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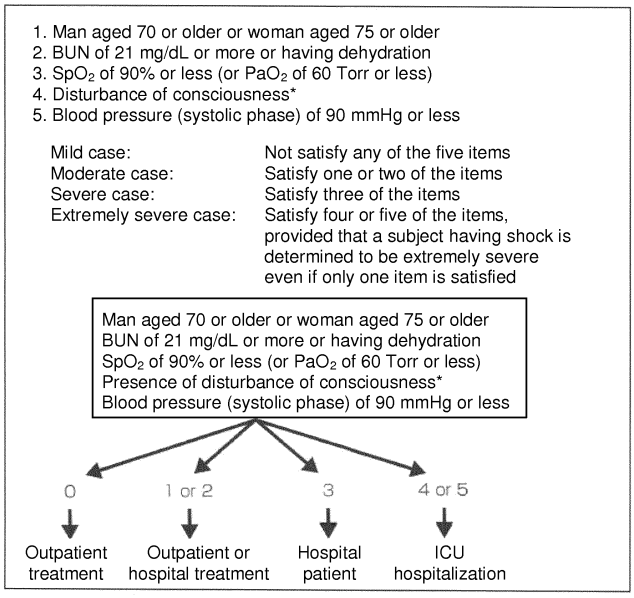
(54) METHOD FOR DETERMINING SEVERITY OF PNEUMOCOCCAL PNEUMONIA

(57) Provided is a method that can more objectively and rapidly determine severity of pneumococcal pneumonia using blood of a subject. The method determines severity of pneumococcal pneumonia, the method including determining the severity based on the presence or absence of a pneumococcal antigen in blood collected from a subject and at least one biochemical value selected from a blood C-reactive protein (CRP) level, a white blood cell (WBC) count and a blood urea nitrogen (BUN) level.

Figure 1



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Description

[Technical Field]

[0001] The present invention relates to a method for determining severity of pneumococcal pneumonia using blood of a subject.

[Background Art]

[0002] Streptococcus pneumoniae (S. pneumoniae) is a bacterium that is most frequently detected as a causative bacterium for community-acquired pneumonia or lower respiratory tract infection and is one of causative bacteria associated with high morbidity and mortality not only in Japan but also in the whole world. Pneumococcal infections show high frequency of occurrence and are also apt to increase in severity. Accordingly, it is important to determine the causative bacteria as early as possible and to select a correct therapeutic drug from the viewpoints of improvement in prognosis, reduction in medical care cost, and prevention of the emergence of resistant bacteria.

[0003] In general, pneumonia is comprehensively diagnosed based on, for example, clinical manifestations, physical findings, general examination findings, and chest radiograph. A patient diagnosed with pneumonia is then subjected to determination of severity in accordance with the A-DROP system shown in "Guidelines for the Management of Community-Acquired Pneumonia in Adults" (Non Patent Document 1). Based on the result, treatment, i.e., outpatient treatment, hospital treatment, or ICU treatment is selected. Here, the A-DROP system determines the severity based on the number of indices that apply to the subject among the following five indices: 1) men aged 70 or older or women aged 75 or older, 2) BUN of 21 mg/dL or more or having dehydration, 3) SpO_2 of 90% or less (or PaO_2 of 60 Torr or less), 4) presence of disturbance of consciousness, and 5) blood pressure (systolic phase) of 90 mmHg or less (see Figure 1).

[0004] Subsequently, an examination for identifying pathogenic bacteria are carried out, and a course of treatment is established depending on the detected pathogenic bacteria. However, the identification of pathogenic bacteria and the drug susceptibility test by culture tests take several days to obtain the results. Recently, a rapid test using a diagnostic agent employing a specific antibody against a pneumococcal antigen in, for example, sputum, epipharynx swab, or urine (e.g., "RAPIRUN (registered trademark) S. pneumoniae" (Otsuka Pharmaceutical Co., Ltd.), "RAPIRUN (registered trademark) S. pneumoniae HS (otitis media/sinusitis)" (Otsuka Pharmaceutical Co., Ltd.), or "BinaxNOW (registered trademark) Streptococcus pneumoniae" (Alere Medical Co., Ltd.)) is performed at an early stage, and a treatment plan is established at an early point.

[0005] It has been reported that bacteremia is observed in 10% of pneumonia patients as complications and that particularly the frequency reaches 60% or more in S. pneumoniae infection. Accordingly, it is important to detect bacteria present in blood. Examples of the method for detecting bacteria in blood include blood culture, RT-PCR, and antigen test. Blood culture is implemented in pneumonia as much as possible. However, blood culture takes time to obtain the test results, and its detection rate is low, about 20%, compared to that of RT-PCR. Accordingly, the method is not effective in urgent situations. RT-PCR has a high detection rate, but it needs a special apparatus and is expensive. Consequently, RT-PCR has not been widely spread in clinical practice. Regarding antigen test, in principle, there are reports on detection of, for example, a C-polysaccharide antigen (C-ps) in serum by, for example, ELISA (Non Patent Documents 2 and 3), but they are detection methods requiring pretreatment or lacking promptness as in ELISA. Consequently, the actual state is that antigen test has not been put to practical use yet.

[0006] Accordingly, a means that can objectively and rapidly determine severity of pneumonia, which is currently judged based on clinical manifestations and information of biochemical markers only, and also can estimate the pathogenic bacteria has been desired.

[Citation List]

[Non Patent Document]

[0007]

[Non Patent Document 1] The JRS Guidelines for the Management of Community-Acquired Pneumonia in Adults, The Japanese Respiratory Society, 2007, pp. 9-12
[Non Patent Document 2] Gillespie S.H., et al., 1995, J. Clin. Pathol., 48: 803-806
[Non Patent Document 3] Coonrod J.D., et al., 1973, J. Lab. Clin. Med., 81: 778-786

[Summary of the Invention]

[Problems to be Solved by the Invention]

[0008] The present invention provides a method capable of more objectively and rapidly determining severity of pneumococcal pneumonia using blood of a subject.

[Means for Solving the Problems]

[0009] The present inventors have investigated methods that can simply and objectively determine severity of pneumococcal pneumonia at the time of initial diagnosis, and have found that when a pneumococcal antigen in blood of a patient is measured and the resulting information is combined with a measured value of a specific biochemical marker (biochemical value), patients with pneumococcal pneumonia that would be determined to be severe or extremely severe by an A-DROP system can be satisfactorily distinguished from patients with pneumococcal pneumonia that would be determined to be mild or moderate by the A-DROP system to thereby detect severe pneumococcal pneumonia. In addition, since this method measures a pneumococcal antigen in blood, pneumococcal bacteremia can also be simultaneously determined.

[0010] The present invention relates to the following aspects 1) to 4):

1) A method for determining severity of pneumococcal pneumonia, comprising determining the severity based on the presence or absence of a pneumococcal antigen in blood collected from a subject and at least one biochemical value selected from a blood C-reactive protein (CRP) level, a white blood cell (WBC) count and a blood urea nitrogen (BUN) level;

2) The method according to aspect 1), wherein the pneumococcal pneumonia is classified as severe or extremely severe when the pneumococcal antigen is positive and the biochemical value is in the following range:

CRP: 10 mg/dL or more,
WBC: 10,000 cells/mm^3 or less, or
BUN: 20 mg/dL or more;

3) The method according to aspect 1) or 2), wherein the pneumococcal antigen is at least one selected from a C-polysaccharide antigen, a capsular antigen, and a cell membrane polysaccharide antigen; and

4) The method according to any one of aspects 1) to 3), wherein pneumococcal bacteremia is simultaneously evaluated.

[Effects of the Invention]

[0011] The method of the present invention can objectively and rapidly judge severity of pneumonia, which have been judged based on clinical manifestations and information of biochemical markers only, and also can decide the place for treatment (outpatient treatment, hospital treatment, or ICU treatment) based on the severity, including estimation of the pathogenic bacteria.

[0012] Consequently, a suitable antibacterial agent can be selected at an early point, which leads to an improvement in prognosis, a reduction in medical care cost, and a prevention of the emergence of resistant bacteria.

[0013] In addition, the method of the present invention can detect a pneumococcal antigen in blood with a sensitivity equivalent to that of RT-PCR and therefore can simultaneously detrmine pneumococcal bacteremia.

[Brief Description of the Drawings]

[0014]

[Figure 1] Figure 1 shows a relationship between severity classification by A-DROP system and place for treatment.

[Figure 2] Figure 2 includes a graph and a table showing the line intensity (pneumococcal antigen detection sensitivity) at each serum dilution rate.

[Figure 3] Figure 3 is a diagram showing the line intensity (pneumococcal antigen detection sensitivity) in each blood sampling method.

[Figure 4] Figure 4 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and CRP with severity.

[Figure 5] Figure 5 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen

and WBC with severity.

[Figure 6] Figure 6 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and BUN with severity.

[Figure 7] Figure 7 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and neutrophils with severity.

[Figure 8] Figure 8 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and Na with severity.

[Figure 9] Figure 9 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and Cr with severity.

[Figure 10] Figure 10 is a graph showing a correlation of a combination of the detection of blood pneumococcal antigen and BNP with severity.

