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(54) **INTERLEUKIN-33 (IL-33) FOR THE
DIAGNOSIS AND PROGNOSIS OF
CARDIOVASCULAR DISEASE**

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(57) **ABSTRACT**

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(63) Continuation of application No. 12/298,613, filed on
Feb. 2, 2009, filed as application No. PCT/US2007/
067626 on Apr. 27, 2007.

(60) Provisional application No. 60/795,473, filed on Apr.
27, 2006.

The present invention includes methods for the use of inter-
leukin-33 (IL-33) in the diagnosis of cardiovascular condi-
tions including acute coronary syndrome (ACS), myocardial
infarction, and/or heart failure, angina, cardiac hypertrophy,
arteriosclerosis, myocarditis, pericarditis, endocarditis,
stroke and/or pulmonary embolism and the determination of
the severity of such conditions (prognosis).

FIG. 1

1 caacagaata ctgaaaaatg aagcctaaaa tgaagtattc aaccaacaaa atttccacag
 61 caaagtggaa gaacacagca agcaaagcct tgtgtttcaa gctgggaaaa tcccaacaga
 121 aggccaaaaga agtttgcccc atgtacttta tgaagctccg ctctggcctt atgataaaaa
 181 aggaggcctg ttacttttagg agagaaacca ccaaaggcc ttcactgaaa acaggtagaa
 241 agcacaaaag acatctggta ctcgctgcct gtcaacagca gtctactgtg gactgctttg
 301 cttttgggtat atcaggggtc cagaaatata ctagagcact tcoatgattca agtatcacag
 361 gaatttcacc tattacagag tatcttgctt ctctaagcac atacaatgat caatccatta
 421 cttttgcttt ggaggatgaa agttatgaga tatatgttga agacttgaaa aaagatgaaa
 481 agaaagataa ggtgttactg agttactatg agtctcaaca cccctcaaat gaatcaggtg
 541 acggtgttga tggtaagatg ttaatggtaa cctgagtc ccataaagtg tgaaaaacca ctgccagacc
 601 atgccaacaa caaggaacac tctgtggagc tccataaagtg tgaaaaacca ctgccagacc
 661 aggccttctt tgtccttcat aatatgcact ccaactgtgt ttcatttgaa tgcaagactg
 721 atcctggagt gtttataggt gtaaaggata atcatcttgc tctgattaaa gtagactctt
 781 ctgagaatth gtgtactgaa aatatcttgt ttaagctctc tgaaacttag ttgatggaaa
 841 cctgtgagtc ttgggttgag taccctaatg ctaccactgg agaaggaatg agagataaag
 901 aaagagacag gtgacatcta agggaaatga agagtgcctt gcatgtgtgg aatgttttcc
 961 atattatgta taaaaatatt ttttctaate ctccagttat tcttttattt cctctgtat
 1021 aactgcatct tcaatacaag tatecagata ttaaataaggg tattggtaaa gaaacgggtca
 1081 acattctaaag gagatacagt ctgaccttta cttttctcta gtttcagtcc agaaagaact
 1141 tcatatttag agctaaggcc actgaggaaa gagccatagc ttaagtctct atgtagacag
 1201 gatccattt taagagcta cttagagaaa taattttcca cagttccaaa cgataggctc
 1261 aaacactaga gctgctagta aaaagaagac cagatgcttc acagaattat cttttttca
 1321 actggaataa aacaccagggt ttgtttgtag atgtcttagg caacactcag agcagatctc
 1381 cttactgtc aggggatatg gaacttcaaa ggcccacatg gcaagccagg taacataaat
 1441 gtgtgaaaaa gtaaagataa ctaaaaaatt tagaaaaata aatccagtat ttgtaaagtg
 1501 aataacttca tttctaattg ttttaattttt aaaattctga tttttatata ttgagtttaa
 1561 gcaaggcatt cttacacgag gaagtgaagt aaattttagt tcagacataa aatttcactt
 1621 attaggaata tgtaacatgc taaaactttt ttttttttaa agagtactga gtcacaacat
 1681 gtttttagagc atccaagtac catataatcc aactatcatg gtaaggccag aatcttcta
 1741 acctaccaga gcttagatga gacaccgat taacattaaa atttcagtaa ctgactgtcc
 1801 tctactocca tggcctacca tccctctgta cctggcttc cagggactta tctcttttaa
 1861 tactcactgt cacattgggc aaagtgtgct ctaatcctta tttccatgt gcacaagtct
 1921 tttgtattc cagcttcctg ataacactgc ttactgtgga atattcattt gacatctgtc
 1981 tcttttcatt tcttttaact accatgccct tgatataatc tttgcacctg ctgaacttca
 2041 tttctgtatc acctgacctc tggatgcaa aacgtttatt ctgctttgtc tgtgtagaa
 2101 ttttagataa agctattaat ggcaatattt ttttgctaaa cgtttttgtt ttttactgtc
 2161 actagggcaa taaaatttat actcaacat ataataacat tttttaacta ctaaaggagt
 2221 agtttttatt ttaaagtctt agcaatttct attacaactt ttcttagact taacacttat
 2281 gataaatgac taacatagta acagaatctt tatgaaatat gaccttttct gaaaatacat
 2341 acttttacat ttctacttta ttgagaccta tttagatgtaa gtgctagtag aatataagat
 2401 aaaagaggct gagaattacc atacaagggt attacaactg taaaacaatt tatctttgtt
 2461 tcattgttct gtcaataatt gttaccaag agataaaaat aaaagcagaa tgtatatcat
 2521 ccatctgaa aaacactaat tattgacatg tgcactctgta caataaactt aaaatgatta
 2581 ttaaataatc aaatataatc actacattgt ttatattatt gaataaagta tttttccaa
 2641 atgt (SEQ ID NO:1)

