(54) **Title:** USE OF THE SOLUBLE FORM OF AXL IN THE DIAGNOSIS AND/OR PROGNOSIS OF HEART FAILURE SYNDROME

FIG. 1
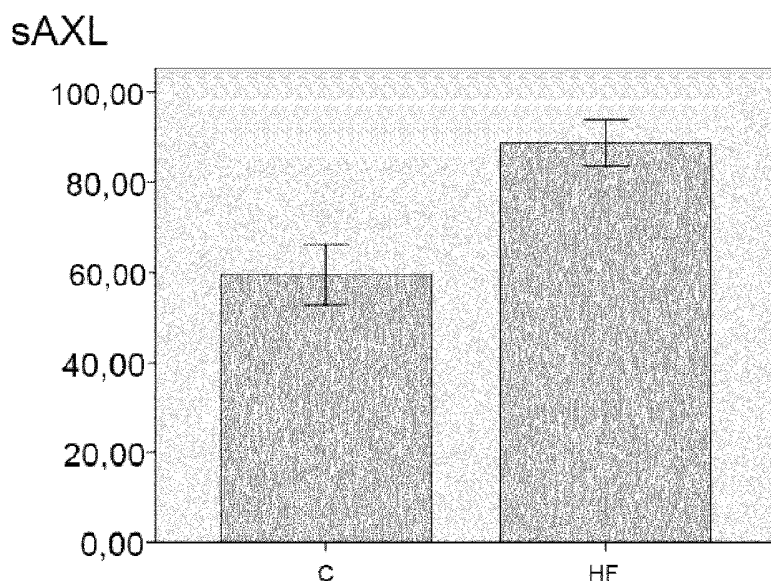
(57) **Abstract:** The present invention refers to a method for diagnosing and/or prognosing a heart failure syndrome (HF), which comprises the step of measuring, in vitro, the amount of the soluble form of AXL (sAXL) in a test sample. The invention furthermore provides methods for deciding or recommending whether to initiate a medical regimen, as well as for determining the efficacy of said medical regimen. The invention also provides the use of sAXL as a marker for the diagnosis and/or prognosis of HF sAXL is stable in serum and is differentially expressed in controls and people suffering from HF.

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Use of the soluble form of AXL in the diagnosis and/or prognosis of heart failure syndrome

The present invention is generally related to diagnostic and prognostic assays. Particularly, the present invention refers to the identification and use of sAXL as a biomarker that is differentially expressed in patients suffering from heart failure syndrome.

BACKGROUND ART

The Heart Failure (hereinafter also referred as "HF") syndrome can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood. HF constitutes the endstage of many cardiopathies and is characterized by a ventricular dysfunction.

In 2008, the European Society of Cardiology (ESC) Guidelines for HF (Dickstein K et al., 2008) pointed out that the prevalence of HF is between 2-3% and rises sharply at 75 years of age, being the prevalence in 70-80 year old people between 10-20%. The overall prevalence of HF is increasing because of the ageing of the population, the success in prolonging survival in patients suffering coronary events, and the success in postponing coronary events by effective prevention. HF is the cause of 5% of acute hospital admissions, is the cause of 10% of patients in hospital beds and accounts for approximately 2% of national cost on health, mostly due to the cost of hospital admissions.

Two classifications of the severity of HF are employed (Dickstein K et al., 2008):

a) The New York Heart Association (NYHA) classification, which is based on symptoms and functional capacity. This classification has been proved to be clinically useful and it is used in most clinical trials. It classifies patients in four different classes:
   Class I, no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation or dyspnoea.
   Class II, slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation or dyspnoea.

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Class III, marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in fatigue, palpitation or dyspnoea.

Class IV, unable to carry on any physical activity without discomfort. Symptoms at rest. If any physical activity is undertaken, discomfort is increased.

b) The American College of Cardiology/American Heart Association (hereinafter also referred as "ACC/AHA") (Jessup M. et al., 2009), which is based on the structural changes and symptoms of HF.

HF diagnosis is complex and requires a clinical examination, an electrocardiogram, a chest X-ray and an echocardiography. Several diagnostic tests are employed routinely to confirm HF but are more sensitive for the detection of patients with reduced ejection fraction than for patients with preserved ejection fraction (Dickstein K et al., 2008).

In this regard, natriuretic peptides, B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) have been proposed as HF diagnostic tools, because elevated levels thereof lend support to a diagnosis of abnormal ventricular function or hemodynamics causing symptomatic HF. Elevated levels of natriuretic peptides should not be used in isolation to confirm or exclude the presence of HF. They may give weight to a suspected diagnosis of HF or trigger consideration of HF when the diagnosis is unknown (Jessup M. et al., 2004)

The main disadvantages for using natriuretic peptides as tools for HF diagnosis is their lability at room temperature and the cost of its measurement (Tang W. H. et al., 2009). Once the blood is retrieved from the patient, a cold cycle for preservation of BNP or NT-proBNP has to be established. Samples should be collected in iced tubes and processed rapidly to avoid *in vitro* degradation. These issues make the measurement of their levels a cumbersome process not available in many clinical settings. All these obstacles make it interesting to devise new methods to help diagnose and categorize HF.

