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(54) **Title:** METHODS OF EMPLOYING VASCULAR LEAKAGE TO DIAGNOSE A CAPILLARY LEAK DISORDER

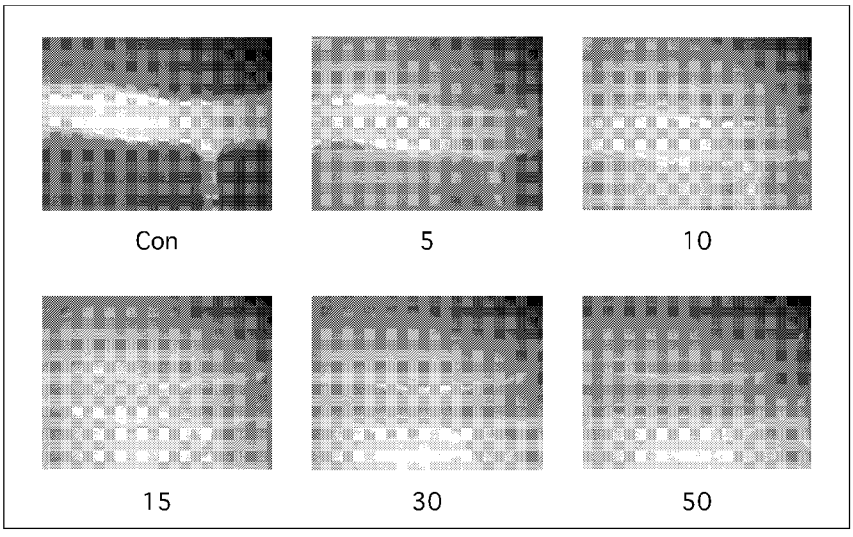

FIG. 1

(57) **Abstract:** A method of diagnosing a capillary leak disorder in a patient includes monitoring the marinobufagenin level in blood and/or urine as indicators of vascular permeability and, if a substantial elevation in marinobufagenin exists with respect to that of a normal person, concluding that a capillary leak disorder exists. The method may be employed to diagnose preeclampsia, as well as illnesses or abnormal conditions selected from the group consisting of acute respiratory distress syndrome, hemorrhagic shock, septic, endotoxemia, septicemia, burns.

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METHODS OF EMPLOYING VASCULAR LEAKAGE TO DIAGNOSE A CAPILLARY LEAK DISORDER

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates to methods of employing vascular leakage to diagnose the presence of a capillary leak disorder in a patient to facilitate prompt and effective treatment of the disorder.

2. Description of the Prior Art

[0002] While the present invention is not so limited, a primary condition of consequence which may be diagnosed by the methods of the present invention is preeclampsia. Preeclampsia is a condition experienced by pregnant women, and which often involves premature birth. Preeclampsia, if untreated, can progress suddenly to eclampsia, which involves coma and/or convulsive seizures which usually are fatal if untreated.

[0003] Preeclampsia is generally characterized by the presence of hypertension, proteinuria, and edema. It is a disorder which generally occurs only in women who are more than twenty weeks pregnant.

[0004] Elevated blood pressure or hypertension has long been recognized as a health problem. It is a very common disease which can have widespread effects on a patient's body and frequently, unlike numerous other diseases, is asymptomatic.

[0005] Despite means of measuring blood pressure of a patient, as by a sphygmomanometer, for example, there is lacking an accurate, reliable means for detecting the presence of volume dependent hypertension.

[0006] U.S. Patent No. 5,773,076 discloses use of a blood or urine specimen in diagnosing hypertension as indication of acute myocardial infarction. It employs plasma and/or levels of marinobufagenin-like immunoreactivity as a marker for hypertension.

[0007] While there is no hard-and-fast rule regarding diagnosis of preeclampsia, several standards have been applied. If a pregnant woman, after twenty weeks of gestation, develops a blood pressure of 140/90 or higher and has edema of the face or hands, and the presence of urinary protein in concentrations greater than 0.3 grams in a twenty-four hour urine collection, this is generally indicative of the presence of preeclampsia.

[0008] There remains a very real and substantial need for a method of effectively diagnosing capillary leak disorders.

SUMMARY OF THE INVENTION

[0009] The present invention has met the need for an effective method for diagnosing capillary leak disorders. The method of the invention involves monitoring marinobufagenin in blood and/or urine to determine if a capillary leak disorder exists. If a meaningful increase in marinobufagenin is determined to exist in blood and/or urine, as compared with a normal patient, it is concluded that a capillary leak disorder exists.

