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(54) Title: HBV IMMUNOCOMPLEXES FOR RESPONSE PREDICTION AND THERAPY MONITORING OF CHRONIC HBV PATIENTS

(57) Abstract: The present invention relates to a method for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, said method comprising the steps of a) determining, in a sample of said subject, the amount of HBV immune complexes, b)comparing the amount of HBV immune complexes obtained in step a) to a reference value, and c) identifying a subject suffering from HBV infection as being susceptible to interferon treatment based on the result of the comparison made in step b). The present invention further relates to the use of the determination of the amount of HBV immune complexes in a sample from a subject suffering from HBV infection and of a detection agent for HBV immune complexes for identifying a subject suffering from HBV infection as being susceptible to interferon treatment. Furthermore, the present invention relates to a device and a kit allowing identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

HBV immunocomplexes for response prediction and therapy monitoring of chronic HBV patients

The present invention relates to the field of diagnostics. In particular, it relates to a method for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, said method comprising the steps of a) determining, in a sample of said subject, the amount of HBV immune complexes, b) comparing the amount of HBV immune complexes obtained in step a) to a reference value, and c) identifying a subject suffering from HBV infection as being susceptible to interferon treatment based on the result of the comparison made in step b). The present invention further relates to the use of the determination of the amount of HBV immune complexes in a sample from a subject suffering from HBV infection and of a detection agent for HBV immune complexes for identifying a subject suffering from HBV infection as being susceptible to interferon treatment. Furthermore, the present invention relates to a device and a kit allowing identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

Hepatitis B is an infectious inflammatory disease of the liver caused by the hepatitis B virus (HBV). The virus is transmitted by exposure to body fluids, transmission by transfusions, dialysis, common use of needles by drug-addicted persons, and perinatal infection being the major modes of transmission.

Acute Hepatitis B virus infection is characterized by liver inflammation, vomiting, and jaundice. Symptoms typically last for a few weeks and then gradually improve while the immune system clears the infection. The probability of full recovery and establishment of protective immunity increases with age: While only 5% adults will suffer from a persistent infection lasting for more than a few weeks, i.e. chronic HBV infection, this rate rises to 70% for young children, and to 95% for newborns infected perinatally.

Chronic HBV is associated with a severe risk of developing cirrhosis and liver cancer, which is why HBV has been classified as a class I carcinogen, i.e. an agent carcinogenic to humans, by WHO's International Agency for the Research on Cancer (IARC). It is thus of outmost importance for the patient to receive a treatment allowing the patient's immune system to clear the infection. Typical treatment schemes include nucleoside or nucleotide analogs inhibiting the viral DNA polymerase, along with Interferon alpha. An important improvement was the introduction of PEGylated interferon (e.g. Pegasys®), providing prolonged half-life of

interferon, into therapy. For review of epidemiology and therapy of HBV infection see J.L. Dienstag N Engl J Med 2008, 359; 1486-1500.

The serology of chronic HBV infection is complex, since on the one hand high anti-HBV titers are directly correlated with the severity of disease, but on the other hand anti-HBV e antigen antibodies were found to be present in patients years before spontaneous seroconversion, i.e. clearance of the HBV e antigen from serum (Maruyama et al. (1993), J. Clin. Invest. 91, 2586-2595). Despite improved therapy, still only a portion of patients will clear the infection under treatment, while others will not. Members of the second group of patients, the non-responders, can frequently clear the infection under a more aggressive therapy regimen, like e.g. increased doses, prolonged treatment, or additional medication. It would, thus, be desirable to be able to predict a patient's response to standard interferon therapy, in order to possibly adjust treatment right from the beginning. In a recent improvement, it was found that determination of a change in HBV soluble antigen (HBsAg) in patient samples after 12 weeks of therapy allows the use of cutoff values for the prediction of therapy outcome. However, cutoff values are only valid for patients being also HBV e antigen (HBeAg) positive, which is the case mainly in patients in the Asian-Pacific area. In HBeAg negative patients, which are the majority e.g. in Europe, cutoff values are much more difficult to define and a decrease of at least 10% between therapy start and week 12 is used as a makeshift.

Recently, it was reported that in HBeAg positive patients, a high amount of HBV complexes comprising HBsAG and anti-HBsAg-IgG before the start of therapy was correlated with a good response to standard interferon therapy. However, this does not allow prediction for HBeAg negative patients. At any rate, at present no method is available that would allow to predict therapy outcome before the therapy is started for all patient groups regardless of their HBeAg status.

Accordingly, the technical problem underlying the present invention can be seen as the provision of means and methods for complying with the aforementioned needs. The technical problem is solved by the embodiments characterized in the claims and herein below.

Therefore, the present invention relates to a method for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, said method comprising the steps of

a) determining, in a sample of said subject, the amount of HBV immune complexes,

b) comparing the amount of HBV immune complexes obtained in step a) to a reference value; and

c) identifying a subject suffering from HBV infection as being susceptible to interferon treatment based on the result of the comparison made in step b).

The method of the present invention, preferably, is an in vitro method. Moreover, it may comprise steps in addition to those explicitly mentioned above. For example, further steps may relate, e.g., to pre-treatment of the sample of step a) or evaluation of the results obtained by the method. Additionally, internal controls, such as sample quality controls or performance controls may be used. The method may be carried out manually or assisted by automation. Preferably, steps (a) to (c) may in total or in part be assisted by automation, e.g. by suitable robotic and sensory equipment for the determining of HBV immune complexes in step (a).

The term "identifying", as used herein, means to allocate a subject into the group of subjects being susceptible to interferon treatment (so-called "responder") or into the group of subjects not being susceptible to interferon treatment (so-called "non-responders"). As will be understood by those skilled in the art, the aforementioned identification is usually not intended to be correct for 100% of the subjects to be analyzed. The term, however, requires that the assessment will be valid for a statistically significant portion of the subjects to be analyzed. Whether a portion is statistically significant can be determined without further ado by the person skilled in the art using various well known statistic evaluation tools, e.g., determination of confidence intervals, p-value determination, Student's t-test, Mann-Whitney test etc.. Details are found in Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York 1983. Preferred confidence intervals are at least 90%, at least 95%, at least 97%, at least 98% or at least 99 %. The p-values are, preferably, 0.1, 0.05, 0.01, 0.005, or 0.0001. Preferably, the probability envisaged by the present invention allows that the differentiation will be correct for at least 60%, at least 70%, at least 80%, or at least 90% of the subjects of a given cohort or population.

Preferably, identifying according to the method of the invention can be accomplished in the absence of knowledge about the HBV e antigen status of the subject, i.e. the identifying leads to a correct allocation to the group of subjects being susceptible to interferon treatment or to the group of subjects not being susceptible to interferon treatment for subjects regardless of the HBeAg status of the investigated subject. Thus, the method can be applied for subjects with detectable amounts of HBeAg in serum samples as well as for subjects lacking detectable amounts of HBeAg in serum samples.

The term "hepatitis B virus (HBV)" refers to a virus species from the family hepadnaviridae which is the causative agent of hepatitis B. HBV virus particles consist of an outer membrane (also called lipid envelope), an icosahedral nucleocapsid and DNA genome. HBV is well known and characterized in the art.

