEHMET ET AL: "Coenzyme Q10 and high-sensitivity C-reactive protein in ischemic and idiopathic dilated cardiomyopathy", CLINICAL CHEMISTRY AND LABORATORY MEDICINE, vol. 46, no. 3, 2008, pages 382-386, XP009185604, ISSN: 1434-6621 the whole document	1-3
X	FOLKERS K ET AL: "Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10.", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA FEB 1985, vol. 82, no. 3, February 1985 (1985-02), pages 901-904, XP002742890, ISSN: 0027-8424 abstract, p. 902, Table 1	1-3
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