In spite of the efforts made, therefore, there is still the need of further HF

diagnostic tools.

SUMMARY OF THE INVENTION

The inventors have revealed that the soluble form of AXL (hereinafter also referred as "sAXL") represents a newly realized biomarker particularly advantageous for predicting, diagnosing and/or prognosing heart failure (HF) syndrome.

In particular, the present inventors have found that the soluble form of the AXL protein (hereinafter also referred as "sAXL") is increased in serum from HF patients compared to the levels detected in serum from subjects with no HF (see FIG. 1).

Furthermore, the inventors have found that the amount of sAXL is increased with NYHA functional classes, being the difference among functional classes statistically significant (see FIG. 2). This is indicative that sAXL provides useful prognosis information: higher sAXL level as more severe is the HF syndrome.

Thus, in a first aspect the present invention provides a method for diagnosing and/or prognosing a heart failure syndrome (HF), said method comprising the step of measuring, *in vitro*, the amount of the soluble form of AXL (sAXL) in a test sample.

As stated above, natriuretic peptides are used in the state of the art to determine HF syndrome. Said peptides are unstable and, if no adequate processing of the sample taken from the subject is made, the peptide is degraded, not being possible its detection. As a consequence of its degradation, the non-detection of said peptides in a test sample gives rise to false negative HF determinations, with the serious implications for the patient suffering the syndrome.

Surprisingly, and contrary to natriuretic peptides, sAXL is stable in serum. This confers two important advantages to the method of the first aspect of the invention when compared to the use of natriuretic peptides. The first one is that no cold preservation is needed for processing the sample; being the

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detection step of the method of the invention simpler. The second one is that using the method of the first aspect of the invention there can be a reduction of the false negative determinations associated with the use of natriuretic peptides as biomarkers, because no degradation of sAXL occurs.

Furthermore, it has been found that the difference in the sAXL expression profile between control subjects and subjects suffering from HF is so noticeable that a skilled doctor, with suitable means, would have a new tool to help in the diagnosis of the syndrome, with the resulting advantages for designing a suitable treatment.

In addition, the fact that the amount of sAXL measured in the test sample is compared with a control sample gives information about the prognosis of the syndrome: the higher the value of sAXL of a patient compared to the upper limit of sAXL reference range, the worse prognosis.

The method object of the first aspect of the invention can be useful in ruling out and ruling in HF in an acute setting with untreated patients; and stratifying the population, among others.

In addition, the method of the present invention is useful in diagnosing and/or prognosing HF associated with other previous cardiovascular diseases such as acute myocardial infarction or atrial fibrillation.

From the experimental data included in the present application, it can also be concluded that sAXL can be used as a marker for determining the risk for developing HF by performing the following steps:

a) determining, *in vitro*, the amount of sAXL in a test sample of the subject; being the subject a person with no heart failure syndrome but suspected that could develop it in the future; and

b) determining whether the sAXL amount determined in step (a) falls within a sAXL concentration reference range obtained from a group of subjects which do not have HF,

wherein, if the amount of sAXL in the test sample is close to the upper limit of

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the sAXL reference range, but without overcoming the upper value of said range, it is indicative that the patient has the risk of developing HF.

In a second aspect, the present invention provides a method of deciding or recommending whether to initiate a medical regimen of a subject suspected of suffering a heart failure syndrome, which method comprises the steps of:

a) determining, *in vitro*, the amount of sAXL in a test sample of the subject; and

b) diagnosing the heart failure syndrome if the sAXL amount in the test sample is higher than the upper limit of the sAXL concentration reference range obtained from a group of subjects which do not have HF, as defined in the method of the first aspect of the invention;

wherein:

i) if the subject is diagnosed of suffering a heart failure syndrome, then the initiation of the medical regimen is recommended, and

ii) if the patient is diagnosed of not suffering the heart failure syndrome, the follow-up of heart function is performed, optionally in consideration of the result of an examination of the patient by a cardiologist.

Determining the sAXL level in a test sample, the skilled person can establish, additionally, which is the most suitable therapy that can be recommended, because the level detected in the sample may reflect the extension (i.e., severity) of the syndrome.

Furthermore, once it has decided to initiate the medical regimen because the subject is suffering from HF syndrome (following the method defined in the second aspect of the invention), it can be monitored how efficient is the regimen: a decrease or return to a normal level of sAXL (i.e., to the level within the sAXL concentration reference range) can indicate that the HF patient has reacted favorably to the medical regimen and, therefore, said regimen is effective; if the level of sAXL does not significantly change or it increases, this can indicate that the medical regimen is not effective. Finally,

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the level of sAXL can be measured at the end of the treatment as part of the monitoring care for controlling relapses.