The terms "hepatitis B virus infection" or "HBV infection" relate to the detectable presence of HBV in a subject. Preferably, HBV presence is diagnosed by the detection of at least one viral polypeptide in a sample from a subject, more preferably, at least one of the viral antigens HBs (HBsAg, Genbank Acc. No.: AAL66340.1 GI:18252577, SEQ ID NO: 1), HBc (HBcAg, Genbank Acc. No.: CAA51257.1 GI:288930, SEQ ID NO: 2), and HBe (HBeAg, Genbank Acc. No.: AAM96930.1 GI:22530876, SEQ ID NO: 3) is detected. It is understood by the skilled person that the HBV polypeptides are referenced as biomarkers, not as specific polypeptides, and that the term HBV encompasses various strains of HBV which may comprise sequence variants of the aforementioned HBV polypeptides. Accordingly, the aforementioned polypeptides having the specific sequences deposited under the Genbank accession numbers are to be understood as exemplary sequences representing a biomarker. Encompassed according to the present invention are also variant polypeptides which vary due to at least one amino acid addition, substitution and/or deletion form the polypeptide having the specific sequence as long as they are also suitable as biomarkers for a HBV infection as discussed above. Preferably, the variant polypeptides are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the specific polypeptides. The term "identical" as used herein refers to sequence identity characterized by determining the number of identical amino acids between two nucleic acid sequences or amino acid sequences wherein the sequences are aligned so that the highest order match is obtained. It can be calculated using published techniques or methods codified in computer programs such as, for example, BLASTP, BLASTN or FASTA (Altschul 1990, J Mol Biol 215, 403). The percent identity values are, in one aspect, calculated over the entire amino acid sequence. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In this context, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. To carry out the sequence alignments, the program PileUp (Higgins 1989, CABIOS 5, 151) or the programs Gap and BestFit (Needleman 1970, J Mol Biol 48; 443; Smith 1981, Adv Appl Math 2, 482), which are part of the GCG software packet (Genetics Computer Group 1991, 575 Science Drive, Madison, Wisconsin, USA 53711), may be used. The sequence identity values recited above in percent (%) are to be determined, in another aspect of the invention, using the program GAP over the entire sequence region with the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000, which, unless otherwise specified, shall always be used as standard settings for sequence alignments.

Also preferably, HBV presence is detected by detecting at least one viral polynucleotide, preferably viral DNA, in a sample from a subject. The nucleotide sequences of the viral polynucleotides or the entire HBV genome are well known in the art. Preferably, HBV nucleotide sequences are deposited under Genbank accession numbers NC_003977.1. It is

understood by the skilled person that the HBV polynucleotides are referenced as biomarkers, not as specific polynucleotides, and that the term HBV encompasses various strains of HBV comprising variant nucleotide sequences. Accordingly, the aforementioned polynucleotides having the specific sequences deposited under the Genbank accession numbers are to be understood as exemplary sequences representing a biomarker. Encompassed according to the present invention are also variant polynucleotides which vary due to at least one nucleotide addition, substitution and/or deletion form the polynucleotides having the specific sequence as long as they are also suitable as biomarkers for a HBV infection as discussed above. Preferably, the variant polynucleotides peptides are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the specific polynucleotides. Identity can be calculated as set forth elsewhere herein for amino acid sequences.

Preferably, the HBV infection referred to herein is a chronic HBV infection, wherein said chronic HBV infection is, preferably, characterized by the detectable presence of HBV in a subject for more than four weeks, more than five weeks, more than six weeks, more than seven weeks, more than eight weeks, more than nine weeks, more than ten weeks, more than eleven weeks, or more than twelve weeks. More preferably, a chronic HBV infection referred to herein follows the definition published by the Center for Disease Control (CDC), according to which a chronic HBV infection is characterized by the following laboratory criteria: IgM antibodies to hepatitis B core antigen (IgM anti-HBc) negative AND a positive result on one of the following tests: hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or hepatitis B virus (HBV) DNA OR HBsAg positive or HBV DNA positive two times at least 6 months apart (any combination of these tests performed 6 months apart is acceptable).

The terms "interferon treatment" and "standard treatment", preferably, relate to a treatment of HBV infection with interferon and, preferably, an inhibitor of the viral DNA polymerase. It is, however, also contemplated by the present invention that interferon treatment preferably is single treatment with interferon and that standard treatment preferably is single treatment with a viral DNA polymerase inhibitor. Preferably, interferon as referred to in this context is an interferon covalently bound to polyethylenglycol (PEG-interferon), more preferably, interferon is interferon 2 alpha, most preferably, interferon is interferon 2 alpha covalently bound to polyethylenglycol (PEG-interferon 2 alpha, commercially available as PEGasys®). Preferably, an inhibitor of the viral DNA polymerase is a nucleotide or nucleoside analogon, more preferably {[2-(6-amino-9H-purin-9-yl)ethoxy]methyl}phosphonic acid (e.g. adefovir®). Thus, standard or interferon treatment, preferably, is treatment with interferon 2 alpha and a nucleotide analogon. More preferably, standard or interferon treatment is treatment with PEG-interferon 2 alpha and a nucleotide analogon and even more preferably standard interferon treatment is treatment with PEG-interferon 2 alpha (e.g. PEGasys®) and adefovir® for less than one year. Most preferably,

standard interferon treatment is treatment with PEG-interferon 2 alpha, e.g. PEGasys®, and adefovir® according to the following therapy plan: Usually, the PEGasys® treatment is carried out by subcutaneous administration (weekly subcutaneous injection) for a duration of 48 weeks whereas the viral DNA polymerase inhibitors (nucleoside/nucleotide analoga) are administrated orally for a longer time, i.e. for more than one year in 80% of the patients. For details see also J.L. Dienstag N Engl J Med 2008, 359; 1486-1500.

The term "being susceptible to interferon treatment" means that a subject can be successfully treated or can at least be successfully treated with a significantly increased likelihood compared to the prevalence for a successful treatment. Subjects which can be successfully treated by interferon treatment as referred to herein are also called responders. Treatment as referred to herein is successful if the HBV infection, at least one symptom associated therewith or at least one complication accompanied therewith are ameliorated to a significant extent and/or cured. Preferably, a successful treatment is also accompanied by a decrease of HBV polypeptide and /or HBV polynucleotide detectable in a sample from a subject as described herein above. More preferably, successful treatment is characterized by the absence of detectable amounts of HBsAG and / or HBeAG in samples from a subject after at most one year, at most 50 weeks, at most 49 weeks, or at most 48 weeks of treatment as determined 60 to 80 weeks, preferably 65 to 75 weeks, or most preferably 72 weeks after start of the treatment.

A subject which can not successfully treated by interferon treatment is also called non-responder. In such subjects, no amelioration or cure of the HBV infection, the at least one symptom or at least one complication associated therewith occurs. Preferably, such a non-responder subject suffers from a severe form of chronic hepatitis B virus infection wherein HBsAG, but not HBeAG, is detectable before and after interferon treatment, or wherein HBsAG and HBeAG are detectable before and after standard interferon treatment. A subject, identified as being not susceptible to interferon treatment shall be regarded for modified treatment measures, e.g. increased doses of interferon and/or polymerase inhibitor, and/or prolonged treatment and/or additional medication.