[0010] A substantial elevation in vascular permeability is deemed to exist when the level of marinobufagenin, as compared with a normal person, is at least about 20 percent greater.

[0011] It is an object of the present invention to provide an accurate, efficient, indirect means for rapidly determining if a capillary leak disorder exists in a patient.

[0012] It is a further object of the present invention to provide a method for indirect determination of vascular leakage by monitoring marinobufagenin levels in blood and/or urine of animals.

[0013] These and other objects of the invention will be more fully understood from the following detailed description of the invention and reference to the illustration appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 illustrates increasing vascular leakage at time increments from 5 seconds to 50 seconds with Con being the control group.

[0015] Figure 2 is a plot of vascular permeability in three groups of test rats with fluorescence intensity being plotted against time with the upper curve being PDS rats, the intermediate curve being the normal pregnant rats ("NP"), and the lowest curve being the control rats.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0016] As employed herein, the term "patient" means members of the animal kingdom, including humans.

[0017] As employed herein, the term "capillary leak disorder" means an illness or an abnormal condition which is characterized by vascular leakage of the fluid portion of the blood from the intravascular into the interstitial compartment and shall expressly include, but not be limited to, acute respiratory distress syndrome, hemorrhagic shock, septic endotoxemia, septicemia, burns, endotoxemia, and other illnesses or abnormal conditions characterized by a substantial elevation in marinobufagenin.

[0018] As employed herein, "substantial elevation" in vascular permeability means an increase in marinobufagenin with respect to that of a normal person not having a capillary leak disorder of at least about 20 percent.

[0019] Figure 1 shows Con being the control and time intervals in minutes related to the extent of leakage. A bolus of 200 nM marinobufagenin was injected intravenously. This shows progressive, large leakage resulting in substantial dye monitored within the extravascular space.

[0020] In normal pregnancy, the hematocrit and hemoglobin values fall. This phenomenon is believed to be the result of the expansion of the extracellular fluid volume in which the fluid portion of the blood increases in excess of the increment in the red cell mass. This results in physiologic anemia.

[0021] Figure 2 shows a plot of vascular permeability in rats. The control rats (C) are the nonpregnant rats, the normal pregnant rats (NP), and the animals rendered preeclamptic (PDS) show that the NP rats developed some low-level vascular leakage with the statistical significance becoming evident at 70 minutes. This was deemed consistent with the edema observed in normal pregnant women. The PDS rats developed a significant change in vascular permeability, which became apparent as early as 10 minutes into the procedure.

[0022] Considering preeclampsia as an example of capillary leak disorders, patients will have expansion of the extracellular fluid volume with the extra salt and water residing largely in the interstitial and not the intravascular compartment. The fluid portion of the blood, including minerals, such as sodium, potassium, and chloride, for example, and other solutes, such as proteins, will therefore, leak from the plasma in the vascular tree into the interstitium. These alterations can lead to impaired blood flow to the fetus, thereby exacerbating the problem of reduced delivery of nutrients and oxygen to the fetus. This contributes, in many cases, to intrauterine growth retardation.

[0023] It has been established that marinobufagenin ("MBG") is at least a biomarker and perhaps an important pathogenetic factor in the etiology of preeclampsia. The present invention has also established that resibufogenin ("RBG") is a compound with a similar structure to that of MBG and acts as an antagonist to MBG.

EXAMPLE 1

[0024] Tests were run on ten rats in the control group, sixteen rats in the normal pregnant group, and fifteen rats in the preeclamptic group ("PDS"). As shown in Table 1, which shows mean values for each group ("NP"), the control group of nonpregnant

rats had hematocrit values of 0.51 +/- 0.02. The second group of rats, which were normal, pregnant rats, had hematocrit values of 0.38 +/- 0.05. While the preeclamptic rats - designated PDS - had hematocrit values of 0.43 +/- 0.03, which is substantially higher than the normal pregnant rat. It is also noted that both the normal, pregnant ("NP") rat hematocrit value and the PDS rats hematocrit value were substantially under the C (control group). In preeclamptic patients, however, the hematocrit rises. It is believed that some of the excess fluid leaks from the intravascular regions into the interstitial compartment. This transudation of the fluid portion at the blood into the space is involved in capillary leak disorders. These results are statistically significantly different from each other.