[Modes for Carrying out the Invention]

[0015] Examples of the subject to which the method of the present invention is applied include patients with pneumococcal pneumonia, patients with suspected pneumococcal pneumonia, patients with suspected pneumococcal respiratory tract infection, patients with suspected systemic pneumococcal infection, patients with suspected pneumococcal inflammation, and healthy subjects who are suspected to have any of pneumococcal diseases represented by the above diseases. The patients mentioned here are not limited to human, and mammals other than human can be subjects of the present invention.

[0016] The "blood" collected from a subject encompasses blood (whole blood), plasma, and serum.

[0017] In measurement of a pneumococcal antigen, serum or plasma is preferably used, and 1- to 10-fold, preferably 2-fold diluted serum or plasma is preferred from the viewpoint of increasing the detection sensitivity.

[0018] Examples of the pneumococcal antigen used in the method of the present invention include a cell wall antigen (C-polysaccharide antigen (C-polysaccharide: C-ps)), a capsular antigen (capsular polysaccharide), and a cell membrane polysaccharide antigen (F-antigen: lipoteichoic acid/teichoic acid). Among these antigens, the C-ps, the capsular antigen, and the cell membrane polysaccharide antigen are preferred, and the C-ps, the capsular antigen, and the cell membrane polysaccharide antigen are more preferably measured or detected together.

[0019] In the method of the present invention, the presence or absence of the pneumococcal antigen in blood is measured, and the measurement encompasses quantitative and non-quantitative measurements. Examples of the non-quantitative measurement include mere measurement of whether or not a pneumococcal antigen is present, measurement of whether or not a certain amount or more of a pneumococcal antigen is present, and measurement of comparing the amount of a pneumococcal antigen to that of another sample (e.g., control sample). Examples of the quantitative measurement include measurement of the concentration of a pneumococcal antigen and measurement of the amount of a pneumococcal antigen.

[0020] In the present invention, that a pneumococcal antigen is positive means that the pneumococcal antigen is present at least in blood.

[0021] Preferred examples of the measurement method of a pneumococcal antigen include immunoassay using an antibody against the pneumococcal antigen. The antibody against the pneumococcal antigen may be any antibody that specifically binds to the pneumococcal antigen, and no limitation is imposed on the source, type (monoclonal or polyclonal) and shape of the antibody. Specifically, a known antibody such as a mouse antibody, a rat antibody, a human antibody, a chimeric antibody, or a humanized antibody can be used.

[0022] Examples of monoclonal antibodies derived from mammals include monoclonal antibodies produced by hybridomas and monoclonal antibodies produced by a host transformed with expression vectors containing antibody genes by genetic engineering. The monoclonal antibody-producing hybridoma can be basically produced through a known technique by performing immunization with a pneumococcal antigen as a sensitized antigen in accordance with a usual immunization method, fusing the resulting immune cells with known parent cells in accordance with a usual cell fusion method, and screening for monoclonal antibody-producing cells by a usual screening method.

[0023] The antibody may be a low-molecular antibody such as an antibody fragment or a modified antibody as long as having the characteristic of recognizing a pneumococcal antigen. Specific examples of the antibody fragment include Fab, Fab', F(ab')2, Fv, and diabody.

[0024] Specific examples of the antibody against a pneumococcal antigen include antibodies against C-ps, such as those described in 1) Nielsen, et al., Antibodies against pneumococcal C-polysaccharide are not protective, Microbial Pathogenesis 14: 299-305 (1993), 2) Gillespie, et al., Detection of C-polysaccharide in serum of patients with Streptococcus pneumoniae bacteremia, J. Clin. Pathol., 48: 803-806 (1995), 3) United States Patent No. 6824997 (1997), or 4) Ehara, et al., A novel method for rapid detection of Streptococcus pneumoniae antigen in sputum and its application in adult respiratory tract infection, Journal of Medical Microbiolog, 57: 820-82 (2008); antibodies against a capsular antigen, such as those described in 1) Coonrod, et al., Detection of type-specific pneumococcal antigens by counterim-

munoelectrophoresis. 11. Etiologic diagnosis of pneumococcal pneumonia, J. Lab. Clin. Med., 81, 778-786 (1973), 2) Feigin, et al., Countercurrent immunoelectrophoresis of urine as well as of CSF and blood for diagnosis of bacterial meningitis, The Journal of Pediatrics, 89, 773-775 (1976), 3) Ajello, et al., Commercial Latex Agglutination Tests for Detection of Haemophilus influenza Type b and Streptococcus pneumoniae Antigens in Patients with Bacteremic Pneumonia, JOURNAL OF CLINICAL MICROBIOLOGY, 25(8), 1388-1391 (1987), 4) Ballaed, et al., Comparison of three latex agglutination kits and counterimmunoelectrophoresis for the detection of bacterial antigens in a pediatric population, Pediatr. Infect. Dis. J., 6(7), 630-634 (1987), or 5) Kobayashi, et al., Evaluation of Streptococcus Pneumoniae-urinary Antigen Detection kit in Patients with Community Acquired Pneumonia, The Journal of the Japanese Association for Infectious Diseases, Vol. 76, No. 12, 995-1002 (2002); and antibodies against a cell membrane polysaccharide antigen, such as those described in Hotomi, et al., Evaluation of a Rapid Immunochromatographic ODK-0901 Test for Detection of Pneumococcal Antigen in Middle Ear Fluids and Nasopharyngeal Secretions, PLoS one, 7(3), e33620 (2012).

[0025] The immunoassay using an antibody against a pneumococcal antigen can be any immunoassay known in the art, and examples thereof include radioimmunoassay (RIA), enzyme immunoassay (EIA) such as ELISA, latex turbidimetric immunoassay (LTIA), and immunochromatography. From the viewpoint of improving detection sensitivity, a sandwich method is preferred. From the viewpoint of simplicity and rapidness, immunochromatography is preferred. The immunochromatography allows diagnosis at bedside or diagnosis within a short time at the time of visit of a patient.

[0026] The label used in the immunoassay may be any label used in the art, and examples thereof include enzymes such as horseradish peroxidase (HRP), alkaline phosphatase, and β-galactosidase; radioisotopes (RI) such as 1251, 32P, 14C, 35S, and 3H; fluorescent materials such as FITC and tetramethyl rhodamine isocyanate; luminescent materials such as chemiluminescent materials; and visualized materials such as gold colloid and colored latex particles. In addition, there may be used a sensitization system using biotin in primary labeling and then avidin labeled with any of the above-mentioned labels or a detection process using a low-molecular material, such as digoxigenin, in primary labeling and then a material, such as an antibody, having affinity to the low-molecular material and labeled with any of the above-mentioned labels.

[0027] The measurement of a pneumococcal antigen in the present invention can also be performed using a commercially available S. pneumoniae detection kit such as "RAPIRUN S. pneumoniae HS (otitis media/sinusitis)" (Otsuka Pharmaceutical Co., Ltd.) and "BinaxNOW Streptococcus pneumoniae" (Alere Medical Co., Ltd.).

[0028] The biochemical value used in the present invention is one or more selected from a blood C-reactive protein (CRP) level, a white blood cell (WBC) count, and a blood urea nitrogen (BUN) level.

(1) C-reactive protein (CRP)

[0029] CRP is a protein that appears in blood when an inflammatory reaction or tissue destruction is occurring in the body and has a property of binding to the C-polysaccharide of S. pneumoniae. CRP is generally used as an inflammatory marker.

[0030] CRP was defined as a parameter for determining severity of pneumonia in "Basic concept for the management of community-acquired pneumonia in adults", which is former guidelines established in 2000. However, in verification in "Guidelines for respiratory infection" established in 2007, there was a report on that CRP does not accurately reflect severity. Accordingly, in new guidelines, CRP was deleted from the items for determining severity (see Non Patent Document 1).

[0031] However, when used in combination with detection (positive) of a pneumococcal antigen, CRP can determine severe and extremely severe pneumococcal pneumonia (see Example 3). In this case, the cut-off value of CRP is 10 to 20 mg/dL.

[0032] That is, in the present invention, the CRP as an index of severe and extremely severe pneumococcal pneumonia is 10 mg/dL or more and preferably 20 mg/dL.

[0033] In the present invention, CRP measurement can be carried out by, for example, but not limited to, latex immunoturbidimetric assay.

(2) White blood cell (WBC) count

[0034] It is known that white blood cell (WBC) count in blood generally increases in bacterial infection, but its level in blood decreases in severe pneumonia due to localization thereof in the lung. WBC was, as in CRP, defined as a parameter for determining severity of pneumonia in the past, but was deleted from the items for determining severity in new guidelines established in 2007 (see Non Patent Document 1).

[0035] However, when used in combination with detection (positive) of a pneumococcal antigen, WBC also can determine severe and extremely severe pneumococcal pneumonia (see Example 3). In this case, the cut-off value of WBC is 6,000 to 10,000 cells/mm^3.

[0036] That is, in the present invention, the WBC as an index of severe and extremely severe pneumococcal pneumonia

is within a distribution range of healthy subjects, or in the case of less than the range, for example, the WBC is 10,000 cells/mm^3 or less and preferably 6,000 cells/mm^3 or less.