Therefore, in a third aspect, the present invention provides a method for determining the efficacy of a medical regimen in a patient already diagnosed of heart failure syndrome, the method comprising the steps of:

(a) *in vitro* measuring the amount of sAXL in a sample from the patient prior to initiating the medical regimen;
(b*) in vitro* measuring the amount of sAXL in a sample from the patient once started the medical regimen; and
(c) comparing the amounts measured in steps (a) and (b), in such a way that if the sAXL amount measured in step (b) is lower than the sAXL amount measured in step (a), it is indicative that the medical regimen is effective in the treatment of HF;

or, alternatively, the method comprising the steps of:

(i) *in vitro* measuring the amount of sAXL in a sample from the patient once initiated the medical regimen; and

(ii) determining whether the amount measured in step (i) falls within a sAXL concentration reference range obtained from a group of subjects which do not have HF,

wherein, if the sAXL amount measured in step (i) is within the sAXL concentration reference range, it is indicative that the medical regimen is effective in the treatment of HF.

In a fourth aspect, the present invention provides the use of sAXL as a marker for the diagnosis and/or prognosis of heart failure syndrome.

In a fifth aspect, the present invention provides the use of means for detecting the presence of sAXL in an isolated sample for the diagnosis and/or prognosis of heart failure syndrome in any of the methods defined above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar representation showing the sAXL levels in serum from controls and HF patients. Particularly, each bar represents the mean sAXL concentration value and the error bars ± 2X standard error. Y-axis: sAXL= Mean sAXL level in serum (ng/ml); X-axis: C= serum from subjects that do not have HF selected for establishing the reference range, HF= serum from HF patients.

FIG. 2 is a bar representation showing the sAXL levels in serum from HF patients (n=149) belonging to NYHA I-IV functional classes. Particularly, each bar represents the mean sAXL concentration value and the error bars ± 2X standard error. Y-axis: sAXL= Mean sAXL level in serum (ng/ml); X-axis: I= patients suffering from NYHA functional Class I, II= patients suffering from NYHA functional Class II, III= patients suffering from NYHA functional Class III, and IV= patients suffering from NYHA functional Class IV.

FIG. 3 shows the sAXL and BNP circulating levels in samples from HF patients, n=156. Y-axis: sAXL= sAXL level in serum (ng/ml); X-axis: BNP= BNP level in plasma (pg/ml).

DETAILED DESCRIPTION OF THE INVENTION

As stated above, the present invention provides a method for diagnosing and prognosing a heart failure syndrome, said method being based on the measurement of the amount of sAXL.

AXL belongs to the receptor tyrosine kinases (RTKs) family. RTKs are membrane proteins recognizing extracellular signals leading to cellular responses as proliferation, arrest or activation. AXL sequence is available in several databases, such as NCBI databases (NP_068713.2) and Uniprot (database accession number: P30530; database version: 133).

In the present invention, the terms "soluble form of AXL" and "sAXL", have the same meaning and are interchangeable. The soluble form of AXL is the extracellular domain of AXL, which is located between the aminoacidic residues 26 to 451 of AXL protein sequence. Particularly, sAXL corresponds

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to SEQ ID NO: 1:

SEQ ID NO: 1

APRGT QAEESPFVGN PGNITGARGL TGTLRCQLQV QGEPPEVHWL
RDGQILELAD STQTQVPLGE DEQDDWIVVS QLRITSLQLS DTGQYQCLVF
LGHQTFVSQP GYVGLEGLPY FLEEPEDRTV AANTPFNLSC
QAQGPPEPVD LLWLQDAVPL ATAPGHGPQR SLHVPGLNKT
SSFSCEAHNA KGVTTSRTAT ITVLPQQPRN LHLVSRQPTE LEVAWTPGLS
GIYPLTHCTL QAVLSNDGMG IQAGEPDPPE EPLTSQASVP PHQLRLGSLH
PHTPYHIRVA CTSSQGPSSW THWLPVETPE GVPLGPPENI
SATRNGSQAF VHWQEPRAPL QGTLLGYRLA YQGQDTPEVL
MDIGLRQEVT LELQGDGSVS NLTVCVAAYT AAGDGPWSLP
VPLEAWRPGQ AQPVHQLVKE PSTPAFSWPW W

The term "heart failure" is to be understood as a syndrome in which the
patients have the following features (Dickstein K et al., 2008): (a) symptoms
such as shortness of breath at rest or during exertion, and/or fatigue; (b)
signs of fluid retention such as pulmonary congestion or ankle swelling; and
(c) objective evidence of an abnormality of the structure or function of the
heart at rest.

The HF syndrome can result from any structural or functional cardiac disorder
that impairs the ability of the ventricle to fill with or eject blood (Hunt S. A. et
al., 2005) and constitutes the endstage of many cardiopathies and is
characterised by a ventricular dysfunction. The most common causes of HF
are acute or chronic ischaemia, hypertension, tachyarrhytmias, valvular
syndrome and cardiomyopathies (such as hypertrophic, dilated, restrictive,
arrhythmogenic right ventricular, myocarditis, among others). HF can also be
induced by myocardial damage by drugs, toxins, nutritional or endocrine
disorders and infiltrative cause such as sarcoidosis, amyloidosis, among
others. Other causes of HF can be Chagas´ disease, HIV infection,
peripartum cardiomyopathy and end-stage renal failure (Dickstein K et al.,
2008).