FIG. 2

1 mkpkmkystn kistakwknt askalcflkg ksqqkakevc pmyfmlrsg lmikkeacyf
 61 rrettkrpsl ktgrkhkrhl vlaacqqst vecfafgisg vqkytralhd ssitgispit
 121 eylaslstyn dqsitfaled esyeiyvedl kkdekkdkvl lsyyesqhps nesgdgvdgk
 181 mlmvtlsptk dfwlhannke hselhkcek plpdqaffvl hnmhsncvsf ecktdpgvfi
 241 gvkdnhlali kvdssenlct enilflkset (SEQ ID NO:2)

INTERLEUKIN-33 (IL-33) FOR THE DIAGNOSIS AND PROGNOSIS OF CARDIOVASCULAR DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 12/298,613, filed on Oct. 27, 2008, which is the U.S. national stage under 35 USC §371 of International Application Number PCT/US2007/067626, filed on Apr. 27, 2007, which claims the benefit under 35 USC §119(e) to U.S. Provisional Patent Application Ser. No. 60/795,473, filed on Apr. 27, 2006, the entire contents of which are hereby incorporated by reference.

TECHNICAL FIELD

[0002] This invention pertains to methods and compositions for the diagnosis and prognosis of cardiovascular conditions.

BACKGROUND

[0003] Despite significant advances in therapy, cardiovascular disease remains the single most common cause of morbidity and mortality in the developed world. Thus, prevention and therapy of cardiovascular conditions such as heart failure, myocardial infarction and stroke is an area of major public health importance. Currently, several risk factors for future cardiovascular disorders have been described and are in wide clinical use in the detection of subjects at high risk. Such screening tests include evaluations of total and HDL cholesterol levels. However, a large number of cardiovascular disorders occur in subjects with apparently low to moderate risk profiles, and ability to identify such patients is limited. Moreover, accumulating data suggests that the beneficial effects of certain preventive and therapeutic treatments for patients at risk for or known to have cardiovascular disorders differs in magnitude among different patient groups.

SUMMARY

[0004] The present invention includes methods for the use of interleukin-33 (IL-33) in the diagnosis and prognosis of cardiovascular conditions including acute coronary syndrome (ACS), coronary artery disease (CAD), myocardial infarction, heart failure, angina, cardiac hypertrophy, arteriosclerosis, myocarditis, pericarditis, endocarditis, stroke and/or pulmonary embolism.

[0005] In one aspect, the invention provides methods for diagnosing a cardiac disease in a subject. The methods include determining a level of IL-33 in a sample from a subject, wherein the level of IL-33 in the sample is indicative of whether the subject has a cardiovascular disease (i.e., correlates to the presence or absence of cardiovascular disease). In some embodiments, the methods include obtaining a biological sample from the subject; determining a level of IL-33 in the sample; and comparing the level of IL-33 in the sample to a reference level of IL-33. The level of IL-33 in the sample as compared to the reference indicates whether the subject has a cardiovascular disease.

[0006] In another aspect, the invention provides methods for determining the severity of a cardiovascular disease in a subject. The methods include determining a level of IL-33 in a sample from a subject, wherein the level of IL-33 in the

sample is indicative of (correlates to) the severity of the cardiovascular disease in the subject.