[0037] In the present invention, WBC measurement can be measured with, for example, but not limited to, an automatic analyzer that can count blood cells.

(3) Blood urea nitrogen (BUN) level

[0038] BUN represents the amount of nitrogen derived from urea in blood and is used as a kidney function marker.

[0039] BUN is known to be high in severe pneumonia patients showing highly enhanced dehydration and catabolism. When used in combination with detection (positive) of a pneumococcal antigen, BUN can determine severe and extremely severe pneumococcal pneumonia with higher accuracy (see Example 3). In this case, the cut-off value of BUN is, for example, about 20 mg/dL.

[0040] That is, in the present invention, the BUN as an index of severe and extremely severe pneumococcal pneumonia is, for example, 20 mg/dL or more.

[0041] In the present invention, BUN can be measured by, for example, but not limited to, a urease GLDH method or a urease ICDH method. Of these, the urease GLDH method is preferably used.

[0042] The method of the present invention measures a pneumococcal antigen in blood and therefore can simultaneously determine pneumococcal bacteremia. Here, the term "Pneumococcal bacteremia" means that S. pneumoniae is present in blood. That is, in the method of the present invention, that a blood pneumococcal antigen is positive suggests pneumococcal bacteremia. Although pneumococcal bacteremia has been conventionally determined by detecting S. pneumoniae in blood culture, the detection rate in blood culture is recognized to be low for not only S. pneumoniae but also microorganisms. Accordingly, in recent years, detection by RT-PCR is increasingly used (Resti, et al., Community-Acquired Bacteremic Pneumococcal Pneumonia in Children: Diagnosis and Serotyping by Real-Time Polymerase Chain Reaction Using Blood Samples, Clinical Infectious Diseases, 51(9): 1042-1049 (2010)). The method of the present invention can infer pneumococcal bacteremia in a simple manner without using RT-PCR.

[Examples]

<Material and method>

[0043] Serum or plasma was separated from whole blood collected from each patient who gave informed consent by usual centrifugation or precipitation method and was stored at -80°C.

(1) Preparation of specimen

[0044] A serum fraction was obtained by collecting blood in a blood-collecting tube containing a serum separator and centrifuging the blood. Plasma was obtained by collecting blood in a blood-collecting tube containing EDTA-2Na or heparin and collecting the supernatant after centrifugation or standing. Both specimens were stored at -80°C.

(2) Evaluation of optimal dilution rates of serum/plasma for measuring pneumococcal antigen

[0045] The optimal dilution rates of serum/plasma were evaluated with "RAPIRUN S. pneumoniae HS (otitis media/sinusitis)" (Otsuka Pharmaceutical Co., Ltd.). Serum/plasma was serially diluted with the specimen extraction reagent included in the kit, and a dilution rate providing the strongest test line intensity was determined. The line after measurement was photographed with a commercially available digital camera and was then evaluated for the intensity with a densitometer (ATTO Co., Ltd.).

(3) Measurement of pneumococcal antigen

[0046] The presence or absence of a pneumococcal antigen in blood was measured with commercially available kits shown below.

1) RAPIRUN S. pneumoniae HS (otitis media/sinusitis) (Otsuka Pharmaceutical Co., Ltd.)

[0047] Two-fold diluted serum/plasma, which had been judged as an optimal dilution rate in the above (2), was dispensed in an amount of 150 μL into a sample cup included in the kit. The sample applying portion of a test stick (the reagent itself) was immersed in the 2-fold diluted serum/plasma, and the subsequent measuring procedure was performed in accordance with the package insert.

2) "BinaxNOW (R) Streptococcus pneumoniae" (Alere Medical Co., Ltd.)

[0048] The cotton swab included in the kit was immersed in the 2-fold diluted serum/plasma prepared in the above 1), and evaluation was performed in accordance with the package insert.

(4) Other measurement items

1) Biochemical marker

[0049] CRP, WBC, neutrophil, BUN, Na, Cr, and BNP of the serum specimen were measured with a Hitachi automated analyzer LABOSPECT 008.

2) Culture

[0050] In order to estimate the pathogenic bacteria, blood and sputum were cultured. Each specimen was cultured in accordance with the standard of Clinical and Laboratory Standards Institute (CLSI).

3) PCR

[0051] S. pneumoniae PspA DNA in serum was quantitatively measured using the PspA gene of S. pneumoniae as a target in accordance with the method in a previous report (Ehara N., et al., 2008, Journal of Medical Microbiology, 57: 820-826). The detection sensitivity in the facility was 200 copies/μg DNA.

Example 1

(1) Severity determination

[0052] Biochemical markers and a pneumococcal antigen in blood were evaluated for the cases shown below that had been subjected to the severity determination using the A-DROP system described in the Guidelines for the Management of Community-Acquired Pneumonia in Adults (Non Patent Document 1). The number of cases of each severity is shown in Table 1.

[Table 1]

	Cases other than pneumonia*	Pneumonia			
		Mild	Moderate	Severe	Extremely severe
Number of specimens	6	16	3	4	3
*: Nontuberculous mycobacterial infection, bronchitis, bronchial asthma, pulmonary emphysema, and dehydration					

(2) Adjustment of optimal specimen concentration

[0053] Different six serum samples were each measured with a kit "RAPIRUN S. pneumoniae HS (otitis media/sinusitis) " as an index before (dilution rate: 1-fold) and after 2-, 6-, and 18-fold dilution with the specimen extraction reagent included in the kit. The line intensities were compared. Figure 2 shows the line intensity at each dilution rate.

[0054] Figure 2 demonstrates that the line intensity showed a maximum value at 2-fold dilution in every serum specimen. The undiluted serum had high viscosity and tended to have a slow flow rate, and thus 2-fold dilution (undiluted serum : specimen extraction solution = 1:1) was believed to be the optimal specimen concentration in both promptness and line intensity.

(3) Difference between blood sampling methods (specimen type)

[0055] Serum and plasma were prepared from blood collected from one patient, wherein the plasma was prepared by four different procedures shown in Figure 3. The line intensities of the serum and plasma were evaluated with "RAPIRUN S. pneumoniae HS (otitis media/sinusitis)". Figure 3 also shows the results.

[0056] In every blood sampling method (serum, EDTA-2Na plasma, or heparin plasma) and in every separation method (centrifugation or standing separation), the line intensities were substantially the same. Thus, the line intensity was

believed not to be affected by the blood sampling method and the separation method. Accordingly, in the subsequent evaluation, serum prepared by centrifugation was used.

(4) Sensitivity and specificity of detection of blood pneumococcal antigen in each patient

[0057] The sensitivity and specificity of detection of blood pneumococcal antigen in each patient were calculated based on the results of a sputum culture test. The sensitivity is the ratio of the number of cases determined to be positive in each measurement method to the number of sputum culture positive cases. The specificity is the ratio of the number of cases determined to be negative in each measurement method to the number of sputum culture negative cases (Table 2).

[Table 2]

		RAPIRUN S. pneumoniae HS		BinaxNOW Streptococcus pneumoniae		RT-PCR	
		Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Cases other than pneumonia		0% (0/3)	100% (3/3)	33% (1/3)	100% (3/3)	0% (0/3)	-
Pneumonia	Mild	25% (3/12)	100% (4/4)	8% (1/12)	100% (4/4)	0% (0/12)	-
	Moderate	-	100% (3/3)	-	100% (3/3)	-	100% (3/3)
	Severe	100% (3/3)	100% (1/1)	100% (3/3)	100% (1/1)	67% (2/3)	100% (1/1)
	Extremely severe	100% (2/2)	0% (0/1)	100% (2/2)	100% (1/1)	100% (2/2)	-

[0058] "RAPIRUN S. pneumoniae HS" and "BinaxNOW Streptococcus pneumoniae" both tended to increase the sensitivity as the severity of pneumonia increases, and the detection rates thereof were equivalent to that of "RT-PCR", which is known as a method of detecting S. pneumoniae with high sensitivity.

Example 2: Results of analysis of severe or extremely severe cases

[0059] The blood pneumococcal antigen of the pneumonia cases determined to be severe or extremely severe was measured with "RAPIRUN S. pneumoniae HS" and "BinaxNOW Streptococcus pneumoniae", and the results were compared to those of blood PCR. As a result, five severe or extremely severe cases in which S. pneumoniae was detected in sputum culture were all positive in both kits. In PCR, four cases, excluding one case, were positive.