In the present invention the "sAXL concentration reference range obtained
from a group of subjects which do not have HF" is to be understood as the
control reference range in order to perform any of the methods object of the
present invention. The subjects (controls) selected to determine the sAXL

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concentration reference range are chosen on the basis of the requirement that such subjects do not have HF. In order to determine the sAXL concentration range, it is determined the sAXL level in a sample taken from each subject (the sample being serum or plasma) and with the results obtained the 95% confidence limits of the reference group is statistically calculated.

The "test sample" refers to a sample obtained for the purpose of diagnosis and prognosis. In certain embodiments, such a sample may be obtained for the purpose of determining the outcome of an ongoing condition or to determine whether a medical regimen should be applied to a subject. Preferably the test sample is selected from the group consisting of: serum, plasma. More preferably, the test sample is serum.

The term "diagnosis" is known to the person skilled in the art. Diagnosing is understood as becoming aware of a particular medical condition, syndrome, complication or risk.

The term "subject" used herein and according to the present invention relates to a healthy individual, an apparently healthy individual, or an individual suffering from a syndrome (patient). The subject can be both a male and a female individual; particularly the patient is suffering from HF.

In the present invention, the expression "medical regimen" is to be understood as encompassing either pharmacological therapies (such as ACE-inhibitors, beta blockers, angiotensin receptor blockers, aldosterone antagonists, and digoxin, among others) as well as other clinical decisions taken by the cardiologist concerning, for instance, hospitalization or discharge decisions, or dietary or social habits pointed by the doctor such as salt ingestion or physical activity.

In the present invention, the expression "optionally in consideration of the result of an examination of the patient by a cardiologist" concerning the method of the second aspect of the invention, encompasses any clinical examination test useful in providing evidence of heart failure, such as electrocardiography, echocardiography, and chest X-ray, among others.

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In a preferred embodiment of the first aspect, the method comprises the steps of:

> (a) measuring, *in vitro*, the amount of sAXL in the test sample; and
> (b) determining whether the amount determined in step (a) falls within a sAXL concentration reference range obtained from a group of subjects which do not have HF,

wherein, if the amount of sAXL in the test sample is higher than the upper limit of the sAXL reference range, it is indicative that the patient is suffering or has a bad prognosis of HF syndrome.

In a preferred embodiment of the method of the first, second, and third aspects of the invention, the amount of sAXL protein present in the sample is determined.

In order to carry out the protein detection, an antibody or a fragment thereof with the ability of binding to sAXL can be used.

In the present invention, the term "antibody or a fragment thereof with the ability of binding to sAXL" is to be understood as any immunoglobulin or fragment thereof able to bind the antigen defined by sAXL. It includes monoclonal and polyclonal antibodies. The term "fragment thereof" encompasses any part of an antibody having the size and conformation suitable to bind an epitope of sAXL. Suitable fragments include F(ab), F(ab') and Fv. An "epitope" is the part of the antigen being recognized by the immune system (B-cells, T-cells or antibodies). Suitable antibodies may be those mentioned in the example below, such as AF154 (R&D systems), which is derived from a mouse myeloma cell line NS0 derived recombinant human AXL Glu33Pro440 (Accession # AAA61243); and PAB2998 (Abnova).

In a preferred embodiment, the antibody or fragment thereof for detecting sAXL is included in a kit. The kit may additionally comprise means (additives, solvents) to visualize the antigen-antibody interactions (dipsticks, chemiluminescent reagents, turbidimetric reagents, etc.). Suitable additives, solvents and reagents to visualize the antigen-antibody interaction are disclosed in the examples.

In another preferred embodiment, the methods of the first, second and third

aspect of the invention, are competitive enzyme linked immunosorbent assay (ELISA) methods. In this embodiment, the *in vitro* measuring of sAXL amount (either in the group of subjects used for determining the sAXL concentration reference range or the test sample) is performed using a sAXL capture antibody, preferably a sAXL polyclonal capture antibody.

Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps. Furthermore, the word "comprise" and its variations encompasses the term "consisting of". Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples and drawings are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

EXAMPLES

1. Patient enrolment and collection of clinical data

This was a prospective study of HF patients with ischemic, idiopathic, valvular and other ethiologies who belong to New York Heart Association (NYHA) functional classes I, II, III or IV. These patients underwent a history and echocardiography examination. Clinical data such as HF evolution time, number of meters walked in the 6 minute walking test, cardiovascular risk factors (hypertension, dyslipidaemia, diabetes mellitus and smoking), and medication were entered for analysis, along with ventricular dilation parameters measured by echocardiography, such as left ventricle end-systolic diameter (LVESD), left ventricle end-dyastolic diameter (LVESD) and EF ("ejection fraction") as a parameter of systolic function.

The Ethical Committee of the affiliated institution approved the protocol and all subjects gave written, informed consent to participate in this study.

Patients were excluded if they were affected by other fibroproliferative diseases such as; kidney failure, cirrhosis, bone metabolic disease,

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hyperthyroidism, pulmonary fibrosis, systemic sclerosis and macular degeneration.