[0007] In some embodiments, the methods include obtaining a biological sample from the subject; determining a level of IL-33 in the sample; and comparing the level of IL-33 in the sample to a reference level of IL-33. The level of IL-33 in the sample as compared to the reference indicates the severity of the cardiovascular disease in the subject.

[0008] In some embodiments of the methods described herein, the sample comprises blood, serum, plasma, urine, or body tissue. In some embodiments, the sample comprises serum.

[0009] In some embodiments of the methods described herein, the reference level represents a level in a subject who does not have cardiovascular disease. In some embodiments, the reference level represents a level in a subject with a preselected severity of cardiovascular disease.

[0010] In some embodiments of the methods described herein, determining a level of IL-33 in the sample includes determining a level of one, two, or all three of mature IL-33, pre-IL-33, and pro-IL-33. Determining a level of IL-33 in the sample can include contacting a binding composition to the sample, wherein the binding composition specifically binds to IL-33, and measuring or determining the specific binding of the binding composition to the sample. Suitable binding compositions include antibodies that bind specifically to IL-33 polypeptide, and oligonucleotide probes that bind specifically to IL-33 polynucleotide.

[0011] In some embodiments, the cardiac disease diagnosed by a method described herein is acute coronary syndrome (ACS), myocardial infarction, heart failure, angina, cardiac hypertrophy, arteriosclerosis, myocarditis, pericarditis, endocarditis, stroke, and/or pulmonary embolism.

[0012] In some embodiments, the methods described herein also include determining a level in the sample of one or more other biomarkers, e.g., biomarkers selected from the group consisting of ST2, NT-proBNP, BNP, NT-proANP, and ANP, troponin, IMA, IL-6, CRP, creatinine, D-dimers, BUN, liver function enzymes, albumin, and bacterial endotoxin.

[0013] In an additional aspect, the invention features kits for diagnosing cardiovascular disease. The kits include an antibody that specifically binds to IL-33, and/or an oligonucleotide probe that specifically binds to a nucleic acid encoding IL-33, and instructions for use in a method described herein.

[0014] "Upregulated," as used herein, refers to increased expression of a gene and/or its encoded polypeptide. "Increased expression" refers to increasing (i.e., to a detectable extent) replication, transcription, and/or translation of IL-33, since upregulation of any of these processes results in an increase in concentration/amount of the polypeptide encoded by the gene. Conversely, "downregulation," or "decreased expression" as used herein, refers to reduced replication, transcription, and/or translation of the IL-33 gene and/or its encoded polypeptide. The upregulation or downregulation of gene expression can be directly determined by detecting an increase or decrease, respectively, in the level of mRNA for the gene, or the level of protein expression of the gene-encoded polypeptide, using any suitable means known to the art, such as nucleic acid hybridization or antibody detection methods, respectively, and in comparison to controls. "Expression," as used herein, refers to nucleic acid and/or polypeptide expression.

[0015] A “cardiac cell,” as used herein, refers to a cardiomyocyte.

[0016] As used herein, a “subject” is a mammal or a non-human mammal. In general, human nucleic acids, polypeptides, and human subjects are preferred for use in diagnosing human patients.

[0017] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

[0018] Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 is a representation of the nucleic acid sequence of human IL-33 (SEQ ID NO:1).

[0020] FIG. 2 is a representation of the amino acid sequence of human IL-33 (SEQ ID NO:2).

DETAILED DESCRIPTION

[0021] It has previously been described that levels of the interleukin-1 receptor-like 1 (IL1RL1) protein can be used to diagnose cardiovascular disease and determine the prognosis for a patient with cardiovascular disease. The ligand for IL1RL1 has been described, and named IL-33 (see, e.g., Schmitz et al., *Immunity* 23(5):479-90 (2005); U.S. Pat. Pub. No. 2005/0203046). The present methods include the measurement of levels of IL-33 for the diagnosis and prognosis of cardiovascular disease.

[0022] General Methodology

[0023] In general, the methods described herein include evaluating levels of IL-33 in a biological sample (e.g., a blood, serum, plasma, urine, or body tissue sample) from a subject, e.g., a mammal, e.g., a human. These levels provide diagnostic information indicating whether the subject has a cardiac disease, as described herein. In some embodiments, the level of IL-33 is determined once, e.g., at presentation. In some embodiments, the level of IL-33 is determined at any one or more of 1, 2, 3, 4, 5, 6, 7, 8, 12, 18, and/or 24 hours, and/or at 1-7 days after the onset of symptoms.