[Table 3]

Specimen No.	Severity of pneumonia	Sputum	Urine	Blood			
		Culture (S. pneumoniae)	Binax	Culture	PCR	Binax	RAPIRUN HS
F-7232	Severe	positive	(+)	negative	2×10^4	(+)	(+)
F-7481	Severe	positive	(+)	negative	$<2\times10^2$	(+)	(+)
F-7164	Severe	positive	(+)	NT	2×10^3	(+)	(+)
F-7506	Extremely severe	positive	(+)	negative	1×10^3	(+)	(+)
F-7322	Extremely severe*	positive	NT	NT	6×10^3	(+)	(+)
F-7210	Severe	negative	negative	negative	$<2\times10^2$	(-)	(-)

(continued)

Specimen No.	Severity of pneumonia	Sputum	Urine	Blood			
		Culture (S. pneumoniae)	Binax	Culture	PCR	Binax	RAPIRUN HS
F-7550	Extremely severe	negative	negative	negative	NT	(-)	(+)

*: The severity was unclear on the measurement day, but the severity was determined to be extremely severe 2 days after.
NT: not tested
RAPIRUN HS: RAPIRUN S. pneumoniae HS, Binax: BinaxNOW Streptococcus pneumoniae

Example 3: Clinical course

[0060] Clinical course of one patient was evaluated. PCR, "RAPIRUN S. pneumoniae HS", and "BinaxNOW Streptococcus pneumoniae" were all negative on the first day of sampling specimens, but all turned to positive 2 days after. Thus, it was judged that the detection efficiencies of PCR, "RAPIRUN S. pneumoniae HS", and "BinaxNOW Streptococcus pneumoniae" were approximately the same (Table 4).

[Table 4]

	Specimen sampling day	Severity of pneumonia	Sputum	Urine	Blood			
			Culture	Binax	PCR	RAPIRUN HS	Culture	Binax
F-7321	1st day	-*	positive	NT	<200	(-)	NT	(-)
F-7322	2 days after	Severe	positive	NT	6000	(+)	NT	(+)
F-7308	4 days after	Extremely severe	negative	(+)	40000	(+)	(-)	(+)
F-7310	4.5 days after	Extremely severe	NT	NT	200000	(+)	NT	(+)
F-7314	5 days after	Extremely severe	NT	(+)	200000	(+)	(-)	(+)
F-7317	6 days after	Extremely severe	negative	NT	70000	(+)	(-)	(+)

RAPIRUN HS: RAPIRUN S. pneumoniae HS, Binax: BinaxNOW Streptococcus pneumoniae
*: Diagnosed with bronchitis

Example 3: Correlation of a combination with measured biochemical values with severity

[0061] (1) Pneumonia cases were classified into a group of severe or extremely severe cases and a group of others and moderate or less cases (including mild cases), and each group was further classified into a subgroup of "RAPIRUN S. pneumoniae HS" (indicated by HS in the drawings) positive cases and a subgroup of negative cases. The measured values of biochemical markers (CRP, WBC, and BUN) were plotted on the vertical axis.

1) CRP (Figure 4)

[0062] In a combination of CRP and "RAPIRUN S. pneumoniae HS", S. pneumoniae PCR positive group could be extracted. It was believed that the optimal cut-off value of CRP in this case was 20 mg/dL.

2) WBC (Figure 5)

[0063] In a combination of WBC and "RAPIRUN S. pneumoniae HS", PCR positive cases could be efficiently detected. It is believed that the upper limit of the distribution range in healthy adults, 10000 cells/mm^3, can be employed as the

cut-off value of WBC in this case.

3) BUN (Figure 6)

[0064] In a combination of BUN and "RAPIRUN S. pneumoniae HS", PCR positive cases could be efficiently detected. It is believed that 20 mg/dL can be employed as the cut-off value of BUN in this case.

Comparative Example

[0065] Pneumonia cases were classified into a group of severe or extremely severe cases and a group of others and moderate or less cases (including mild cases), and each group was further classified into a subgroup of "RAPIRUN S. pneumoniae HS" (indicated by HS in the drawings) positive cases and a subgroup of negative cases. The measured values of biochemical markers (neutrophil, Na, Cr, and BNP) were plotted on the vertical axis.

[0066] The results are shown in Figures 7 to 10. When neutrophil or Na was employed, there was no significant difference between the groups. Regarding Cr and BNP, high value cases were observed in the subgroup of RAPIRUN S. pneumoniae HS positive cases of the group of severe cases, which was limited to a part of the cases and did not show clinical significance.

[Industrial Applicability]

[0067] In diagnosis using a biochemical marker alone, though the severity of a case suspected with pneumonia can be predicted to some extent, it is impossible to distinguish from not only S. pneumoniae but also from other infectious diseases. It has been reported that about 50% to 80% of the microorganisms causing community-acquired pneumonia in adults are bacteria and, among them, 12% to 27% of the pneumonia are pneumococcal pneumonia. Pneumococcal pneumonia is particularly apt to become severe, and discrimination of S. pneumoniae from other pathogenic microorganisms is clinically important. Furthermore, in order to prevent various pathogenic bacteria from becoming resistant to drugs, it is believed that selection of an optimal antibacterial agent by rapidly determining pathogenic bacteria is important also for shortening the duration of treatment. In the case of S. pneumoniae, the guidelines require that the first choice is a penicillin antibiotic. Accordingly, a combination of a biochemical marker and a blood pneumococcal antigen detection system can rapidly determine severe pneumococcal pneumonia, which cannot be judged by the biochemical marker alone, and can simultaneously determine severity of pneumonia, which cannot be determined by the pneumococcal antigen detection system alone, and therefore has significance for selecting an optimal antibacterial agent and judging necessity of hospital treatment.

Claims

1. A method for determining severity of pneumococcal pneumonia, comprising determining the severity based on the presence or absence of a pneumococcal antigen in blood collected from a subject and at least one biochemical value selected from a blood C-reactive protein (CRP) level, a white blood cell (WBC) count and a blood urea nitrogen (BUN) level.

2. The determination method according to Claim 1, wherein the pneumococcal pneumonia is classified as severe or extremely severe when the pneumococcal antigen is positive and the biochemical value is in the following range:

   CRP: 10 mg/dL or more,
   WBC: 10,000 cells/mm^3 or less, or
   BUN: 20 mg/dL or more.

3. The method according to Claim 1 or 2, wherein the pneumococcal antigen is at least one selected from a C-polysaccharide antigen, a capsular antigen, and a cell membrane polysaccharide antigen.

4. The method according to any one of Claims 1 to 3, wherein pneumococcal bacteremia is simultaneously evaluated.

Figure 1

1. Man aged 70 or older or woman aged 75 or older
2. BUN of 21 mg/dL or more or having dehydration
3. SpO_2 of 90% or less (or PaO_2 of 60 Torr or less)
4. Disturbance of consciousness*
5. Blood pressure (systolic phase) of 90 mmHg or less

Mild case:	Not satisfy any of the five items
Moderate case:	Satisfy one or two of the items
Severe case:	Satisfy three of the items
Extremely severe case:	Satisfy four or five of the items, provided that a subject having shock is determined to be extremely severe even if only one item is satisfied

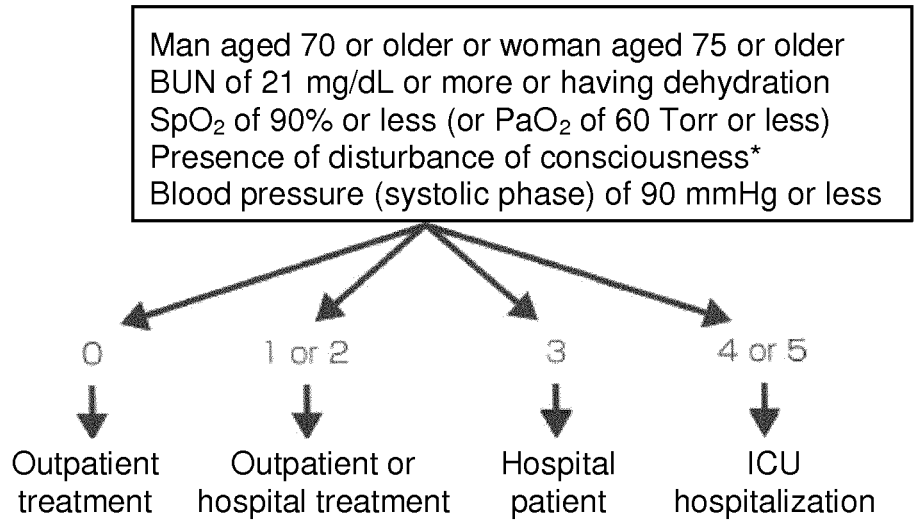
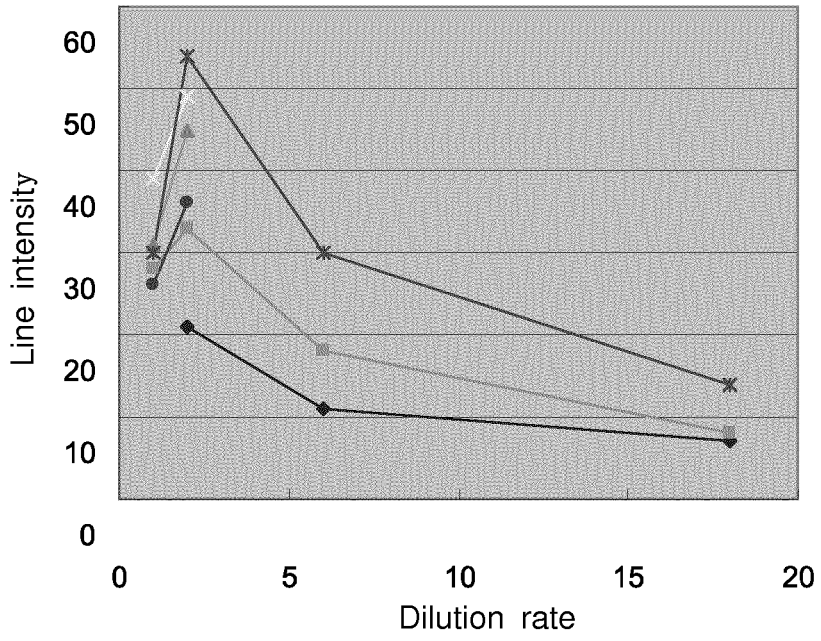