Table 1	
Hematocrit Values in Rat Preeclampsia Studies	
Control (nonpregnant)	0.51 +/- 0.02
Normal pregnant	0.38 +/- 0.05
PDS rats	0.43 +/- 0.03

EXAMPLE 2

[0025] In order to confirm the fact that capillary leak disorders, such as preeclampsia, can be diagnosed by monitoring vascular permeability, a series of tests was performed on rats. Fifteen Sprague-Dawley rats were obtained. Five of the rats were normal, non-pregnant animals and were used as a control group. Five rats were normal pregnant rats (NP). As MBG has a deleterious effect on vascular integrity, and MBG is increased slightly in normal pregnancy and by a great deal in preeclampsia, one would expect an increased vascular leakage in a normal, pregnant rat, as compared with a normal rat, and a further increase in vascular leakage in a preeclamptic rat ("PDS"), as compared with a normal pregnant rat.

[0026] In the experimental procedure, single venules were visualized by way of a videomicroscopic technique, which permits viewing blood flow directly. The rats were injected with albumin that had been tagged with fluorescein dye. The distribution of the albumin in the omental vessels of the rat was then observed with a computer being employed to repeatedly measure the dye in the blood vessel and in the interstitium next to the blood vessel, as well as a remote site, which would serve as the control.

[0027] A substantial elevation in vascular permeability is deemed to exist if the marinobufagenin in a patient's blood or urine specimen was increased over that of a normal patient by at least about 20 percent.

[0028] While for simplicity of disclosure, emphasis has been placed on one of the methods to diagnose preeclampsia, it will be appreciated that it may be employed to diagnose other capillary leak disorders.

[0029] It will be appreciated that the present invention provides an efficient, accurate, and simple means for determining whether a patient has a capillary leak disorder.

[0030] Whereas particular embodiments of the invention have been described herein for purposes of illustration, it will be evident to those skilled in the art that numerous variations of the details may be made without departing from the invention as set forth in the appended claims.

WHAT IS CLAIMED IS:

1. A method of diagnosing a capillary leak disorder in a patient comprising:

monitoring the blood and/or urine level of marinobufagenin in said patient, and

if a substantial elevation in marinobufagenin as compared with a normal patient exists, concluding that a capillary leak disorder is present.

2. The method of Claim 1 including

employing said method to diagnose the presence of at least one illness or abnormal condition selected from the group consisting of acute respiratory distress syndrome, hemorrhagic shock, septic, endotoxemia, septicemia, and burns.

3. The method of Claim 1 including employing said method to diagnose the presence of preeclampsia.

4. The method of Claim 2 including said patient is a human being.

5. The method of Claim 1 including employing said method on human beings, and

determining that a capillary leak disorder exists if there is an elevation in marinobufagenin level of at least 20 percent as compared with a normal person.