[0024] In embodiments where the level of IL-33 is determined more than once, the highest level can be used, or the change in levels can be determined and used. Levels of IL-33 can also be determined multiple times to evaluate a subject's response to a treatment. For example, a level of IL-33 taken after administration of a treatment, e.g., one or more doses or rounds of a treatment, can be compared to levels of IL-33 before the treatment was initiated, e.g., a baseline level. The change in IL-33 levels would indicate whether the treatment was effective; e.g., a reduction in IL-33 levels would indicate that the treatment was effective.

[0025] In some embodiments, the level of IL-33 can be measured in patients previously diagnosed with a cardiovascular condition who are undergoing treatment on an outpa-

tient basis, to monitor the condition of the patient and determine whether their condition is improving, stable or deteriorating. Levels of IL-33 can be measured at one or more times and compared to previous measurements, where an increase in IL-33 relative to an earlier measurement would indicate that the patient's condition is deteriorating, suggesting that the current treatment should be adjusted or possibly that the patient should be admitted to a hospital for inpatient treatment.

[0026] Evaluating circulating levels of IL-33 in a subject typically includes obtaining a biological sample, e.g., serum or blood, from the subject. Levels of IL-33 in the sample can be determined by measuring levels of polypeptide, either the pre-IL33, pro-IL-33 or IL-33 peptide, in the sample, using methods known in the art and/or described herein, e.g., immunoassays such as enzyme-linked immunosorbent assays (ELISA). Alternatively, levels of IL-33 mRNA can be measured, again using methods known in the art and/or described herein, e.g., by quantitative PCR or Northern blotting analysis.

[0027] For example, a method as described herein, e.g., for diagnosis or prognosis of cardiac disease, can include contacting a sample from a subject, e.g., a sample including blood, serum, plasma, urine, or body tissue from the subject, with a binding composition (e.g., an antibody or oligonucleotide probe) that specifically binds to a polypeptide or nucleic acid of IL-33. The methods can also include contacting a sample from a control subject, normal subject, or normal tissue or fluid from the test subject, with the binding composition, e.g., to provide a reference or control. Moreover, the method can additionally include comparing the specific binding of the composition to the test subject with the specific binding of the composition to the normal subject, control subject, or normal tissue or fluid from the test subject. Expression or activity of IL-33 in a test sample or test subject can also be compared with that in a control sample or control subject. A control sample can include, e.g., a sample from a non-affected subject, or a subject who has a cardiac disease of known severity. Expression or activity from a control subject or control sample can be provided as a predetermined value, e.g., acquired from a statistically appropriate group of control subjects.

[0028] An antibody that “binds specifically to” an antigen, binds preferentially to the antigen in a sample containing other proteins. The term “antibody” as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The antibody can be polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human, non-human, e.g., murine, monospecific, or single chain antibody. In some embodiments it has effector function and can fix complement.

[0029] An “oligonucleotide probe” (also referred to simply as a “probe”) is a nucleic acid that is at least 10, and less than 200 (typically less than about 100 or 50) base pairs in length. A probe that “binds specifically to” a target nucleic acid hybridizes to the target under high stringency conditions. As used herein, the term “hybridizes under high stringency conditions” describes conditions for hybridization and washing. As used herein, high stringency conditions are 0.5M sodium phosphate, 7% SDS at 65° C., followed by one or more washes at 0.2×SSC, 1% SDS at 65° C. Methods for perform-

ing nucleic acid hybridization assays are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

[0030] Detection can be facilitated by coupling (e.g., physically linking) the antibody or probe to a detectable substance (e.g., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, quantum dots, or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[0031] Diagnostic assays can be used with biological matrices such as live cells, cell extracts, cell lysates, fixed cells, cell cultures, bodily fluids, or forensic samples. Conjugated antibodies useful for diagnostic or kit purposes, include antibodies coupled to dyes, isotopes, enzymes, and metals, see, e.g., Le Doussal et al., *New Engl. J. Med.* 146:169-175 (1991); Gibellini et al., *J. Immunol.* 160:3891-3898 (1998); Hsing and Bishop, *New Engl. J. Med.* 162:2804-2811 (1999); and Everts et al., *New Engl. J. Med.* 168:883-889 (2002). Various assay formats exist, such as radioimmunoassays (RIA), ELISA, and lab on a chip (U.S. Pat. Nos. 6,176,962 and 6,517,234).

[0032] Known techniques in biochemistry and molecular biology can be used in the methods described herein (see, e.g., Maniatis et al., *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1982); Sambrook and Russell, *Molecular Cloning, 3rd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001); Wu, *Recombinant DNA*, Vol. 217, Academic Press, San Diego, Calif. (1993); and Ausbel et al., *Current Protocols in Molecular Biology*, Vols. 1-4, John Wiley and Sons, Inc. New York, N.Y. (2001)).