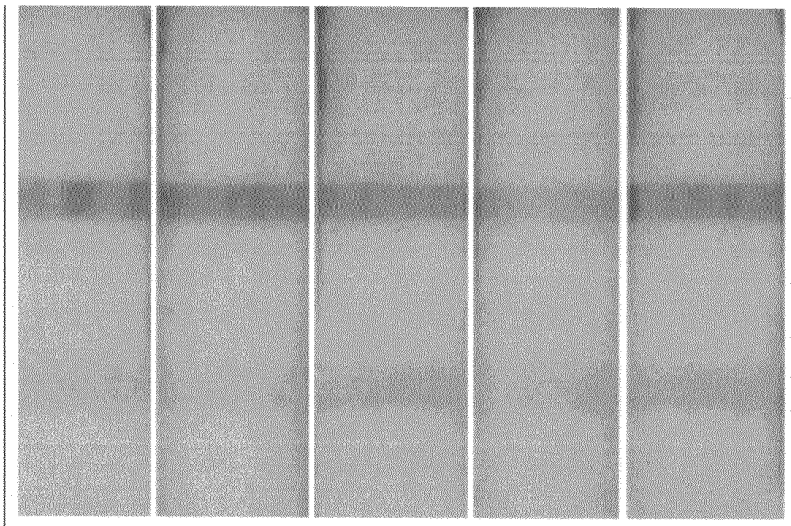
Figure 2



Line intensity at each dilution rate

Dilution rate	F-7232	F-7308-1	F-7308-2	F-7310	F-7314	F-7317
1	-	28	31	39	30	26
2	21	33	45	49	54	36
6	11	18	-	-	30	-
18	7	8	-	-	14	-

Figure 3



Line intensity	13	11	20	13	18
Specimen type	Serum	Plasma			
		Standing EDTA·2Na	Standing Heparin	Centrifugation EDTA·2Na	Centrifugation Heparin

Figure 4

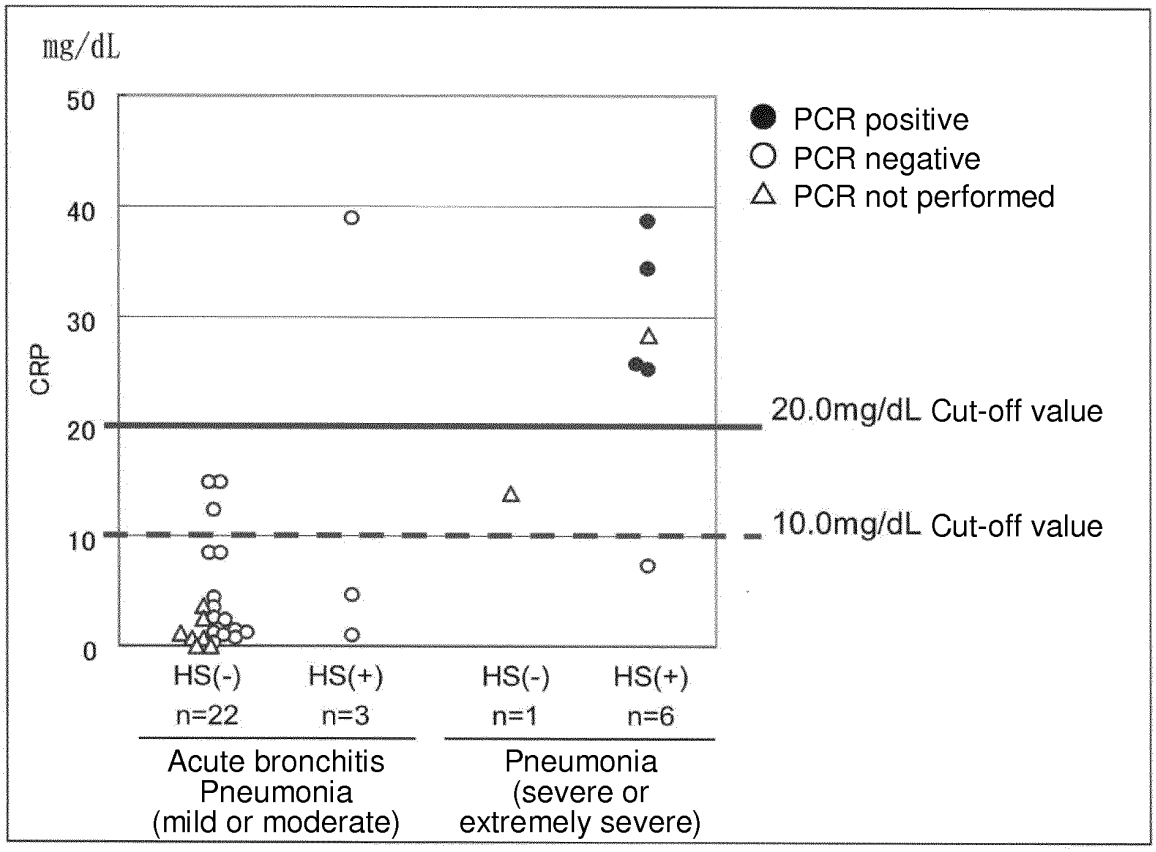

mg/dL
50
40
30
20
10
0
CRP
PCR positive
PCR negative
PCR not performed
20.0mg/dL Cut-off value
10.0mg/dL Cut-off value
HS(-)
n=22
HS(+)
n=3
HS(-)
n=1
HS(+)
n=6
Acute bronchitis
Pneumonia
(mild or moderate)
Pneumonia
(severe or
extremely severe)

Figure 5

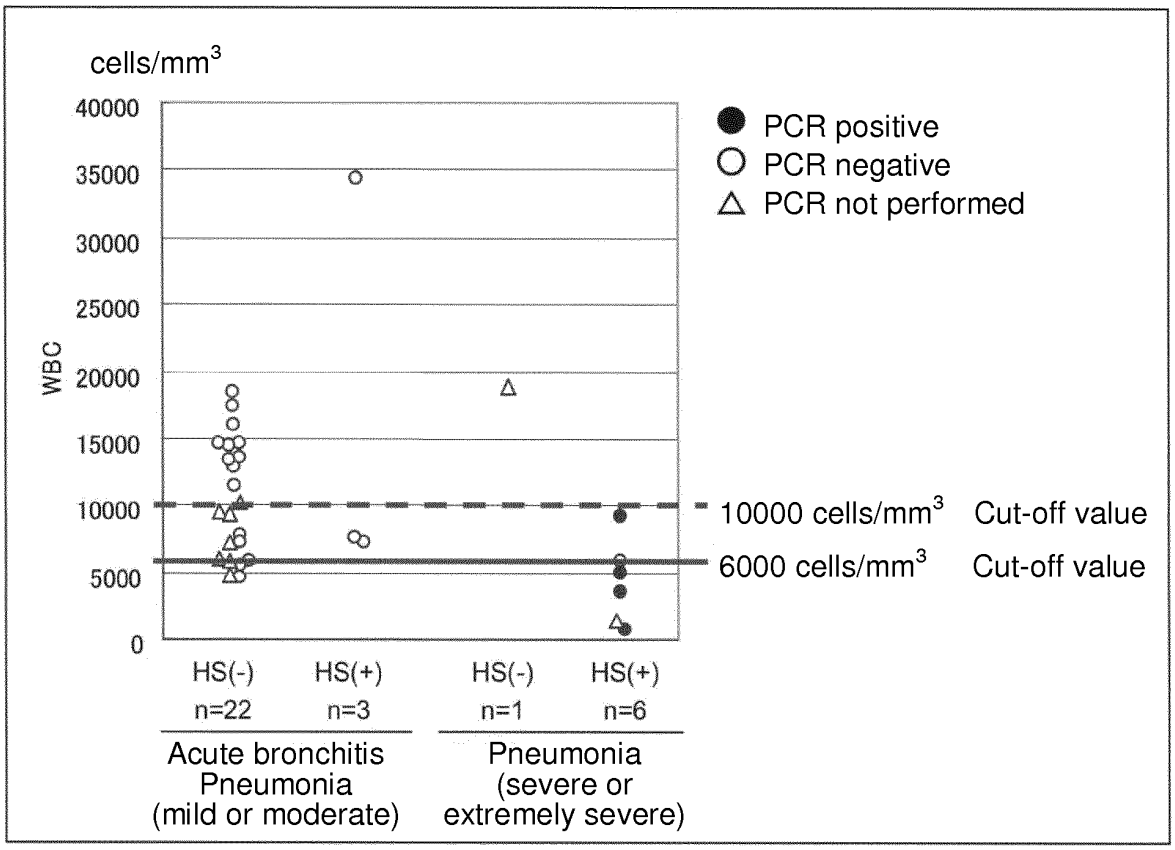

cells/mm3
WBC
40000
35000
30000
25000
20000
15000
10000
5000
0
PCR positive
PCR negative
PCR not performed
10000 cells/mm3 Cut-off value
6000 cells/mm3 Cut-off value
HS(-) n=22
HS(+) n=3
HS(-) n=1
HS(+) n=6
Acute bronchitis Pneumonia (mild or moderate)
Pneumonia (severe or extremely severe)