All subjects selected for determining the sAXL reference range (hereinafter also referred "control subjects") have no heart failure. A subgroup of these subjects did not present any heart failure symptoms and another subgroup of these subjects were clinically evaluated by a cardiologist to rule out HF.

2. Collection of serum samples

Blood samples were collected from an antecubital vein from 188 HF patients and from 66 controls.

Serum samples for sAXL analysis were kept at room temperature for at least 30 minutes after blood extraction, to let the clot to be formed and were later centrifuged at a relative centrifugal force (RCF) 1800 x g for 10 minutes at room temperature. The supernatant was collected, aliquoted and kept at −80 °C until analysis.

To measure circulating levels of Brain Natriuretic Peptide (BNP) whole blood was collected in a chilled tube with the anticoagulant EDTA. The sample was promptly put on ice after blood extraction and was centrifuged at RCF 1800 x g for 10 minutes at 4 °C. The supernatant was collected, aliquoted and kept at −80 °C until analysis.

3. Collection and data analysis

Statistics were calculated with the SPSS software, version 18.0 (SPSS Institute Inc, Cary, NC, USA). The non-parametric Mann-Whitney test was applied to compare the sAXL values between controls and HF patients. The non-parametric Kruskal Wallis test was applied to analyze for sAXL differences among functional classes in HF patients. The Spearman correlation coefficient was used to analyse for putative association among the circulating sAXL and BNP levels. Numerical values in the text are mean sAXL values ± standard error of the mean. In all cases, a p value<0.05 was considered to be statistically significant.

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In this study an ELISA method to quantify soluble AXL levels in serum was designed. For the determination of sAXL, the polyclonal antibody AF154 (R&D systems) was used as capture antibody, and the biotinylated BAF154 (R&D Systems) was used as detection antibody.

Multiwell plates (96 wells, Rubilabor) were coated with the capture antibody AF154 at a concentration of 2.0 μg/mL in phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2, filtered through a 0.2 μm pore). The plate is sealed and left overnight at room temperature. The plates were washed three times with wash buffer (PBS with 0,05% Tween20) and then plates were blocked for 1 hour at room temperature with PBS containing 1 % BSA (Sigma).

Serum samples were diluted 200 times in PBS containing 1% bovine serum albumin (BSA). A standard curve was made by serial dilution of a purified sAXL protein produced by recombinant expression of the extracellular part of AXL obtained from R&D systems (154-AL-100). A negative control without serum sample was used and pooled-plasma samples were used in each plate. After sample addition, the plates were washed three times with wash buffer (PBS with 0,05% Tween20). Then, 100 μL of the BAF154, diluted in PBS containing 1% BSA was added at a concentration of 50 ng/mL and incubated 2 hours at room temperature. The aspiration/wash steps were repeated and 100 μL of the working dilution of Streptavidin-HRP (Sigma) were added to each well. Cover the plate and incubate for 20 minutes at room temperature. The aspiration/wash steps are repeated and a solution of tetramethyl-benzidine (slow kinetic form; Sigma) was added to the well. The reaction was stopped with sulfuric acid at 1M concentration. The absorbance of wells was determined with an automatic plate reader at 450 nm.

4. Results:

In FIG. 1 it is shown that sAXL levels in serum from HF patients is statistically higher (88.5±2.55 ng/ml, n=188) than in serum from control subjects (59.4±3.4 ng/ml, n=66, p<0.001*, Mann-Whitney test). This is indicative that sAXL is a good diagnostic marker of HF syndrome.

In FIG. 2 it is shown the mean sAXL values ± 2x standard error of the mean

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for each one of the four classes distinguished by NYHA:

Class I: 65.4±26.7 ng/ml (n=2);
Class II: 78.7±2.2 ng/ml (n=98),
Class III: 108.4±7.1 ng/ml (n=45), and
Class IV: 117.2±19 ng/ml (n=5).

From the results obtained, it can be concluded that sAXL levels increase with NYHA functional classes and, therefore, with the severity of the syndrome. The difference among functional classes is statistically significant (p<0.001*, Kruskal Wallis test).

Therefore, it can be concluded that sAXL is a prognosis marker of HF: the higher the value of sAXL of a patient compared to sAXL levels in controls, the worse the prognosis.

In FIG. 3 it is shown a significant correlation between sAXL and BNP circulating levels in HF patients (n= 156, p<0.001, R= 0.304, Spearman Test).

sAXL levels correlate with BNP levels, the current marker of HF. Due to this correlation, it can be concluded that sAXL is a marker of diagnosis and prognosis of HF, providing technical advantages to BNP measurements due to its stability in serum (in contrast to BNP, which is unstable).

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CLAIMS

1.- A method for diagnosing and/or prognosing a heart failure syndrome (HF), said method comprising the step of measuring, *in vitro*, the amount of the soluble form of AXL (sAXL) in a test sample.