[0033] Once a level of IL-33 has been determined, the level can be compared to a reference level. In some embodiments, e.g., where the level of IL-33 is determined using an ELISA, the reference level will represent a threshold level, above which the subject can be diagnosed with cardiac disease. The reference level chosen may depend on the methodology used to measure the levels of IL-33.

[0034] In some embodiments, the level of IL-33 is used to determine the level of severity of cardiac disease in a subject. The level of IL-33 typically increases with the severity of the cardiac disease, therefore, higher levels indicate more severe disease. Thus, the reference levels can represent levels of IL-33 in subjects with cardiac disease of known severity.

[0035] In some embodiments, the reference level is a range of levels.

[0036] In some embodiments, both levels of Interleukin 1 Receptor-Like 1 (IL1RL1) and IL-33 are determined, and the information from the comparison of both biomarkers with their respective reference levels provides cumulative information regarding the presence of cardiac disease in the subject, and/or the presence of a severe disease in the subject. In

some embodiments, the ratio of IL1RL1 to IL-33 may be determined, and the ratio compared to a reference ratio that represents a threshold ratio above which the subject has cardiac disease.

[0037] Also included herein are kits that include a reagent for the detection of one or more of the IL-33 polypeptide(s) or nucleic acid, e.g., an anti-IL-33 antibody (i.e., an antibody that binds specifically to IL-33), or a nucleic acid probe complementary to all or part of the IL-33 nucleic acid, and instructions for use.

[0038] Interleukin-33 (IL-33)

[0039] IL-33 was recently identified as the ligand for IL1RL1, and the presence of increased levels of IL-33 in various inflammatory disorders has been described (see Schmitz et al., *Immunity* 23(5):479-90 (2005); U.S. Pat. Pub. No. 2005/0203046).

[0040] IL-33 protein is expressed as an inactive molecule, pre-IL-33, that is activated after cleavage by Caspase 1 resulting in the active IL-33 peptide as well as the cleavage peptide product, pro-IL-33. Therefore, the methods described herein can include measuring one, two, or all three of mature IL-33, pre-IL-33, and/or pro-IL-33, all of which are included in the term "IL-33" as used herein.

[0041] The nucleic acid sequence of human IL-33 can be found at GenBank Acc. No. NM_033439.2 (FIG. 1, SEQ ID NO:1), and the polypeptide sequence is at GenBank Acc. No. NP_254274.1 (FIG. 2, SEQ ID NO:2). Additional information is available in the public databases at GeneID: 90865, MIM ID #*608678, and UniGene No. Hs.348390. IL-33 is also known as Chromosome 9 Open Reading Frame 26 (C9ORF26); Nuclear Factor from High Endothelial Venules (NFHEV); and Interleukin 33. See also Baekkevold et al., *Am. J. Path.* 163: 69-79 (2003).

[0042] Methods for measuring levels of IL-33 polypeptide and nucleic acid are known in the art, see, e.g., Schmitz et al., *Immunity* 23(5):479-90 (2005); U.S. Pat. Pub. No. 2005/0203046.

[0043] Interleukin 1 Receptor-Like 1 (IL1RL1)

[0044] In the methods described herein, IL1RL1 can be measured in addition to IL-33. The ratio of IL1RL1 to IL-33 can also be determined. The IL gene is a member of the interleukin-1 receptor family, whose protein product exists both as a trans-membrane form, as well as a soluble receptor that is detectable in serum (Kieser et al., *FEBS Lett.* 372(2-3):189-93 (1995); Kumar et al., *J. Biol. Chem.* 270(46):27905-13 (1995); Yanagisawa et al., *FEBS Lett.* 302(1):51-3 (1992); Kuroiwa et al., *Hybridoma* 19(2):151-9 (2000)). ST2 was recently described to be markedly up-regulated in an experimental model of heart failure (Weinberg et al., *Circulation* 106(23):2961-6 (2002)), and preliminary results suggest that ST2 concentrations may be elevated in those with chronic severe HF (Weinberg et al., *Circulation* 107(5):721-6 (2003)) as well as in those with acute myocardial infarction (MI) (Shimpo et al., *Circulation* 109(18):2186-90 (2004)).