Figure 6

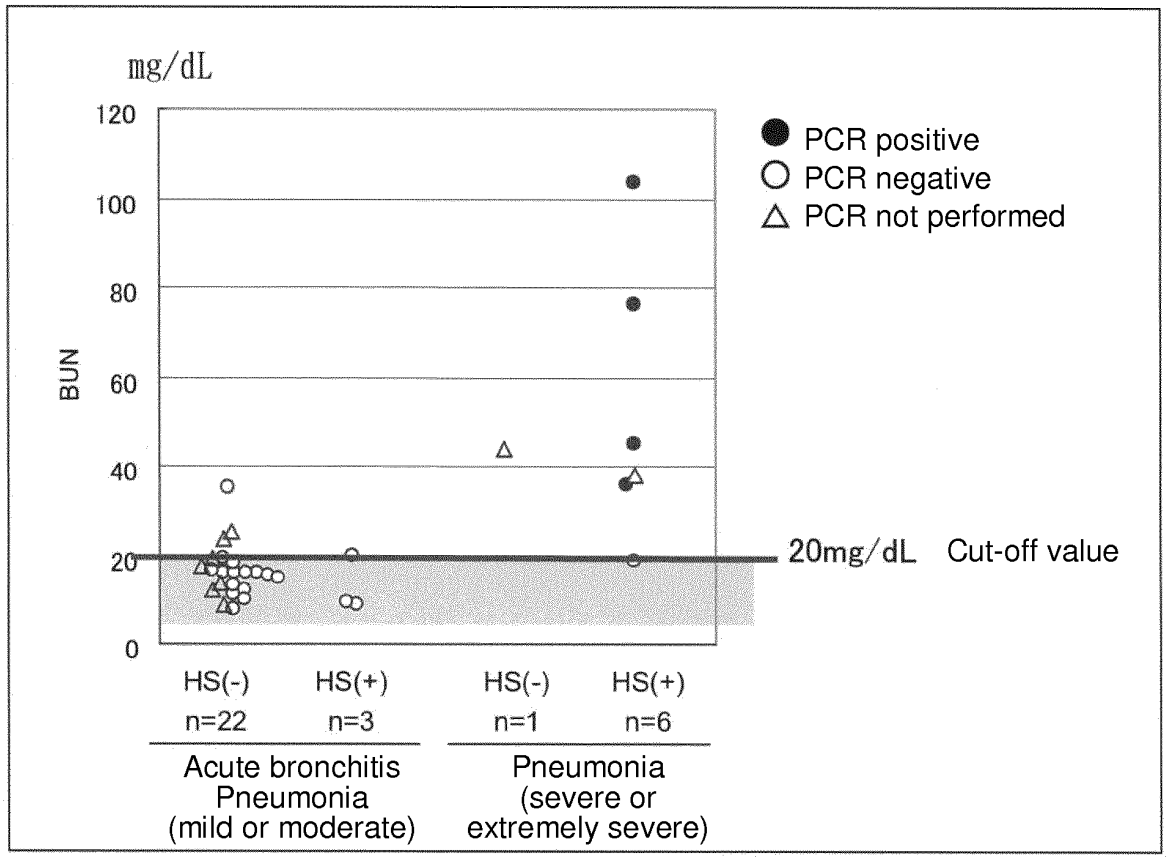

mg/dL
120
100
80
60
40
20
0
BUN
PCR positive
PCR negative
PCR not performed
20mg/dL Cut-off value
HS(-)
n=22
HS(+)
n=3
HS(-)
n=1
HS(+)
n=6
Acute bronchitis
Pneumonia
(mild or moderate)
Pneumonia
(severe or
extremely severe)

Figure 7

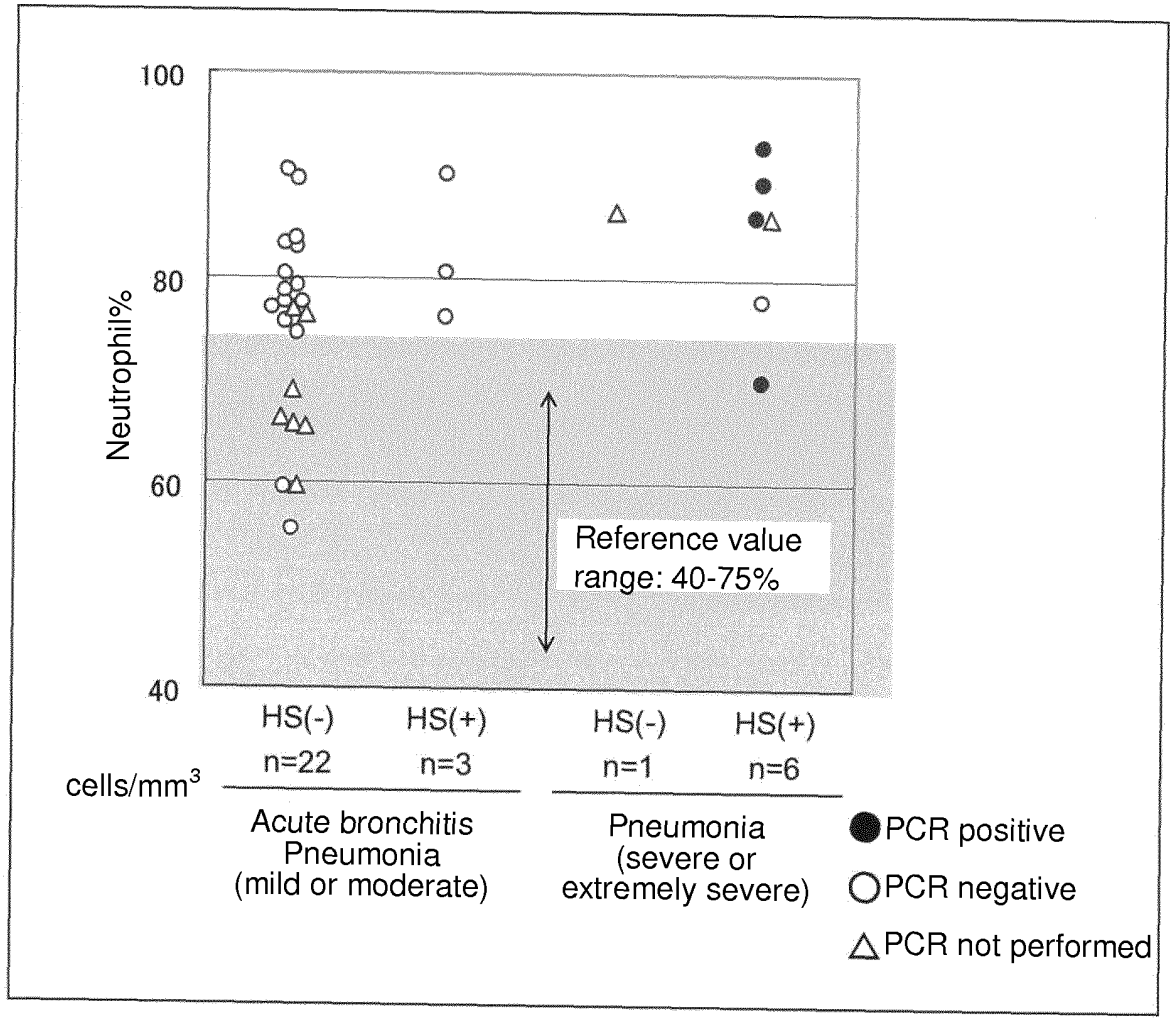

100
80
60
40
Neutrophil%
Reference value
range: 40-75%
HS(-)
n=22
HS(+)
n=3
HS(-)
n=1
HS(+)
n=6
cells/mm3
Acute bronchitis
Pneumonia
(mild or moderate)
Pneumonia
(severe or
extremely severe)
PCR positive
PCR negative
PCR not performed