As used herein, the term "HBV immune complex" relates to a complex formed between at least one HBV polypeptide and at least one immunoglobulin as described herein below directed against said at least one HBV polypeptide, i.e. an anti-HBV immunoglobulin. Preferably, the complex is formed between at least one HBV polypeptide and more than one, more than two, more than three, more than four, more than five, more than ten, more than twenty, more than fifty, or more than a hundred anti-HBV immunoglobulin molecules directed against said at least one HBV polypeptide. Preferably, the HBV polypeptide is elected from the list of HBV antigens related to herein above, more preferably, the HBV polypeptide is the HBsAG. The anti-HBV

immunoglobulin, preferably, is a soluble immunoglobulin present in at least one of a subject's body fluids, preferably blood or serum. Preferably, the anti-HBV immunoglobulin is an IgG, more preferably a serum IgG. Most preferably, the anti-HBV immunoglobulin comprised in the HBV immune complex is an IgG directed against the HBsAg, i.e. an anti-HBsAg IgG. It is to be understood that the HBV immune complex may comprise other molecules than the ones expressly detailed in this specification, it is, however, preferred that the anti-HBV immunoglobulin and the HBV polypeptide of the present invention form a substantial part of the HBV immune complex. Thus, the anti-HBV immunoglobulin and the HBV polypeptide constitute, preferably, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, or at least 75% of the mass of the HBV immune complex. More preferably, HBV immune complex essentially consists of the anti-HBV immunoglobulin and the HBV polypeptide, i.e. the anti-HBV immunoglobulin and the HBV polypeptide constitute at least 80%, at least 90%, or at least 95% of the mass of the HBV immune complex. Even more preferably, HBsAg and anti-HBsAg IgG constitute a substantial part of the HBV immune complex. Most preferably, the HBV immune complex essentially consists of HBsAg and anti-HBsAg IgG.

The terms "immunoglobulin" or "Ig" are understood by the skilled person. They relate to a member molecule of the family of immunoglobulins, characterized in that they are soluble polypeptides produced by the immune system of an individual in response to contact with a foreign antigen. Preferably, the immunoglobulin is an immunoglobulin from a mammal and, more preferably, the immunoglobulin is a human immunoglobulin. Also preferably, the immunoglobulin is elected from the group consisting of IgA, IgD, IgE, IgG, and IgM. More preferably, the immunoglobulin is an IgG, most preferably a human IgG. Human IgGs are characterized by a molecular mass of approximately 150 kDa and by a structure consisting of two identical heavy chains of about 50 kDa and two identical light chains of approximately 25 kDa. The two heavy chains are linked to each other and to a light chain by disulfide bonds to form the typical Y-shaped molecule. Preferably, the IgG are glycosylated, more preferably IgG are glycosylated by N-glycosylation. Preferably, the IgG is of one of the subgroups IgG1, IgG2, IgG3, or IgG4.

The term "anti-immunoglobulin antibody" relates, preferably, to a soluble molecule from the protein family of antibodies recognizing an immunoglobulin, preferably recognizing a human immunoglobulin, i.e. the anti-immunoglobulin antibody of the present invention preferably is an anti-human-immunoglobulin antibody, more preferably recognizing a human IgG, i.e. an anti-human-IgG antibody. The anti-immunoglobulin antibody according to the present invention is preferably an antibody with low to medium affinity and with high avidity as detailed herein below. The affinity of an antibody for an epitope is defined as the strength of all non-covalent interactions between the antigen-recognition site on said antibody and the epitope. An antibody

with a high affinity binds strongly to an antigen via many and / or strong non-covalent interactions and, thus, remains bound to the antigen for a relatively long period of time. An antibody with a low affinity, on the other hand, interacts with few and / or weak non-covalent interactions and thus dissociates rapidly from the antigen. It is known to the person skilled in the art that the affinity of an antibody can be described by it dissociation constant (K_d). Preferably, a dissociation constant of 10^{-10} mol/l to 10^{-9} mol/l is indicative of a very high affinity, a dissociation constant of 10^{-8} mol/l is indicative of a high affinity, a dissociation constant of 10^{-7} mol/l is indicative of a low affinity, and a dissociation constant of 10^{-6} mol/l and higher is indicative of a very low affinity. Thus, the dissociation constant of the anti-immunoglobulin antibody, preferably, is 10^{-6} mol/l to 10^{-8} mol/l, more preferably the dissociation constant is 10^{-7} to 10^{-8} mol/l. In molecules comprising more than one binding site, like e.g. antibodies, the interaction of a first binding site increases the probability of a further binding site to interact. The strength of such multiple interactions between a molecule comprising more than one binding site and a molecule to be bound is known to the skilled artisan as avidity. A high avidity can thus compensate for a relatively low affinity of the single binding site. Thus, the anti-immunoglobulin antibody, preferably, has more than two antigen recognition sites, more preferably more than four antigen recognition sites. Most preferably, the anti-immunoglobulin antibody has ten or more antigen recognition sites. Preferably, the anti-immunoglobulin antibody is an IgG, IgD, IgE, more preferably the anti-immunoglobulin antibody is an antibody comprising more than two antigen recognition sites, e.g. an IgA, most preferably, the anti-immunoglobulin antibody is an antibody comprising more than four antigen recognition sites in one molecule, e.g. an IgM. The anti-immunoglobulin antibody is polyclonal or monoclonal and is produced in a mammal or in a mammalian cell suited for antibody production. Preferably, the anti-immunoglobulin antibody specifically binds to an epitope of an immunoglobulin of the present invention. More preferably, the anti-immunoglobulin antibody is an anti-human-immunoglobulin antibody, i.e. binds to the human immunoglobulin of the present invention, even more preferably, the binding is specific for human immunoglobulin. Most preferably, the anti-immunoglobulin antibody is an anti-human-IgG antibody, i.e. specifically binds to human IgG. It is, however, also envisaged by the present invention that the anti-immunoglobulin antibody specifically binds to a neoepitope formed by the binding of an immunoglobulin to the HBV polypeptide in the HBV immune complex. Methods of how to produce antibodies are well known in the art, and include, e.g., immunization of live animals according to known protocols or the production of monoclonal or polyclonal antibodies in cell culture systems (Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual). Preferably, the anti-immunoglobulin antibody is produced in mouse, rat, rabbit, pig, cow, donkey, goat, sheep, or the like or in eggs. It is, however, also considered by the present invention that the anti-immunoglobulin antibody is produced in a transgenic plant. Preferably, the anti-immunoglobulin antibody is an IgM, most preferably a monoclonal IgM. More preferably, the anti-immunoglobulin antibody is a anti-(aggregated-humanIgG) IgM, i.e.

an IgM with a low affinity and a high avidity for human IgG as described herein above, e.g. the <h-agg.IgG> IgM as described in WO2008/135274, most preferably, the anti-human-immunoglobulin antibody is. MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873), MAb <h-Agg.-IgG>M-1.010.2-IgM and MAb <h-Agg.-IgG>M-1.1.7-IgM (shown in Table 1), MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873) being most preferred.

Preferably, the anti-immunoglobulin antibody carries a label. The term "label" as used herein relates to any substance capable of producing a detectable signal. Preferably, the label is a chromogen, a fluorescent, chemiluminescent or electrochemiluminscent compound, a catalyst, an enzyme, a an enzymatic substrate, a dye, a colloidal metal or nonmetallic particle, or an organic polymer particle, or the like.

The term "subject", as used herein, relates to a mammal and, preferably, to a human. The subject, preferably, suffers from HBV infection. More preferably, the subject has a chronic HBV infection. Moreover, the subject has, preferably, an unknown HBV e antigene status.