2.- The method of claim 1 comprising the steps of

> (a) measuring, *in vitro*, the amount of sAXL in the test sample; and
> (b) determining whether the amount determined in step (a) falls within a sAXL concentration reference range obtained from a group of subjects which do not have HF,

wherein, if the amount of sAXL in the test sample is higher than the upper limit of the sAXL reference range, it is indicative that the patient is suffering or has a bad prognosis of HF syndrome.

3.- The method of any of the preceding claims, wherein it is determined the protein amount of sAXL in the test sample.

4.- The method of any of the preceding claims, wherein the test sample is selected from the group consisting of: serum, and plasma.

5.- A method of deciding or recommending whether to initiate a medical regimen of a subject suspected of suffering a heart failure syndrome, which method comprises the steps of:

a) determining, *in vitro*, the amount of sAXL in a test sample of the subject; and

b) diagnosing the heart failure syndrome if the sAXL is higher than the upper limit of the sAXL concentration reference range obtained from a group of subjects which do not have HF;

wherein:

i) if the subject is diagnosed of suffering a heart failure syndrome, then the

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initiation of the medical regimen is recommended, and

ii) if the patient is diagnosed of not suffering the heart failure syndrome, the follow-up of heart function is performed optionally in consideration of the result of an examination of the patient by a cardiologist.

6.- A method for determining the efficacy of a medical regimen in a patient already diagnosed of heart failure syndrome, the method comprising the steps of:

(a) *in vitro* measuring the amount of sAXL in a sample from the patient prior to the medical regimen:
(b*) in vitro* measuring the amount of sAXL in a sample from the patient once started the medical regimen; and
(c) comparing the amounts measured in steps (a) and (b), in such a way that if the sAXL amount measured in step (b) is lower than the sAXL amount measured in step (a), it is indicative that the medical regimen is effective in the treatment of HF;

or, alternatively, the method comprising the steps of:

(i) *in vitro* measuring the amount of sAXL in a sample from the patient once started the medical regimen; and

(ii) determining whether the amount measured in step (i) falls within a sAXL concentration reference range obtained from a group of subjects which do not have HF,

wherein, if the sAXL amount measured in step (i) is within the sAXL concentration reference range, it is indicative that the medical regimen is effective in the treatment of HF.

7.- The methods of any of claims 5-6, wherein it is determined the protein amount of sAXL in the test sample.

8.- The methods of any of claims 5-7, wherein the test sample is selected from the group consisting of: serum and plasma.

9. The method of any of claims 1-8, which is a competitive enzyme linked immunosorbent assay (ELISA) method.

10. The method of claim 9, wherein the *in vitro* measuring of sAXL amount is performed using a sAXL capture antibody.

11.- Use of sAXL as a marker for the diagnosis and/or prognosis of heart failure syndrome.

12.- Use of means for detecting the presence of sAXL in an isolated sample for the diagnosis and/or prognosis of heart failure syndrome in any of the methods of claims 1 to 10.

13.- The use of claim 12, wherein the means is an antibody or a fragment thereof which specifically binds to sAXL.