Figure 8

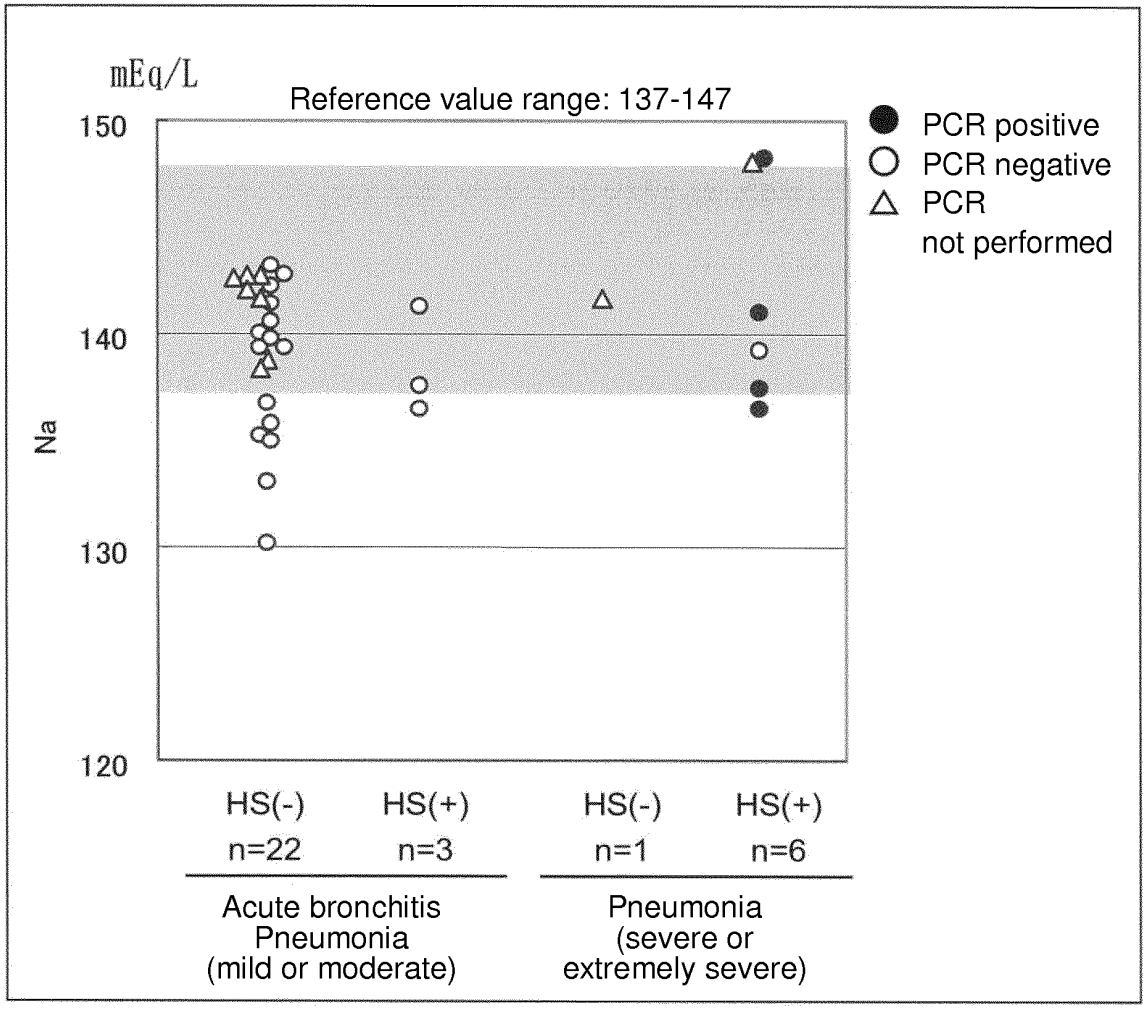

mEq/L
Reference value range: 137-147
150
140
130
120
Na
PCR positive
PCR negative
PCR not performed
HS(-)
n=22
HS(+)
n=3
HS(-)
n=1
HS(+)
n=6
Acute bronchitis Pneumonia (mild or moderate)
Pneumonia (severe or extremely severe)

Figure 9

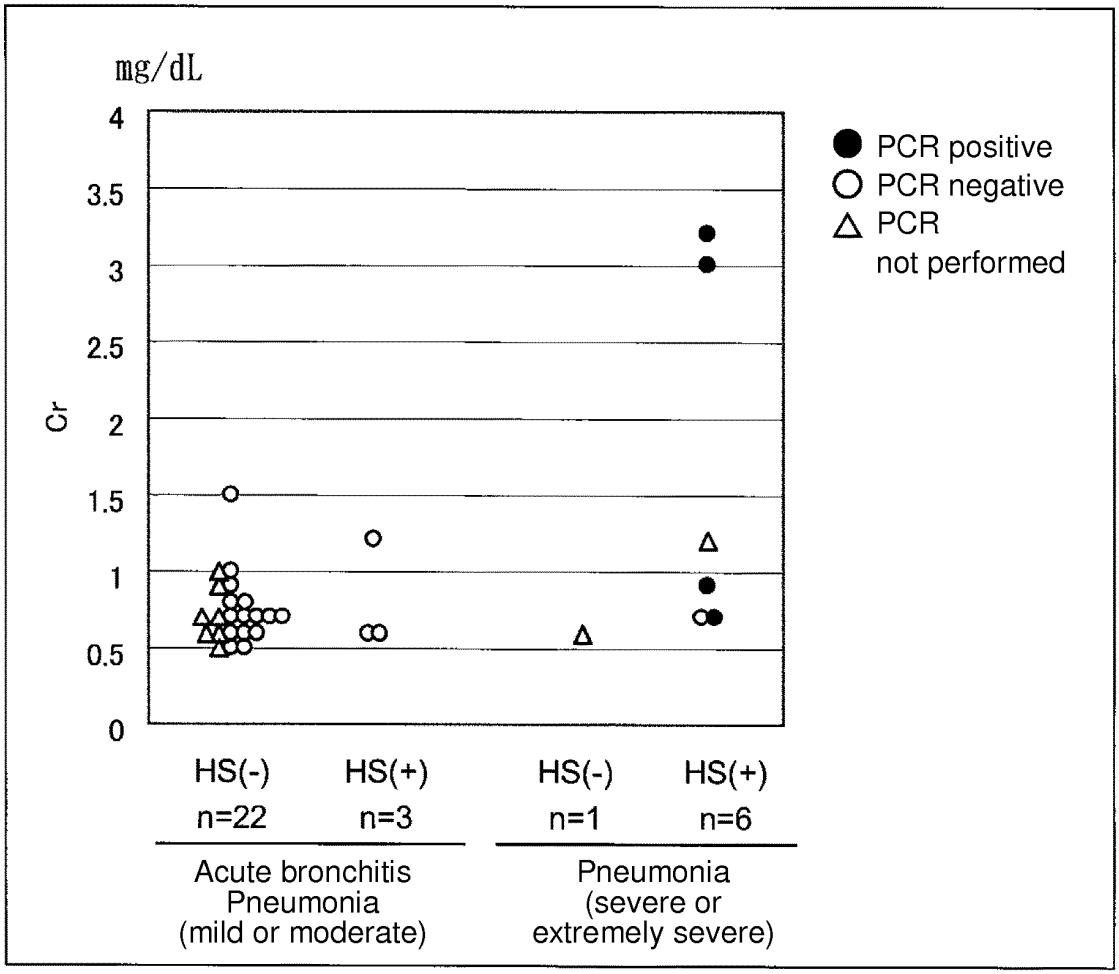
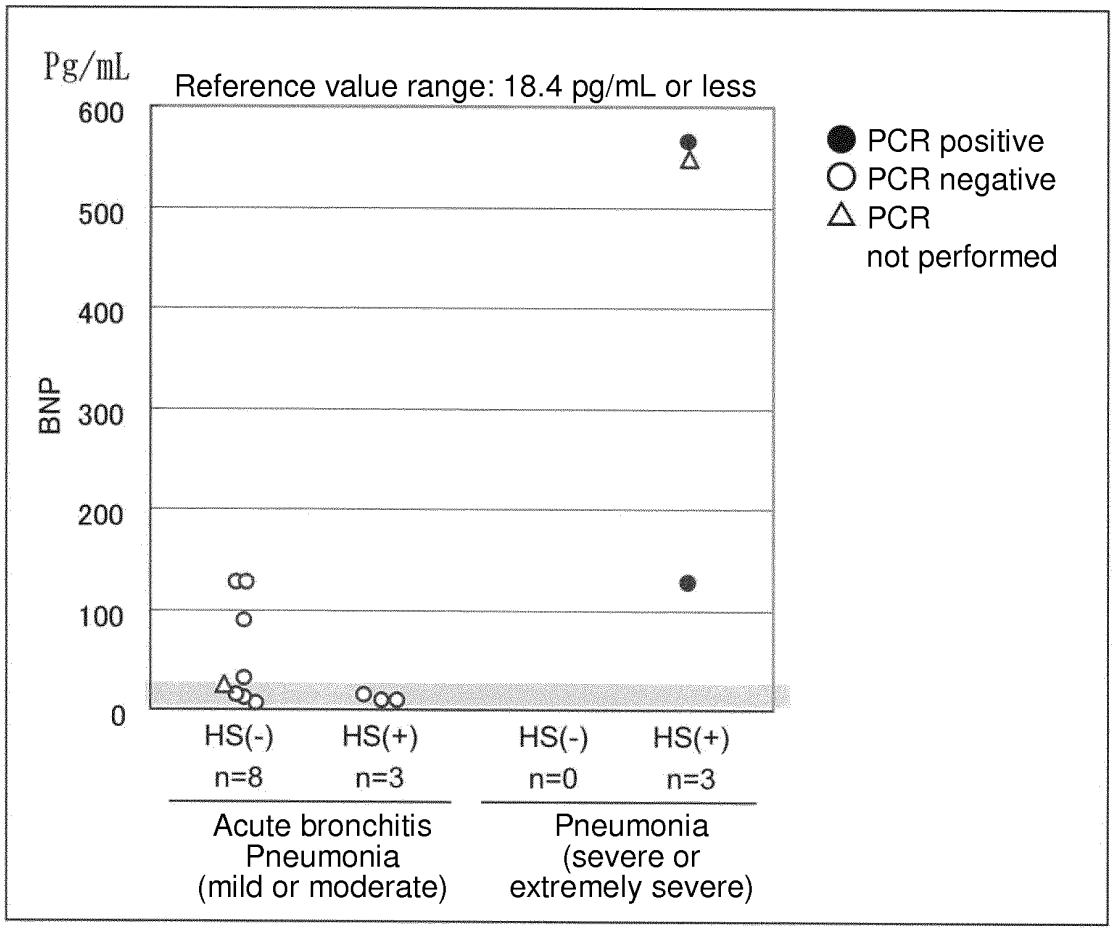

mg/dL
Cr
4
3.5
3
2.5
2
1.5
1
0.5
0
PCR positive
PCR negative
PCR not performed
HS(-) n=22
HS(+) n=3
HS(-) n=1
HS(+) n=6
Acute bronchitis Pneumonia (mild or moderate)
Pneumonia (severe or extremely severe)