The term "sample" refers to a sample of a body fluid, to a sample from a tissue or an organ or to a sample of wash/rinse fluid obtained from an outer or inner body surface. The sample preferably comprises polypeptides, more preferably HBV antigens, most preferably HBsAg. Samples of blood, plasma, serum, urine, saliva, or lacrimal fluid are encompassed by the method of the present invention. Such samples can be obtained by use of brushes, (cotton) swabs, spatula, rinse/wash fluids, punch biopsy devices, puncture of cavities with needles or surgical instrumentation. However, samples obtained by well known techniques including, preferably, scrapes, swabs or biopsies from the urogenital tract, perianal regions, anal canal, the oral cavity, the upper aerodigestive tract and the epidermis are also included as samples of the present invention. Cell-free fluids may be obtained from the body fluids or the tissues or organs by separating techniques such as filtration or centrifugation. Preferably, samples are obtained from body fluids known to comprise HBV polypeptides in subjects infected with HBV, i.e., preferably, blood, serum, saliva, or the like. It is to be understood that the sample may be further processed in order to carry out the method of the present invention. Particularly, cells might be removed from the obtained sample by methods and means known in the art. Moreover, HBV immune complexes might be extracted and/or purified from the obtained sample by methods and means known in the art. Thus, the term sample also may relate to HBV immune complexes purified and/or extracted from any sample as mentioned above.

The term "determining" relates to the quantification of the amount of HBV immune complex present in a sample, i.e. measuring the amount or concentration of said HBV immune complex, preferably semi-quantitatively or quantitatively. Measuring can be done directly or indirectly.

The determining of the amount of immune complexes can be accomplished in a variety of ways known to the skilled person, e.g. gel filtration chromatography followed by western blotting, co-immunoprecipitation, or the like. Preferably, the amount of HBV immune complexes is determined by the methods described in the examples herein below. Advantageously, it was found that the sandwich-ELISA used in the examples allows reliable quantification of HBV immune complexes. Moreover, it was found that the use of aggregated antibodies, e.g. aggregated IgG, as anti-human-Ig antibodies, leads to a highly improved signal-to-noise ratio.

In accordance with the present invention, determining the amount of the HBV immune complexes can be achieved by all known means for determining the amount of a polypeptide or peptide in a sample, provided that they are adapted to specifically detect the HBV immune complexes of the present invention. Preferably, detection agents are to be used which specifically bind to and, thus, allow for the detection of the HBV immune complexes. Detection agents, preferably, encompass antibodies or fragments thereof that specifically bind to the complexes, aptameres, anticalins, or Designed Ankyrin Repeat Proteins (DARPins) that specifically bind to the complexes. Preferably, double-specificity immunoassays are applied, i.e. assays wherein the presence or the intensity of a signal will depend on the presence of both kinds of molecules comprised in the HBV immune complexes, i.e. the HBV polypeptide and the anti-HBV immunoglobulin. Said means comprise immunoassay devices and methods which may utilize labeled molecules in various sandwich, competition, or other assay formats. Said assays will develop a signal which is indicative of the presence or absence of the HBV immune complexes. Moreover, the signal strength can, preferably, be correlated directly or indirectly (e.g. reverse-proportional) to the amount of HBV immune complexes present in a sample. Said methods comprise, preferably, biosensors, optical devices coupled to immunoassays, biochips, analytical devices such as mass-spectrometers, NMR-analyzers, or chromatography devices. Further, methods include micro-plate ELISA-based methods, fully-automated or robotic immunoassays (available for example on multi parameter biochip platforms or ElecsysTM analyzers), CBA (an enzymatic Cobalt Binding Assay, available for example on Roche-HitachiTM analyzers), and latex agglutination assays (available for example on Roche-HitachiTM analyzers).

The term "amount" as used herein encompasses the absolute amount of the HBV immune complexes referred to herein, the relative amount or concentration of the HBV immune complexes referred to herein as well as any value or parameter which correlates thereto. Such values or parameters comprise intensity signal values from all specific physical or chemical properties obtained from the HBV immune complexes referred to herein by measurements, e.g., expression levels determined from biological read out systems in response to the polypeptides referred to herein or intensity signals obtained from specifically bound ligands. It is to be

understood that values correlating to the aforementioned amounts or parameters can also be obtained by all standard mathematical operations.

"Comparing" as used herein encompasses comparing the amount of the HBV immune complexes referred to herein which are comprised by the sample to be analyzed with an amount of the said HBV immune complexes in a suitable reference sample as specified elsewhere herein in this description. Also encompassed is comparing the ratio of the amount of the HBV immune complexes to the amount of HBV antigen, preferably HBsAg, in the sample to a suitable reference ratio. It is to be understood that comparing as used herein refers to a comparison of corresponding parameters or values, e.g., an absolute amount of the HBV immune complexes as referred to herein is compared to an absolute reference amount of said HBV immune complexes; a concentration of the HBV immune complexes as referred to herein is compared to a reference concentration of said HBV immune complexes; an intensity signal obtained from the HBV immune complexes as referred to herein in a test sample is compared to the same type of intensity signal of said HBV immune complexes in a reference sample; or a ratio of the amount of the HBV immune complexes to the amount of HBV antigen as referred to herein is compared to a corresponding reference ratio. The comparison referred to in the methods of the present invention may be carried out manually or computer assisted. For a computer assisted comparison, the value of the determined amount or ratio may be compared to values corresponding to suitable references which are stored in a database by a computer program. The computer program may further evaluate the result of the comparison by means of an expert system. Accordingly, the result of the identification referred to herein may be automatically provided in a suitable output format.

The term "reference value" as used herein refers to an amount of HBV immune complexes, which allows assessing if being susceptible to interferon treatment or not being susceptible to interferon treatment is to be assumed for the subject from which the sample is derived. A suitable reference value may be determined from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the sample.

Reference amounts can, in principle, be calculated for a group or cohort of subjects as specified herein based on the average or mean values for a given HBV immune complex by applying standard methods of statistics. In particular, accuracy of a test such as a method aiming to diagnose an event, or not, is best described by its receiver-operating characteristics (ROC) (see especially Zweig 1993, Clin. Chem. 39:561-577). The ROC graph is a plot of all of the sensitivity versus specificity pairs resulting from continuously varying the decision threshold over the entire range of data observed. The clinical performance of a diagnostic method depends on its accuracy, i.e. its ability to correctly allocate subjects to a certain prognosis or diagnosis.

The ROC plot indicates the overlap between the two distributions by plotting the sensitivity versus 1-specificity for the complete range of thresholds suitable for making a distinction. On the y-axis is sensitivity, or the true-positive fraction, which is defined as the ratio of number of true-positive test results to the product of number of true-positive and number of false-negative test results. This has also been referred to as positivity in the presence of a disease or condition. It is calculated solely from the affected subgroup. On the x-axis is the false-positive fraction, or 1-specificity, which is defined as the ratio of number of false-positive results to the product of number of true-negative and number of false-positive results. It is an index of specificity and is calculated entirely from the unaffected subgroup. Because the true- and false-positive fractions are calculated entirely separately, by using the test results from two different subgroups, the ROC plot is independent of the prevalence of the event in the cohort. Each point on the ROC plot represents a sensitivity/-specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions of results) has an ROC plot that passes through the upper left corner, where the true-positive fraction is 1.0, or 100% (perfect sensitivity), and the false-positive fraction is 0 (perfect specificity). The theoretical plot for a test with no discrimination (identical distributions of results for the two groups) is a 45° diagonal line from the lower left corner to the upper right corner. Most plots fall in between these two extremes. If the ROC plot falls completely below the 45° diagonal, this is easily remedied by reversing the criterion for "positivity" from "greater than" to "less than" or vice versa. Qualitatively, the closer the plot is to the upper left corner, the higher the overall accuracy of the test. Dependent on a desired confidence interval, a threshold can be derived from the ROC curve allowing for the diagnosis or prediction for a given event with a proper balance of sensitivity and specificity, respectively. Accordingly, the reference to be used for the methods of the present invention can be generated, preferably, by establishing a ROC for said cohort as described above and deriving a threshold amount there from. Dependent on a desired sensitivity and specificity for a diagnostic method, the ROC plot allows deriving suitable thresholds.