[0045] The transmembrane form of IL1RL1 is thought to play a role in modulating responses of T helper type 2 cells (Lohning et al., *Proc. Natl. Acad. Sci. U.S.A.* 95(12):6930-5 (1998); Schmitz et al., *Immunity* 23(5):479-90 (2005)), and may play a role in development of tolerance in states of severe or chronic inflammation (Brint et al., *Nat. Immunol.* 5(4): 373-9 (2004)), while the soluble form of IL1RL1 is up-regulated in growth stimulated fibroblasts (Yanagisawa et al., 1992, supra). Experimental data suggest that the IL1RL1

gene is markedly up-regulated in states of myocyte stretch (Weinberg et al., 2002, supra) in a manner analogous to the induction of the BNP gene (Bruneau et al., *Cardiovasc. Res.* 28(10):1519-25 (1994)).

[0046] Tominaga, *FEBS Lett.* 258:301-304 (1989), isolated murine genes that were specifically expressed by growth stimulation in BALB/c-3T3 cells; they termed one of these genes St2 (for Growth Stimulation-Expressed Gene 2). The St2 gene encodes two protein products: ST2, which is a soluble secreted form; and ST2L, a transmembrane receptor form that is very similar to the interleukin-1 receptors. The HUGO Nomenclature Committee designated the human homolog, the cloning of which was described in Tominaga et al., *Biochim. Biophys. Acta.* 1171:215-218 (1992), as Interleukin 1 Receptor-Like 1 (IL1RL1). The two terms are used interchangeably herein.

[0047] The mRNA sequence of the shorter, soluble isoform of human ST2 can be found at GenBank™ Acc. No. NM_003856.2, and the polypeptide sequence is at GenBank Acc. No. NP_003847.2; the mRNA sequence for the longer form of human ST2 is at GenBank Acc. No. NM_016232.4; the polypeptide sequence is at GenBank Acc. No. NP_057316.3. Additional information is available in the public databases at GeneID: 9173, MIM ID # 601203, and UniGene No. Hs.66. In general, in the methods described herein, the soluble form of ST2 polypeptide is measured.

[0048] Methods for detecting and measuring ST2 are known in the art, e.g., as described in U.S. Pat. Nos. 2003/0124624, 2004/0048286 and 2005/0130136, the entire contents of which are incorporated herein by reference. Kits for measuring ST2 polypeptide are also commercially available, e.g., the ST2 ELISA Kit manufactured by Medical & Biological Laboratories Co., Ltd. (MBL International Corp., Woburn, Mass.), no. 7638. In addition, devices for measuring ST2 and other biomarkers are described in U.S. Pat. Pub. No. 2005/0250156.

[0049] In some embodiments, the methods include determining the identity of the nucleotide sequence at RefSNP ID: rs1041973.

[0050] Other Biomarkers

[0051] The methods described herein can also include measuring levels of other biomarkers in addition to IL-33. Suitable biomarkers include NT-proBNP, BNP, NT-proANP, and ANP troponin, CK-MB, Myo, IMA, IL-6, CRP, creatinine, D-dimers, and/or BUN. Methods for measuring these biomarkers are known in the art, see, e.g., U.S. Pat. Nos. 2004/0048286 and 2005/0130136 to Lee et al.; Dhalla et al., *Mol. Cell Biochem.* 87:85-92 (1989); Moe et al., *Am. Heart J.* 139:587-95 (2000), the entire contents of which are incorporated herein by reference.

[0052] In these embodiments, levels of IL-33 and one or more additional biomarkers are determined, and the information from the comparison of the biomarkers with their respective reference levels provides additional information regarding the presence of cardiovascular disease in the subject, and/or the level of severity of the disease in the subject.

[0053] Cardiovascular Disease

[0054] The methods described herein are useful in the diagnosis and prognosis of subjects with cardiovascular disease, e.g., MI, ACS, CAD, HF and/or stroke. In subjects with cardiovascular disease, a diagnosis is often made, and the extent of any cardiac tissue damage determined, using one or more of the following methods: electrocardiogram (ECG)—single or repeated over several hours; echocardiography;

coronary angiography; nuclear ventriculography (e.g., radio-nuclide ventriculography (RNV) or multiple gate acquisition scan (MUGA)).

[0055] In addition, some biomarkers have proven to be by-products of heart damage or in conjunction with coronary artery disease (CAD), and therefore useful for diagnosis of cardiac disease. These biomarkers include Troponin I (TnI) and troponin T (TnT); creatine phosphokinase (CPK) and CPK-MB; ischemia modified albumin (IMA) and serum myoglobin. The present invention provides additional methods of diagnosing cardiovascular disease, and prognostic methods for determining the severity of disease in a subject. Thus, the methods described herein can include determining IL-33 levels as part of a diagnostic effort, e.g., to determine whether a subject has cardiovascular disease, and/or can include determining IL-33 levels in a subject for whom a diagnosis of cardiovascular disease has already been made, e.g., to determine the severity of the disease.