Figure 10


Pg/mL
Reference value range: 18.4 pg/mL or less
600
500
400
300
200
100
0
BNP
PCR positive
PCR negative
PCR not performed
HS(-) n=8
HS(+) n=3
HS(-) n=0
HS(+) n=3
Acute bronchitis Pneumonia (mild or moderate)
Pneumonia (severe or extremely severe)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/059564

A. CLASSIFICATION OF SUBJECT MATTER
G01N33/569(2006.01)i, *G01N33/53*(2006.01)i, *C12Q1/04*(2006.01)n, *C12Q1/68*(2006.01)n

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)
G01N33/569, G01N33/53, C12Q1/04, C12Q1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2013
Kokai Jitsuyo Shinan Koho 1971-2013 Toroku Jitsuyo Shinan Koho 1994-2013

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JSTPlus/JMEDPlus/JST7580(JDreamIII)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Yuzuru ITO et al., "Haien Kyukinsei Haien no Jushodo no Hantei ni Okeru CRP-chi no Yuyosei", The Journal of the Japanese Respiratory Society, 2006, vol.44 special extra issue, page 279, PP165	1-4
Y	S H Gillespie, Detection of C-polysaccharide in serum of patients with Streptococcus pneumoniae bacteraemia, J Clin Pathol., 1995, Vol.48, p803-806, particularly, abstract, page 804, right column, 4th line from the bottom to page 805, left column, line 5	1-4
A	Nobuyasu ISHIKAWA et al., "Ketsueki Kensa Kekka yori Jushorei to Hantei sareta Haien no Kento", Japanese Society of Pediatric Pulmonology Zasshi, 2007, vol.18, no.1, pages 78 to 81	1-4

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"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
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"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
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 May, 2013 (22.05.13)	04 June, 2013 (04.06.13)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2013/059564

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Kazuma NAGATA et al., "Analysis of blood cultures in patients presenting with community-acquired pneumonia at the emergency room", The journal of the Japanese Respiratory Society, 2010, vol.48, no.9, pages 661 to 667	1-4
P,Y	Kiyoyasu FUKUSHIMA et al., "Ketsuekichu no Haien Kyukin Kogen o PCR-ho to Rapirun Haien Kyukin HS Kit o Mochiite Keijiteki ni Sokutei shita Ichishorei - HS ni yoru Kinketsusho no Soki Shindan no Kokoromi-", Dai 60 Kai Japanese Society of Chemotherapy Nishinihon Shibu Sokai, Dai 55 Kai The Japanese Association for Infectious Diseases Nakanihon Chihokai Gakujutsu Shukai, Dai 82 Kai The Japanese Association for Infectious Diseases Nishinihon Chihokai Gakujutsu Shukai Kyodo Kaisai 2012 Nen Program Koen Shorokushu, 2012.10, page 287, 159	1-4
P,Y	Kiyoyasu FUKUSHIMA et al., "Rapirun Haien Kyukin HS Kit o Mochiita Kesseichu no Haien Kyukin Kogen no Kento", Dai 61 Kai Higashinihon Branch of Japanese Association for Infectious Diseases Gakujutsu Shukai, Dai 59 Kai Higashinihon Branch of Japan Society of Chemotherapy Sokai Godo Gakkai Program Shorokushu, 2012.09, page 298, O140	1-4

Form PCT/ISA/210 (continuation of second sheet) (July 2009)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 6824997 B **[0024]**

Non-patent literature cited in the description

- RAPIRUN (registered trademark) S. pneumoniae. Otsuka Pharmaceutical Co., Ltd, **[0004]**
- RAPIRUN (registered trademark) S. pneumoniae HS (otitis media/sinusitis). Otsuka Pharmaceutical Co., Ltd, **[0004]**
- BinaxNOW (registered trademark) Streptococcus pneumoniae. Alere Medical Co., Ltd, **[0004]**
- The JRS Guidelines for the Management of Community-Acquired Pneumonia in Adults. *The Japanese Respiratory Society,* 2007, 9-12 **[0007]**
- **GILLESPIE S.H. et al.** *J. Clin. Pathol.,* 1995, vol. 48, 803-806 **[0007]**
- **COONROD J.D. et al.** *J. Lab. Clin. Med.,* 1973, vol. 81, 778-786 **[0007]**
- **NIELSEN et al.** Antibodies against pneumococcal C-polysaccharide are not protective. *Microbial Pathogenesis,* 1993, vol. 14, 299-305 **[0024]**
- **GILLESPIE et al.** Detection of C-polysaccharide in serum of patients with Streptococcus pneumoniae bacteremia. *J. Clin. Pathol.,* 1995, vol. 48, 803-806 **[0024]**
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- **FEIGIN et al.** Countercurrent immunoelectrophoresis of urine as well as of CSF and blood for diagnosis of bacterial meningitis. *The Journal of Pediatrics,* 1976, vol. 89, 773-775 **[0024]**
- **AJELLO et al.** Commercial Latex Agglutination Tests for Detection of Haemophilus influenza Type b and Streptococcus pneumoniae Antigens in Patients with Bacteremic Pneumonia. *JOURNAL OF CLINICAL MICROBIOLOGY,* 1987, vol. 25 (8), 1388-1391 **[0024]**
- **BALLAED et al.** Comparison of three latex agglutination kits and counterimmunoelectrophoresis for the detection of bacterial antigens in a pediatric population. *Pediatr. Infect. Dis. J.,* 1987, vol. 6 (7), 630-634 **[0024]**
- **KOBAYASHI et al.** Evaluation of Streptococcus Pneumoniae-urinary Antigen Detection kit in Patients with Community Acquired Pneumonia. *The Journal of the Japanese Association for Infectious Diseases,* 2002, vol. 76 (12), 995-1002 **[0024]**
- **HOTOMI et al.** Evaluation of a Rapid Immunochromatographic ODK-0901 Test for Detection of Pneumococcal Antigen in Middle Ear Fluids and Nasopharyngeal Secretions. *PLoS one,* 2012, vol. 7 (3), e33620 **[0024]**
- *Basic concept for the management of community-acquired pneumonia in adults,* 2000 **[0030]**
- *Guidelines for respiratory infectio,* 2007 **[0030]**
- **RESTI et al.** Community-Acquired Bacteremic Pneumococcal Pneumonia in Children: Diagnosis and Serotyping by Real-Time Polymerase Chain Reaction Using Blood Samples. *Clinical Infectious Diseases,* 2010, vol. 51 (9), 1042-1049 **[0042]**
- **EHARA N. et al.** *Journal of Medical Microbiology,* 2008, vol. 57, 820-826 **[0051]**

专利名称(译)	确定肺炎球菌肺炎严重程度的方法		
公开(公告)号	EP2833148A4	公开(公告)日	2015-07-22
申请号	EP2013769634	申请日	2013-03-29
申请(专利权)人(译)	大冢PHARMACEUTICAL CO.，LTD.		
当前申请(专利权)人(译)	大冢PHARMACEUTICAL CO.，LTD.		
[标]发明人	FUKUSHIMA KIYOYASU TANAKA YUMI		
发明人	FUKUSHIMA, KIYOYASU TANAKA, YUMI		
IPC分类号	G01N33/569 G01N33/53 C12Q1/04 C12Q1/68		
CPC分类号	C12Q1/689 G01N33/5308 G01N33/56911 G01N33/56944 G01N33/56972 G01N2333/3156		
优先权	2012081038 2012-03-30 JP		
其他公开文献	EP2833148A1		
外部链接	Espacenet		

摘要(译)

提供了一种能够使用受试者的血液更客观且快速地确定肺炎球菌肺炎的严重性的方法。该方法确定肺炎球菌肺炎的严重程度，该方法包括基于从受试者收集的血液中是否存在肺炎球菌抗原来确定严重性，以及选自血液C-反应蛋白（CRP）水平的至少一种生化值，a白细胞（WBC）计数和血尿素氮（BUN）水平。