Preferably, the reference amount as used herein is derived from samples of subjects obtained before treatment, but for which it is known if their donors responded to treatment or not. This reference amount level may be a discrete figure or may be a range of figures. Evidently, the reference level or amount may vary between individual species of HBV immune complexes. The measuring system therefore, preferably, is calibrated with a sample or with a series of samples comprising known amounts of HBV immune complex or HBV immune complexes. More preferably, the system is calibrated with a series of mixtures comprising defined volumes of HBsAg-only serum, i.e. serum comprising high amounts of HBsAg, but no anti-HBV immunoglobulin or HBV immune complexes, and anti-HBsAg immunoglobulin-only serum, e.g. serum comprising high titers of anti-HBsAg IgG, but no HBsAg or HBV immune complexes. It is understood by the skilled person that in such case the amount of HBV immune complex will

preferably be expressed as arbitrary units (AU). Thus, preferably, the amounts of HBV immune complex or HBV immune complexes is or are determined by comparing the signal obtained from the sample to signals comprised in a calibration curve.

The reference amount applicable for an individual subject may vary depending on various physiological parameters such as age, gender, or subpopulation. Thus, a suitable reference amount may be determined by the methods of the present invention from a reference sample to be analyzed together, i.e. simultaneously or subsequently, with the test sample. Moreover, a threshold amount can be preferably used as a reference amount. Preferably, an amount of HBV immune complexes which is above the threshold amount is indicative of a mild form of HBV infection; and an amount of HBV immune complexes which is equal or below the threshold amount will be indicative for a severe form of HBV infection.. It is to be understood that the aforementioned amounts may vary due to statistics and errors of measurement.

In one embodiment of the method of the present invention, it has been found that an increased amount of HBV immune complexes is, preferably, indicative of a subject being susceptible to interferon treatment while a decreased amount for HBV immune complexes is indicative for a subject being not susceptible to interferon treatment. In this case, the reference amounts are, preferably, those which are the average or mean amounts found in a subjects suffering from HBV prior to the treatment for a given population or cohort of subjects. A decrease or an increase of the HBV immune complex amounts referred to herein is, preferably, a statistically significant decrease or increase.

A reference amount may, preferably, be derived from a sample of a subject or group of subjects suffering from HBV which is/are known to be susceptible to interferon treatment. In such a case, a determined amount of the HBV immune complexes which is essentially identical or increased compared to the reference amount shall be indicative for a subject being susceptible to interferon treatment. An amount which is decreased shall be indicative for subject which is not susceptible for interferon treatment.

A reference amount may, preferably, also be derived from a sample of a subject or group of subjects suffering from HBV which is/are known not to be susceptible to interferon treatment. In such a case, a determined amount of the HBV immune complexes which is essentially identical or decreased compared to the reference amount shall be indicative for a subject not being susceptible to interferon treatment. An amount which is increased shall be indicative for subject which is not susceptible for interferon treatment.

Advantageously, it was found that the determining the amount of HBV immune complexes present in a sample from a subject allows for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to standard interferon treatment. As is detailed herein in the examples, preferably, a increased amount of HBV immune complexes is indicative of a subject being susceptible to interferon treatment, and a decreased amount of HBV immune complexes is indicative of a subject not being susceptible to interferon treatment. Meaning, preferably, that a subject with a high amount of HBV immune complexes has a high probability to respond to standard interferon treatment, and that a subject with a low amount of HBV immune complexes has a low probability to respond to standard interferon treatment. It is thus possible, using the method of the present invention, to decide before a therapy is started, if a subject should be treated with standard interferon therapy or if the subject should preferably receive a modified treatment. Also advantageously, it was found that the knowledge of the HBeAg status and, thus, the determination of the HBeAg status of a subject is irrelevant for the method of the present invention in order to correctly identify a subject susceptible to interferon treatment.

The definitions made above apply *mutatis mutandis* to the following:

The present invention also contemplates a method for treating a subject suffering from HBV infection by an interferon treatment comprising identifying the subject as being susceptible for the interferon treatment, preferably, by the aforementioned method of the invention, and administering to a subject identified as being susceptible for the interferon treatment a therapeutically effective amount of the interferon treatment specified elsewhere herein.

The present invention also relates to a method for differentiating in a subject suffering from HBV infection between a mild and a severe form of HBV infection, said method comprising the steps of:

a) determining, in a sample of said subject, the amount of HBV immune complexes,

b) comparing the amount of HBV immune complexes obtained in step a) to a reference value; and

c) differentiating between mild and severe HBV infection based on the result of the comparison made in step b).

In this case, a severe form of HBV infection is characterized by a decreased amount of HBV immune complexes as set forth elsewhere herein while a mild form of HBV infection is characterized by an increased amount of HBV immune complexes. In this case, the reference

amounts are, preferably, those which are the average or mean amounts found in a subjects suffering from HBV prior to the treatment for a given population or cohort of subjects.

A "mild form" of HBV infection is, preferably, a form which can be treated by interferon treatment as set forth elsewhere herein while a "severe form" is, preferably, a chronic HBV infection which can not be treated by interferon treatment.

The present invention relates also to the use of the amount of HBV immune complexes in a sample from a subject suffering from HBV infection or a detection agent for the HBV immune complexes in such a sample for identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

Suitable detection agents which can be used for determining the HBV complexes present in a sample are described elsewhere herein in detail.

Moreover, the present invention also relates to a device for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, comprising an analyzing unit for determining the amount of HBV immune complexes and an evaluation unit for comparing said amount to a reference amount and for identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

The term "device" as used herein relates to a system of means comprising at least the aforementioned means operatively linked to each other as to allow the differentiation. Preferred means for determining the amount of the said HBV immune complexes and means for carrying out the comparison are disclosed above in connection with the methods of the invention. How to link the means in an operating manner will depend on the type of means included into the device. For example, where means for automatically determining the amount of the HBV immune complexes are applied, the data obtained by said automatically operating means can be processed by, e.g., a computer program in order to establish a diagnosis (i.e. identifying a subject being susceptible for the interferon treatment). Preferably, the means are comprised by a single device in such a case. Said device may accordingly include an analyzing unit for the measurement of the amount of the HBV immune complexes in a sample and an evaluation unit for processing the resulting data for the diagnosis. Alternatively, where means such as test stripes are used for determining the amount of the HBV immune complexes, the means for diagnosing may comprise control stripes or tables allocating the determined amount to an amount known to be accompanied with response to standard interferon treatment or with non-response to interferon treatment. Preferred means for detection are disclosed in connection with embodiments relating to the methods of the invention above. In such a case, the means are operatively linked in that the

user of the system brings together the result of the determination of the amount and the diagnostic value thereof due to the instructions and interpretations given in a manual. The means may appear as separate devices in such an embodiment and are, preferably, packaged together as a kit. The person skilled in the art will realize how to link the means without further inventive skills. Preferred devices are those which can be applied without the particular knowledge of a specialized clinician, e.g., test stripes or electronic devices which merely require loading with a sample. The results may be given as output of parametric diagnostic raw data, preferably, as absolute or relative amounts. It is to be understood that these data will need interpretation by the clinician. However, also envisaged are expert system devices wherein the output comprises processed diagnostic raw data the interpretation of which does not require a specialized clinician. Further preferred devices comprise the analyzing units/devices (e.g., biosensors, arrays, solid supports coupled to ligands specifically recognizing the polypeptides, Plasmon surface resonance devices, NMR spectro-meters, mass- spectrometers etc.) or evaluation units/devices referred to above in accordance with the methods of the invention.