6. The method of Claim 1 including monitoring said marinobufagenin in blood.

7. The method of Claim 1 monitoring said marinobufagenin in urine.

8. The method of Claim 1 monitoring said marinobufagenin in both blood and urine.

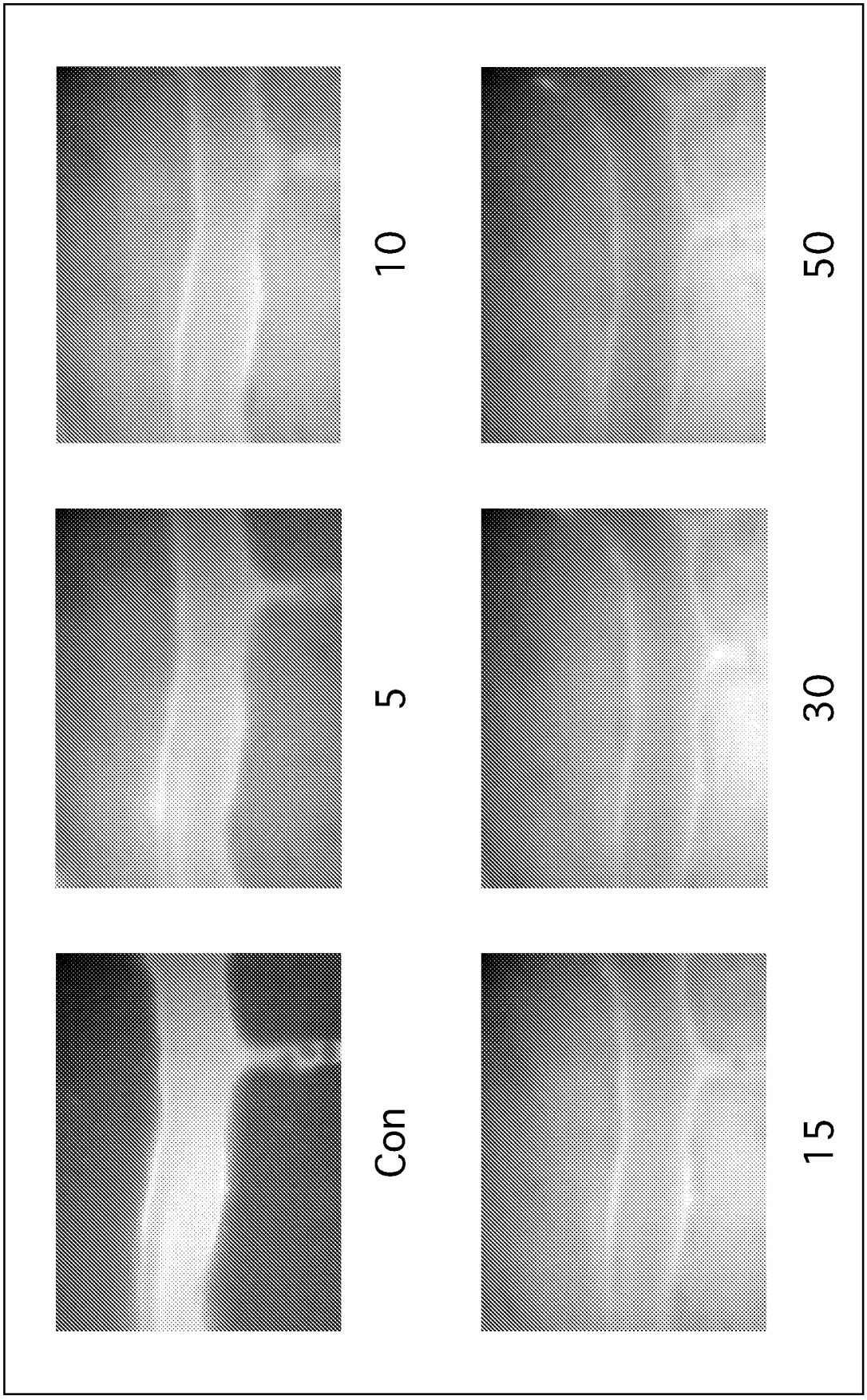

FIG. 1

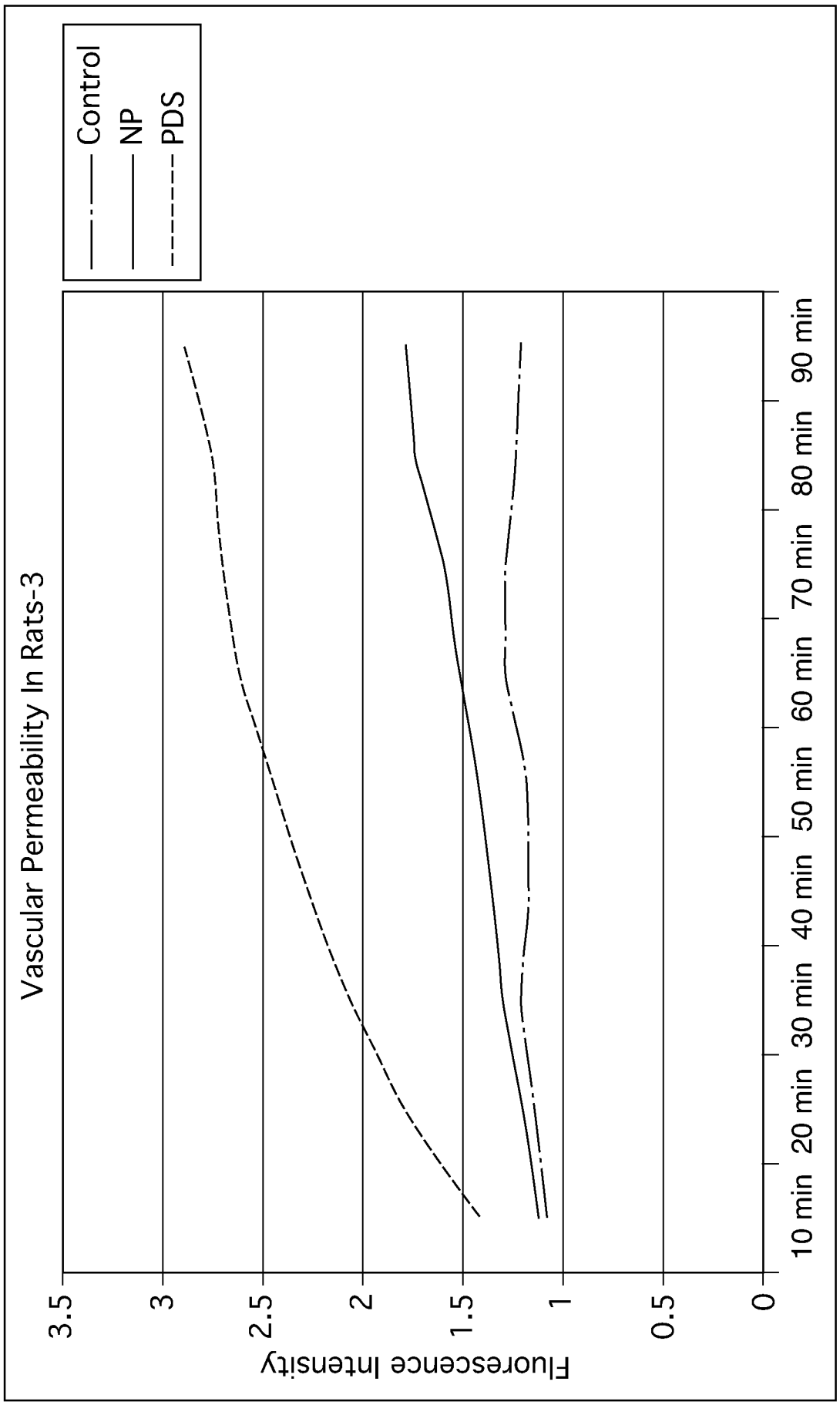
Vascular Permeability In Rats-3
Control
NP
PDS
Fluorescence Intensity
3.5
3
2.5
2
1.5
1
0.5
0
10 min
20 min
30 min
40 min
50 min
60 min
70 min
80 min
90 min

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/65088

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/50; A61B 5/00 (2008.04)
USPC - 436/87; 600/573
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)
USPC: 436/87; 600/573

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)
PubWEST (PGPB, USPT, USOC, EPAB, JPAB); DialogPro; Google (incl. Patents, Scholar) - capillary, leak, (intra)vascular, marinobufagenin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0263891 A1 (PUSCHETT) 23 November 2006 (23.11.2006), para [0024], [0027], [0030]-[0033]	1-8