14.- The use of claim 13, wherein the antibody or fragment thereof, forms part of a kit.
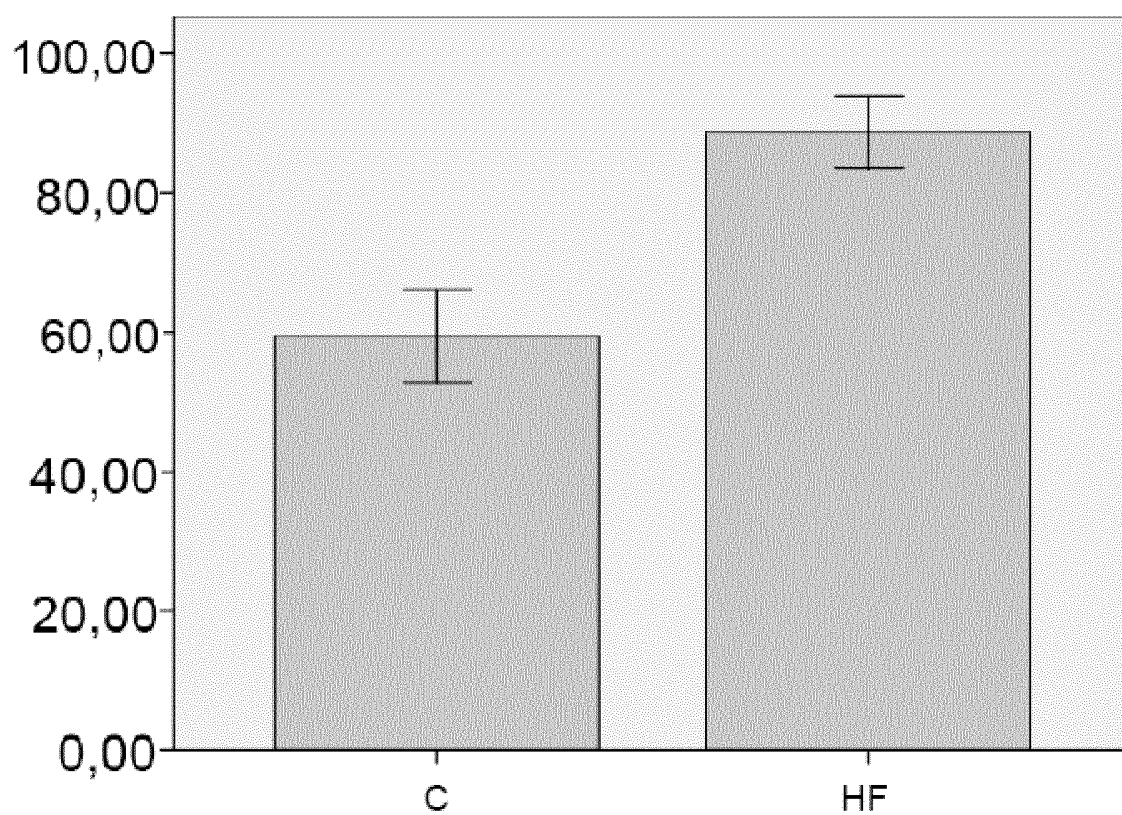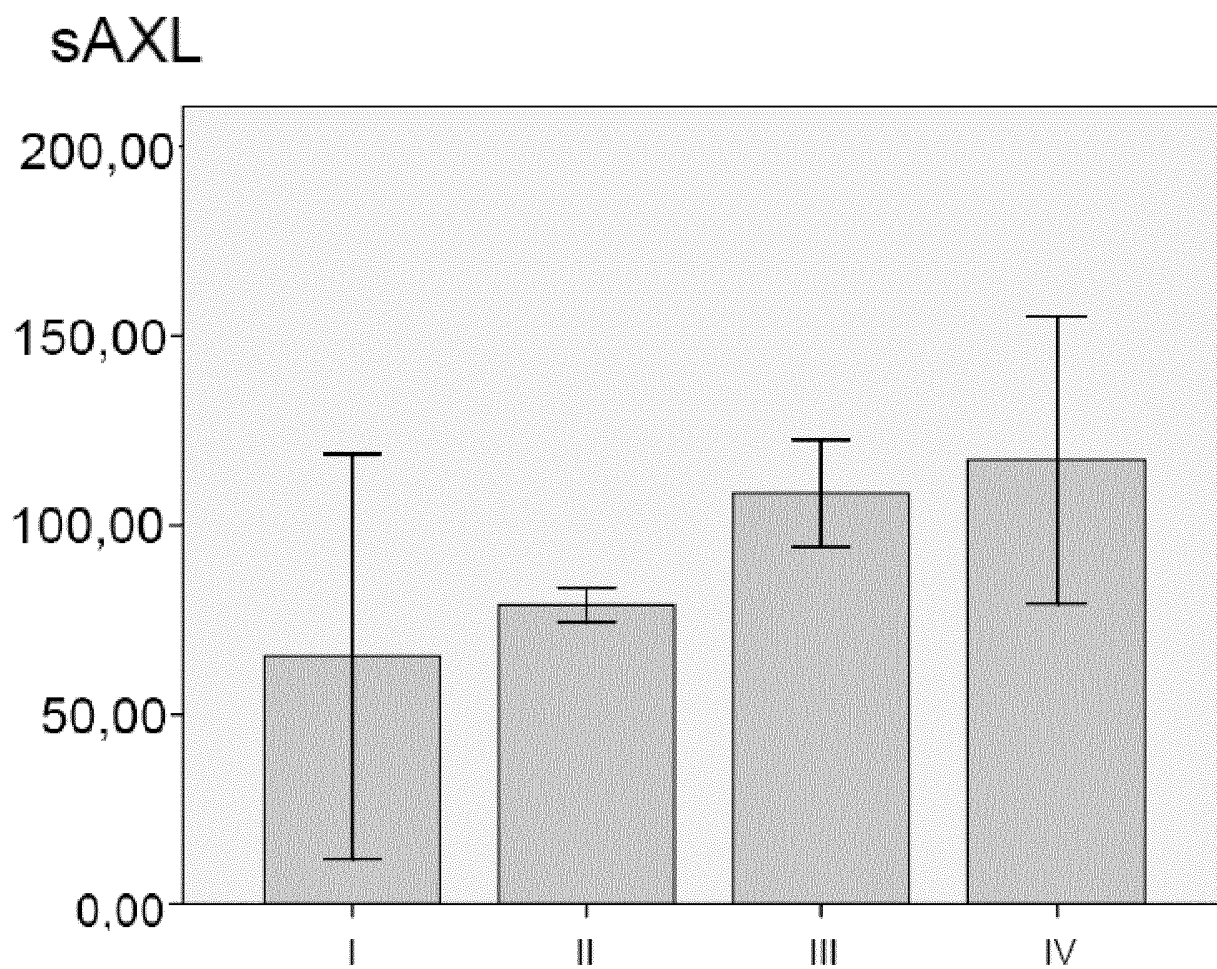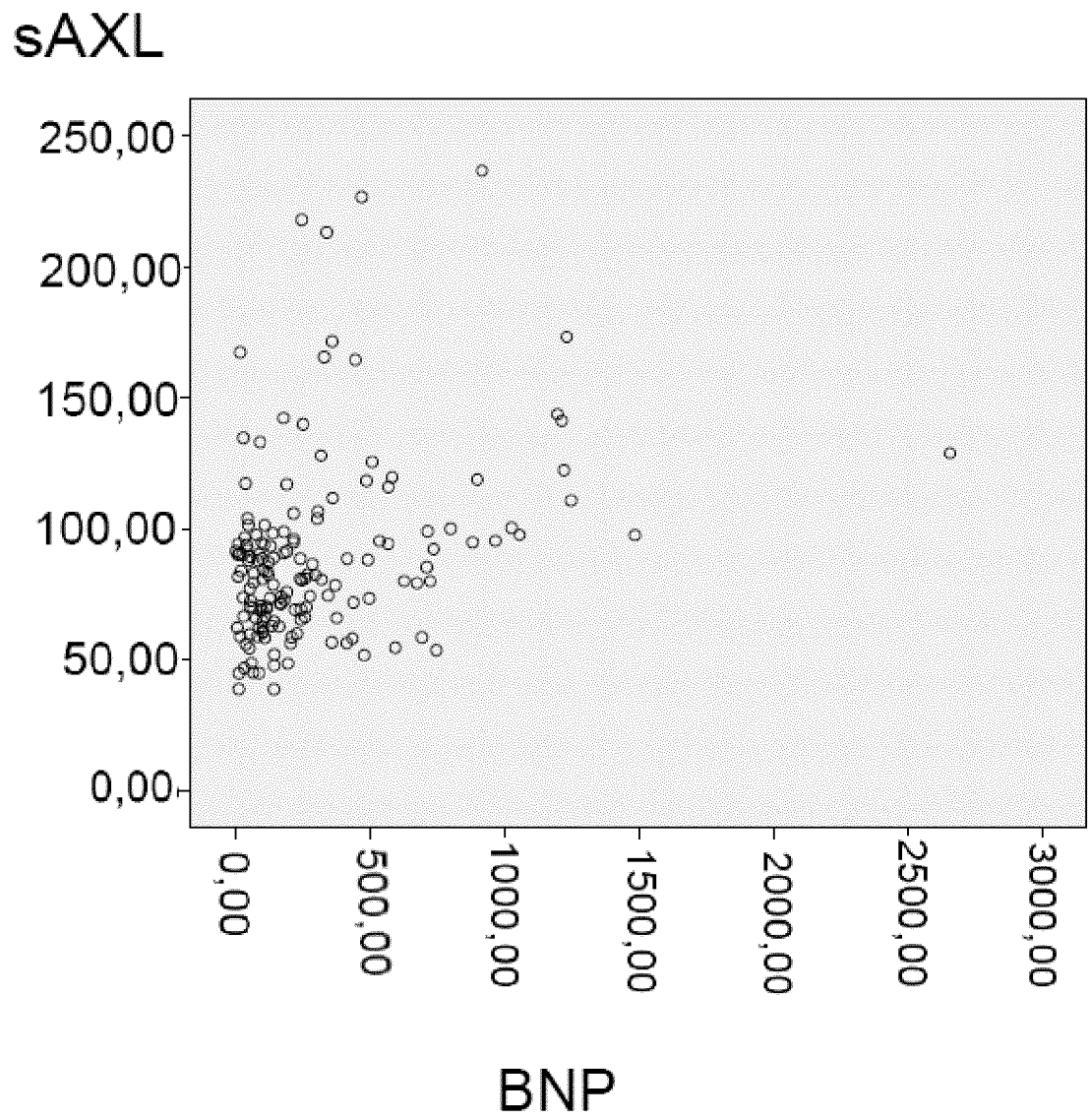
FIG. 1

sAXL

FIG. 2

sAXL

FIG. 3

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N33/68
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, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/060871 A1 (MAYO FOUNDATION [US]; BROZOVICH FRANK V [US]; OGUT OZGUR [US]; CHEN FR) 22 May 2008 (2008-05-22) claims 1,2 ----- -/--	1-14

[X] Further documents are listed in the continuation of Box C.

[X] 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 March 2013	20/03/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gundlach, Björn

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EKMAN C ET AL: "Plasma concentrations of Gas6 and soluble Axl correlate with disease and predict mortality in patients with critical limb ischemia", CLINICAL BIOCHEMISTRY, ELSEVIER INC, US, CA, vol. 43, no. 10-11, 1 July 2010 (2010-07-01), pages 873-876, XP027085691, ISSN: 0009-9120 [retrieved on 2010-06-13] abstract page 874, column 1, paragraph 2 page 876, column 1, paragraph 3 -----	12-14
A	US 5 468 634 A (LIU EDISON T [US]) 21 November 1995 (1995-11-21) column 6, line 25 - column 8, paragraph 2 -----	12-14
A	US 2003/157573 A1 (MOR ORNA [IL]) 21 August 2003 (2003-08-21) paragraph [0045] claim 28 -----	12-14

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