The present invention contemplates a kit comprising instructions to carry out the method of the present invention, a detection agent for determining the amount of an HBV immune complex, and, preferably, standards for reference amounts allowing identifying a subject suffering from HBV infection as being susceptible to standard interferon treatment.

The term "kit" as used herein refers to a collection of the aforementioned components, preferably, provided separately or within a single container. The container, also preferably, comprises instructions for carrying out the method of the present invention. Examples for such the components of the kit as well as methods for their use have been given in this specification. The kit, preferably, contains the aforementioned components in a ready-to-use formulation. Preferably, the kit may additionally comprise instructions, e.g., a user's manual for interpreting the results of any determination(s) with respect to the diagnoses provided by the methods of the present invention. Particularly, such manual may include information for allocating the amounts of the determined HBV immune complex to the kind of diagnosis. Details are to be found elsewhere in this specification. Additionally, such user's manual may provide instructions about correctly using the components of the kit for determining the amount(s) of the respective biomarker. A user's manual may be provided in paper or electronic form, e.g., stored on CD or CD ROM. The present invention also relates to the use of said kit in any of the methods according to the present invention.

All references cited in this specification are herewith incorporated by reference with respect to their entire disclosure content and the disclosure content specifically mentioned in this specification.

FIGURES

Fig. 1: Principle of HBV immune complex detection; Anti-HBsAg capture antibodies (Biotin aHBS1, Biotin aHBS2) are bound to a solid surface. HBV immune complexes (comprising HBsAg and anti-HBsAg IgG) are captured to the surface and detected by a monoclonal IgM with a low affinity, but a high avidity to human IgG conjugated to Digoxigenin (anti-<aggregated human IgG> IgM-Digoxigenin.

Fig. 2: Schematic drawing of the study design.

Fig. 3: Amount of HBV immune complexes vs. response to standard treatment in a cohort not differentiated according to HBeAg status; A) and B): concentration of HBsAg at week 0 (i.e. before start of therapy, A) and at week 12 (B) of therapy in serum samples from patients responding (R) or non-responding (NR) to standard therapy. Differences between responders and non-responders are not statistically significant. C) and D): concentration of HBsAg/anti-HBsAg IgG complex at week 0 (C) and at week 12 (D) of therapy in serum samples from patients responding (R) or non-responding (NR) to standard therapy. Differences between responders and non-responders are statistically significant at week 0 ($p=0.0093$) and at week 12 ($P=0.0179$).

Fig. 4: ROC curves for the data obtained in Fig. 3.

EXAMPLES

The following Examples shall merely illustrate the invention. They shall not be construed, whatsoever, to limit the scope of the invention.

Example 1
Production of monoclonal mouse IgM antibodies with rheumatoid factor-like specificity

Immunogen: H-IgG polymer:

10 mg human IgG1 (Sigma Company) is dissolved in 0.6 ml 25 mM bicarbonate buffer pH 9.5. After adding 3.5 µl 12.5% glutardialdehyde solution, it is incubated for 2 hours at room temperature. Subsequently it is cooled in an ice bath, adjusted to pH 8.3 with 50 mM triethanolamine solution pH 8.0 and 0.15 ml freshly prepared sodium boron hydride solution (8 mg boron hydride/ml water) is added. After 2.5 hours at 0°C the preparation is dialysed for 16 hours at 4°C against 10 mM potassium phosphate buffer/0.2 M NaCl, pH 7.5. The dialysate containing IgG polymer is stored in aliquots at 80°C or used for immunization and for specificity tests in culture supernatants of hybridoma cells. H-IgG3 polymer is produced in a similar manner starting from human IgG3 (Sigma Company).

Immunization of mice:

12 week old, female Balb/c mice are firstly immunized intraperitoneally with 100 µg H-IgG1 or IgG3 polymer together with the adjuvant CFA (complete Freund's adjuvant). After 8 days a further immunization is carried out with 100 µg of the respective IgG polymer in CFA. 13 days after the initial immunization, 200 µg of the respective polymer is administered intraperitoneally without adjuvant, 14 and 15 days after the initial immunization 100 µg was administered in each case intraperitoneally and intravenously. The fusion is carried out after 16 days.

Production of hybridoma clones:

Fusion and cloning:

Spleen cells of an immunized mouse are fused with myeloma cells following the method of Galfré, G., Methods in Enzymology 73 (1981) 3-46. Approximately 1 x 10^8 spleen cells of the immunized mouse are mixed with 2 x 10^7 myeloma cells (P3X63-Ag8-653, ATCC CRL 1580) and centrifuged (10 min at 300 g and 4°C). The cells are then washed once with RPMI-1640 medium without foetal calf serum (FCS) and again centrifuged at 400 g in a 50 ml conical tube. 1 ml PEG (polyethylene glycol) (molecular weight 4000, Merck, Darmstadt) is added and mixed by pipetting. After 1 min in a water bath at 37°C, 5 ml RPMI 1640 without FCS is added dropwise, mixed, filled up to 50 ml with medium (RPMI 1640 + 10% FCS) and subsequently centrifuged. The sedimented cells are taken up in RPMI 1640 medium containing 10% FCS and sown in hypoxanthine-azaserine selection medium (100 mmol/l hypoxanthine, 1 µg/ml azaserine in RPMI 1640 + 10% FCS). Interleukin 6 (100 U/ml) is added to the medium as a growth factor. After about 10 days the primary cultures were tested for specific antibody synthesis. Primary cultures which show a positive reaction with aggregated human IgG1 but no cross-reaction with monomeric IgG are cloned by means of a fluorescence-activated cell sorter in 96-well cell culture plates. Interleukin 6 (100 U/ml) is added to the medium as a growth additive.

The following hybridoma clones were obtained in this manner:

Table 1:

Monoclonal antibody name	Immunogen	Subclass specificity polymer
MAb<h-Agg.-IgG>M-3.022.5-IgM	h-IgG1 polymer	IgG1>IgG3>IgG4>IgG2
MAb <h-Agg.-IgG>M-1.010.2-IgM	h-IgG1 polymer	IgG1>IgG3>IgG4>IgG2
MAb <h-Agg.-IgG>M-1.1.7-IgM	h-IgG3 polymer	IgG1>IgG3>IgG2>IgG4

Screening test for monoclonal antibodies having specificity for aggregated human IgG.

Streptavidin-coated microtitre plates (MTPs) are coated with biotinylated human IgG1 or IgG3. Afterwards they are incubated with the monoclonal antibody in the cell culture supernatant. Subsequently the bound antibodies are detected in the usual manner using an anti-<mouse-IgM>-peroxidase (POD) by reaction with a POD substrate.