[0056] Risk factors for cardiovascular disease include smoking, hypertension, high fat diet, poor blood cholesterol levels, especially high LDL (“bad”) cholesterol and low HDL (“good”) cholesterol, diabetes, male gender, age, heredity, and being overweight/obese. Biomarkers for increased risk include elevated homocysteine, C-reactive protein, and fibrinogen levels.

[0057] Myocardial Infarction (MI)

[0058] A myocardial infarction (MI) occurs when an area of heart muscle becomes necrotic (dies) or is permanently damaged because of an inadequate supply of oxygen to that area (ischemia). Most heart attacks are caused by a clot that blocks one of the coronary arteries. Clots usually form in a coronary artery that has been previously narrowed from changes related to atherosclerosis; atherosclerotic plaque (buildup) inside the arterial wall sometimes cracks, and this triggers the formation of a clot, also called a thrombus.

[0059] A clot in the coronary artery interrupts the flow of blood and oxygen to the heart muscle, resulting in ischemia of the tissue leading to the death of heart cells in that area (necrosis). The damaged heart muscle loses its ability to contract, and the remaining heart muscle needs to compensate for that weakened area.

[0060] Acute Coronary Syndrome (ACS)

[0061] ACS is a term that is used to cover any group of clinical symptoms associated with acute myocardial ischemia. Patients with ACS include those whose clinical presentations cover the following range of diagnoses: unstable angina, non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI). Myocardial ischemia is most often due to atherosclerotic plaques, as described above for MI.

[0062] Heart Failure (HF)

[0063] Heart failure is a pathophysiologic state in which the heart, via an abnormality of cardiac function (detectable or not), fails to pump blood at a rate commensurate with the requirements of the metabolizing tissues, and/or pumps only from an abnormally elevated diastolic filling pressure.

[0064] Heart failure may be caused by myocardial failure but may also occur in the presence of near-normal cardiac function under conditions of high demand, e.g., stress. Inadequate adaptation of the cardiac myocytes to increased wall stress to maintain adequate cardiac output following myocardial injury, is generally the inciting event in CHF. HF may have an acute onset or may occur over several months to

years, due to a primary disturbance in myocardial contractility or an excessive hemodynamic burden placed on the ventricle, or both.

[0065] Special Populations

[0066] Certain populations of subjects may benefit particularly from the methods described herein. These subjects include people for whom BNP or NT-proBNP is less useful, such as in those with impaired renal function (Anwaruddin et al., *J. Am. Coll. Cardiol.* 47(1):91-7 (2006); McCullough et al., *Am. J. Kidney Dis.* 41(3):571-9 (2003)), or in those who are overweight (Body Mass Index (BMI) of 25-29) or obese (BMI \geq 30) (Krauser et al., *Am. Heart J.* 149(4):744-50 (2005); McCord et al., *Arch. Intern. Med.* 164(20):2247-52 (2004)). It is known and accepted in the field that patients with a high BMI usually have levels of natriuretic peptide that are lower than expected relative to a normal body mass patient for the same level of disease; the exact mechanism for this phenomenon is not known. IL-33 levels may not be affected by renal failure or high BMI. The methods described herein can include determining a subject's BMI, and if the subject is overweight or obese, selecting the patient for determination of IL-33 levels, as described herein.

EXAMPLES

[0067] The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1

Detection and Measurement of IL-33 in the Serum

[0068] A blood sample is collected from a subject, and serum is prepared from the sample using standard methods. A labeled monoclonal antibody to IL-33 (e.g., as described in U.S. Pat. App. Pub. No. 2005/0203046, incorporated herein by reference in its entirety) is added to the sample and incubated for a sufficient amount of time for binding to occur. The antibody/IL-33 complexes are then detected using standard methods, and the amount of IL-33 present is quantified.

Other Embodiments

[0069] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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Trp	Leu	His	Ala	Asn	Asn	Lys	Glu	His	Ser	Val	Glu	Leu	His	Lys	Cys
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			260					265					270		

What is claimed is:

1. A method of diagnosing a cardiovascular disease in a subject, the method comprising determining a level of IL-33 in a sample from a subject, wherein the level of IL-33 in the sample is indicative of whether the subject has a cardiovascular disease.

2. A method of determining the severity of a cardiovascular disease in a subject, the method comprising determining a level of IL-33 in a sample from a subject, wherein the level of IL-33 in the sample is indicative of the severity of the cardiovascular disease in the subject.