Y	US 5,704,358 (ZIKRIA) 6 January 1998 (06.01.1998), Col. 4, ln. 26-46; Col. 6, ln. 23-58; Col. 7, ln. 14-34; FIG. 1-2	1-8

☐ Further documents are listed in the continuation of Box C. ☐

* 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
1 August 2008 (01.08.2008)	08 AUG 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

专利名称(译)	采用血管渗漏诊断毛细血管渗漏障碍的方法		
公开(公告)号	EP2160602A1	公开(公告)日	2010-03-10
申请号	EP2008756441	申请日	2008-05-29
[标]申请(专利权)人(译)	史考特与怀特纪念医院＆SCOTT SHERWOOD＆布林德利FOUND		
申请(专利权)人(译)	SCOTT和白色纪念医院和SCOTT SHERWOOD和BRINDLEY基金会		
当前申请(专利权)人(译)	SCOTT和白色纪念医院和SCOTT SHERWOOD和BRINDLEY基金会		
[标]发明人	PUSCHETT JULES B		
发明人	PUSCHETT, JULES B.		
IPC分类号	G01N33/50 A61B5/00		
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代理机构(译)	PARRY，SIMON JAMES		
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其他公开文献	EP2160602A4		
外部链接	Espacenet		

摘要(译)

诊断患者毛细血管渗漏障碍的方法包括监测血液和/或尿液中的marinobufagenin水平作为血管通透性的指标，并且如果相对于正常人存在marinobufagenin的显着升高，则得出结论毛细血管渗漏。存在障碍。该方法可用于诊断先兆子痫，以及选自由急性呼吸窘迫综合征，失血性休克，败血症，内毒素血症，败血症，烧伤组成的组的疾病或异常病症。