Determination of the subclass specificity using human IgG bound to a solid 10 phase:

In order to determine the specificity of the antibodies in the culture supernatant of the hybridoma cells, MTPs coated with recombinant streptavidin (MicroCoat Company, Order No. 12-K 96 N) are coated with 1 µg/ml biotinylated h-IgG (=h-IgG-Bi) of subclass 1 or 2 or 3 or 4 in incubation buffer. Since IgG bound via biotin to a solid phase behaves like aggregated polymeric IgG, this experimental approach can be used to determine the subclass specificity. For this 100 µl h-IgG-Bi solution per well is incubated for 60 minutes at room temperature while shaking and subsequently washed 3 times with 0.9% NaCl / 0.05% Tween ® 20. In the next step 100 µl of the antibody solution to be examined (culture supernatant) is added to a coated well and incubated for 1 hour at room temperature while shaking. After washing 3 times with 0.9% sodium chloride / 0.05% Tween® 20, 100 µl of a POD-labeled Fab fragment of a polyclonal antibody from the goat against mouse IgM (Dianova Company, Order No. 115-036-075, concentration used 0.16 µg/ml incubation buffer) is added in each case to detect bound antibody from the sample, incubated for 1 hour at room temperature while haking and subsequently washed 3 times with 0.9% sodium chloride / 0.05% Tween® 20. Finally 100 µl / well ABTS® substrate (Roche Diagnostics GmbH, Order No. 1684 302) is added and the absorbance at 405/492 nm is measured after 30 min at room temperature in an MR700 Microplate reader from the Dynatech Company.

Incubation buffer: 40 mM Na phosphate, pH 7.4, 200 mM Na tartrate, 0.1% Tween® 20, 0.2% bovine serum albumin.

Determination of the reactivity / cross-reaction with monomeric, human IgG1:

In order to determine the reactivity / cross-reaction with monomeric, non-aggregated H-IgG1, the monoclonal antibody to be examined is pre-incubated in the test described above with monomeric, non-aggregated IgG1 in increasing concentrations or in excess. If the measured signal remains unchanged at a high level, there is no cross-reaction. If the measured signal decreases, a cross-reaction has occurred.

For this microtitre plates (MTP) (MicroCoat Company, Order No. 12-K 96 N) coated with recombinant streptavidin are coated with 1 µg/ml biotinylated HIgG1 (=H-IgG1-Bi) in incubation buffer. 100 µl of the H-IgG1-Bi solution is used per well and incubated for 60 min at room temperature while shaking and subsequently washed 3 times with 0.9% NaCl / 0.05% Tween® 20. The monoclonal antibody to be tested for cross-reaction is pre-incubated with serial concentrations of up to 1 µg/ml monomeric, non-aggregated IgG1. The pre-incubation takes place in uncoated 96-well MTPs for 1 hour at room temperature while shaking.

In the next step 100 µl of this solution (antibody + non-aggregated, monomeric IgG1 in excess) is added to a coated well and incubated for 1 hour at room temperature while shaking. After washing 3 times with 0.9% sodium chloride / 0.05% Tween® 20, 100 µl of a POD-labeled Fab fragment of a polyclonal antibody from the goat against mouse IgM (Dianova Company, Order No. 115-036-075, concentration used 0.16 µg/ml incubation buffer) is added in each case to detect bound antibody from the sample, incubated for 1 hour at room temperature while shaking and subsequently washed 3 times with 0.9% sodium chloride / 0.05% Tween® 20.

Finally 100 µl / well ABTS® substrate (Roche Diagnostics GmbH, Order No. 1684 302) is added and the absorbance at 405/492 nm is measured after 30 min at room temperature in an MR700 Microplate reader from the Dynatech Company. The monoclonal rheumatoid factor-like binding antibodies that are suitable in the sense of the invention recognize all human IgG subclasses and exhibit less than 10% cross-reaction with monomeric h-IgG in a competition test. If HIgG1 polymer is used to determine the reactivity, the measured signal is greatly reduced. Table 1 shows the major properties of the monoclonal antibodies that were found.

Fermentation of hybridoma clones to isolate monoclonal antibodies:

The hybridoma cells that are obtained are sown at a density of 1 x 10^5 cells per ml in RPMI 1640 medium containing 10% FCS and propagated for 7 days in a fermenter (Thermodux Company, Wertheim/_Main, model MCS-104XL, Order No. 144-050). Average concentrations of 100 µg monoclonal antibody per ml are reached in the culture supernatant.

Isolation of monoclonal MAb <h-Agg.-IgG>M-3.022.5-IgM:

5 mg MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873) is adjusted to a total volume of 2 ml with 0.1 M sodium phosphate buffer, pH 8.6. 50 µl of a 1.11 mM solution of digoxigenin-3-O-methyl-carbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester in dimethyl sulfoxide is added to this solution and subsequently stirred for 60 min at 25°C. The ratio of IgM to activated digoxigenin is 1:10. The IgM-digoxigenin that forms is dialysed against 20 mM potassium phosphate buffer / 0.1 M NaCl / 3% sucrose, pH 7.5. The dialysed IgM-Dig is stored in aliquots at -80°C.

Example 2
Fully automated immunoassay on a multi parameter biochip platform (IMPACT)

A multiparameter biochip platform is described in Hornauer, H. et al., BIOspectrum, Special Proteomics 10 (2004) 564-565 and Hornauer, H. et al., Laborwelt 4 (2004) 38-39. To determine complex levels an array-based assay was used (IMPACT - Immunological Multi-Parameter Chip Technology, Roche Diagnostics).

A streptavidin coating is applied over the whole area of a test area of about 2.5 x 6 mm on a black-stained polystyrene support (solid phase). Lines of identical spots of approximately 10 to 20 per line consisting of biotinylated fragments of the therapeutic antibody are applied to the test area in an ink-jet procedure; the diameter per spot is about 150 µm.

The following test-specific reagents were used:

Sample dilution buffer and detection antibody buffer:
50 mM Tris, pH 6.6; 30 mM MES; 50 mM NaCl; 0.1% detergent (polydocanol); 5mM EDTA; 0.5% Casein; 0.2% preservative (oxypyrion and methylisothiazolone hydrochloride (MIT))

If samples showed very high concentrations of HBV-immunocomplexes and results were not within the measuring range, additional manual sample dilutions were performed using Elecsys

Diluent MultiAssay (Ident No. 03609987). Samples were diluted further in 10-fold steps (e.g. 1:10, 1:100, 1:1000)

Wash buffer: 10 mM Tris, 0.01% polydocanol, 0.001% oxypyrion, 0.001% MIT

Samples: human sera from chronic HBV patients before and during treatment with Peginterferon alpha-2a (PEGASYS) and Adefovir (Fig. 2, Table 2).

Two different biotinylated antibodies specifically binding to HBs antigen were used as biotinylated capture antibodies. Immune complexes of HBs antigen and anti-HBs antibodies were bound to the solid phase-bound capture antibodies. Immune complexes were detected by a monoclonal IgM antibody specific for human aggregated IgG (MAb<h-Agg.-IgG>M-3.022.5-IgM)(Fig. 1).

The samples were diluted 1:5 with the sample dilution buffer for the measurement. The diluted samples were incubated for 12 min at 37°C. After aspirating the sample and washing the test field with wash buffer, they were incubated with the MAb <h-Agg.-IgG>M-3.022.5-IgM (DSM ACC2873), an antibody labeled with digoxin (Dig-labeled monoclonal antibody <h-Agg.-IgG>), for 6 min at 37°C with a subsequent washing step. After incubation with a fluorescently labeled <Dig> antibody for 3 min at 37°C and subsequently washing and suction drying the test field, the signals were detected by a CCD camera (Fig. 3). The concentration could be calculated with suitable complex-calibrators.