3. The method of claim **1**, wherein determining a level of IL-33 in the sample comprises determining a level of one, two, or all three of mature IL-33, pre-IL-33, and pro-IL-33.

4. The method of claim **1**, wherein the sample comprises blood, serum, plasma, urine, or body tissue.

5. The method of claim **1**, further comprising comparing the level of IL-33 in the sample to a reference level, wherein the reference level represents a level in a subject who has or does not have cardiovascular disease.

6. The method of claim **2**, further comprising comparing the level of IL-33 in the sample to a reference level, wherein the reference level represents a level in a subject with a preselected severity of cardiovascular disease.

7. The method of claim **1**, wherein determining a level of IL-33 in the sample comprises contacting a binding composition to the sample, wherein the binding composition spe-

cifically binds to IL-33, and measuring or determining the specific binding of the binding composition to the sample.

8. The method of claim **7**, wherein the binding composition comprises an antibody that binds specifically to IL-33 polypeptide.

9. The method of claim **8**, wherein the binding composition comprises an oligonucleotide probe that binds specifically to IL-33 polynucleotide.

10. The method of claim **1**, wherein the cardiovascular disease is acute coronary syndrome (ACS), myocardial infarction, heart failure, angina, cardiac hypertrophy, arteriosclerosis, myocarditis, pericarditis, endocarditis, stroke or pulmonary embolism.

11. The method of claim **1**, further comprising determining a level in the sample of one or more other biomarkers.

12. A method of monitoring the condition of a subject previously diagnosed with a cardiovascular condition, the method comprising:

determining a first level of IL-33 in a sample from a subject;

determining at least one subsequent level of IL-33 in a sample from the subject; and

comparing the level of IL-33 in the subsequent sample to the level of IL-33 in the first sample,

wherein a difference in the IL-33 level in the subsequent sample relative to the level in the first sample is correlated with a change in the patient's condition.

13. The method of claim **12**, wherein the subject is undergoing treatment on an outpatient basis.

14. The method of claim **12**, wherein an increase in IL-33 in the subsequent sample relative to the level in the first sample indicates that the patient's condition is deteriorating.

15. The method of claim **12**, wherein an increase in IL-33 in the subsequent sample relative to the level in the first sample indicates that the current treatment should be adjusted.

16. The method of claim **15**, wherein an increase in IL-33 in the subsequent sample relative to the level in the first sample indicates that the patient should be admitted to a hospital for inpatient treatment.

17. A kit for diagnosing cardiovascular disease, the kit comprising an antibody that specifically binds to IL-33, or an

oligonucleotide probe that specifically binds to a nucleic acid encoding IL-33, and instructions for use in the method of claim **1**.

18. A kit for determining the severity of a cardiovascular disease in a subject, the kit comprising an antibody that specifically binds to IL-33, or an oligonucleotide probe that specifically binds to a nucleic acid encoding IL-33, and instructions for use in the method of claim **2**.

19. The method of claim **2**, wherein determining a level of IL-33 in the sample comprises determining a level of one, two, or all three of mature IL-33, pre-IL-33, and pro-IL-33.

20. The method of claim **2**, wherein the sample comprises blood, serum, plasma, urine, or body tissue.

* * * * *

专利名称(译)	白细胞介素-33 (IL-33) 用于心血管疾病的诊断和预后		
公开(公告)号	US20110053170A1	公开(公告)日	2011-03-03
申请号	US12/943676	申请日	2010-11-10
[标]申请(专利权)人(译)	SNIDER詹姆斯五世 JACOBSON SVEN		
申请(专利权)人(译)	SNIDER詹姆斯五世 JACOBSON SVEN		
当前申请(专利权)人(译)	急救诊断, INC.		
[标]发明人	SNIDER JAMES V JACOBSON SVEN		
发明人	SNIDER, JAMES V. JACOBSON, SVEN		
IPC分类号	C12Q1/68 G01N33/566 G01N33/53		
CPC分类号	C12Q1/6883 Y10T436/143333 G01N2800/32 G01N33/6869		
优先权	60/795473 2006-04-27 US PCT/US2007/067626 2007-04-27 WO		
外部链接	Espacenet USPTO		

摘要(译)

本发明包括使用白细胞介素-33 (IL-33) 诊断心血管疾病的方法, 包括急性冠状动脉综合征 (ACS), 心肌梗塞和/或心力衰竭, 心绞痛, 心脏肥大, 动脉硬化, 心肌炎, 心包炎, 心内膜炎, 中风和/或肺栓塞以及确定此类病症的严重程度 (预后)。

FIG. 1

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FIG. 2

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