Table 2: study population

HBeAg positive (n=29)			HBeAG negative (n=21)	
HBsAg seroconversion	HBeAg seroconversion	non responder	HBsAg seroconversion	non-responder
n=4	n=10	n=15	n=5	n=16

Table 3: Statistical values for the ROC analysis (Fig. 4)

test	auc	std	LCL	UCL
complex (HBsAg/antiHBsAG IgG)	0.7258	0.0768	0.5753	0.8764
HBsAg	0.5054	0.0920	0.3250	0.6858
ratio HBsAg / complex	0.7093	0.0776	0.5572	0.8613

Claims

1. A method for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, said method comprising the steps of:
   a) determining, in a sample of said subject, the amount of HBV immune complexes,
   b) comparing the amount of HBV immune complexes obtained in step a) to a reference amount, and
   c) identifying a subject suffering from HBV infection as being susceptible to interferon treatment based on the result of the comparison made in step b),
   wherein the HBV e antigen status of the subject is unknown.

2. The method of claim 1, wherein said HBV infection is a chronic HBV infection.

3. The method of any one of claims 1 to 2, wherein the interferon treatment comprises treatment with PEG-interferon and an inhibitor of the viral DNA polymerase.

4. The method of claim 3, wherein said PEG-interferon treatment is PEGasys® and said inhibitor of the viral DNA polymerase is Adefovir®.

5. The method of any one of claims 1 to 4, wherein the HBV immune complexes comprise hepatitis B soluble antigen (HBsAg) and an anti-HBsAG IgG.

6. The method of any one of claims 1 to 5, wherein an increased amount of HBV immune complexes is indicative of a subject being susceptible to interferon treatment while a decreased amount for HBV immune complexes is indicative for a subject being not susceptible to interferon treatment.

7. The method of any one of claims 1 to 6, wherein the sample is a serum sample.

8. The method of any one of claims 1 to 7, wherein the amount of HBV immune complexes is detected with an anti-immunoglobulin antibody.

9. The method of any one of claims 1 to 8, wherein i) the HBV immune complexes comprise anti-HBsAg IgG, anti-HBeAg IgG, or anti-HBcAg IgG and wherein ii)

the amount of HBV immune complexes is detected with an anti-(aggregated-humanIgG) IgM.

10. The method of any one of claims 1 to 9, wherein the amount of HBV immune complex is determined with an enzyme-linked immunosorbent assay (ELISA).

11. Use of the amount of HBV immune complexes in a sample from a subject suffering from HBV infection or a detection agent for the HBV immune complexes in such a sample for identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

12. A device for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, comprising an analyzing unit for determining the amount of HBV immune complexes and an evaluation unit for comparing said amount to a reference amount and for identifying a subject suffering from HBV infection as being susceptible to interferon treatment.

13. A kit comprising instructions to carry out the method of the present invention, a detection agent for determining the amount of an HBV immune complex, and, preferably, standards for reference amounts allowing identifying a subject suffering from HBV infection as being susceptible to standard interferon treatment.

14. The use of claim 11, the device of claim 12, or the kit of claim 13, wherein the HBV e antigen status of the subject is unknown.

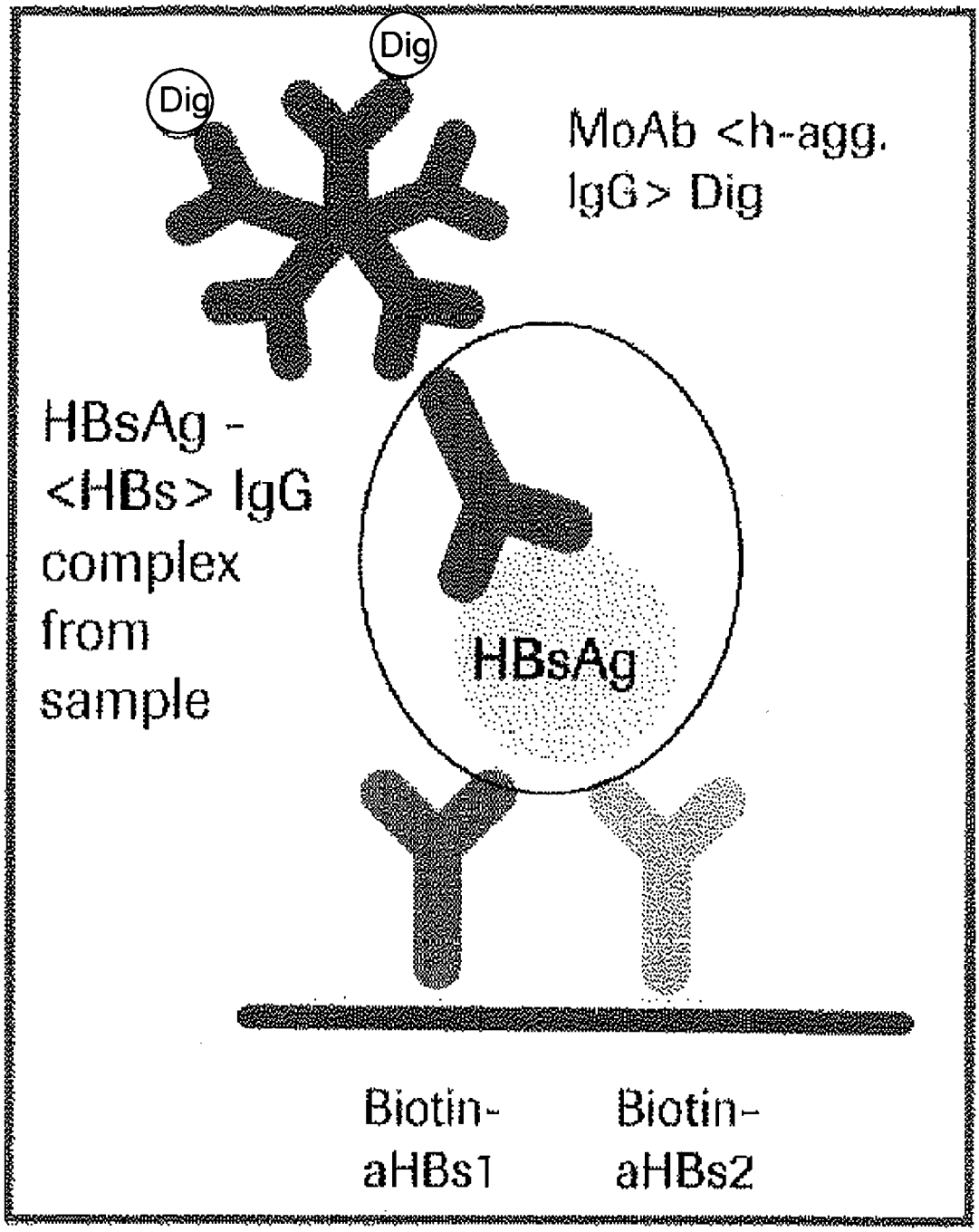
Dig
Dig
MoAb <h-agg. IgG> Dig
HBsAg - <HBs> IgG complex from sample
HBsAg
Biotin-aHBs1
Biotin-aHBs2

Fig. 1

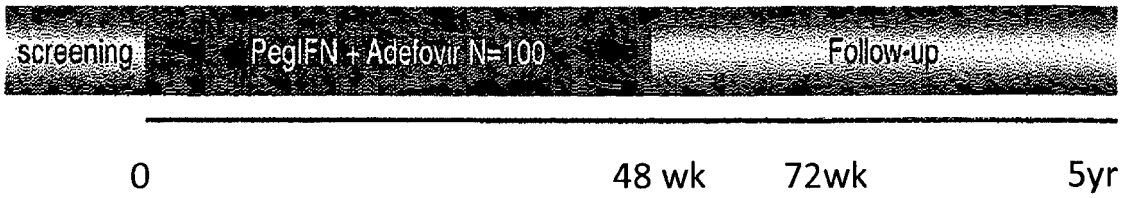

Fig. 2

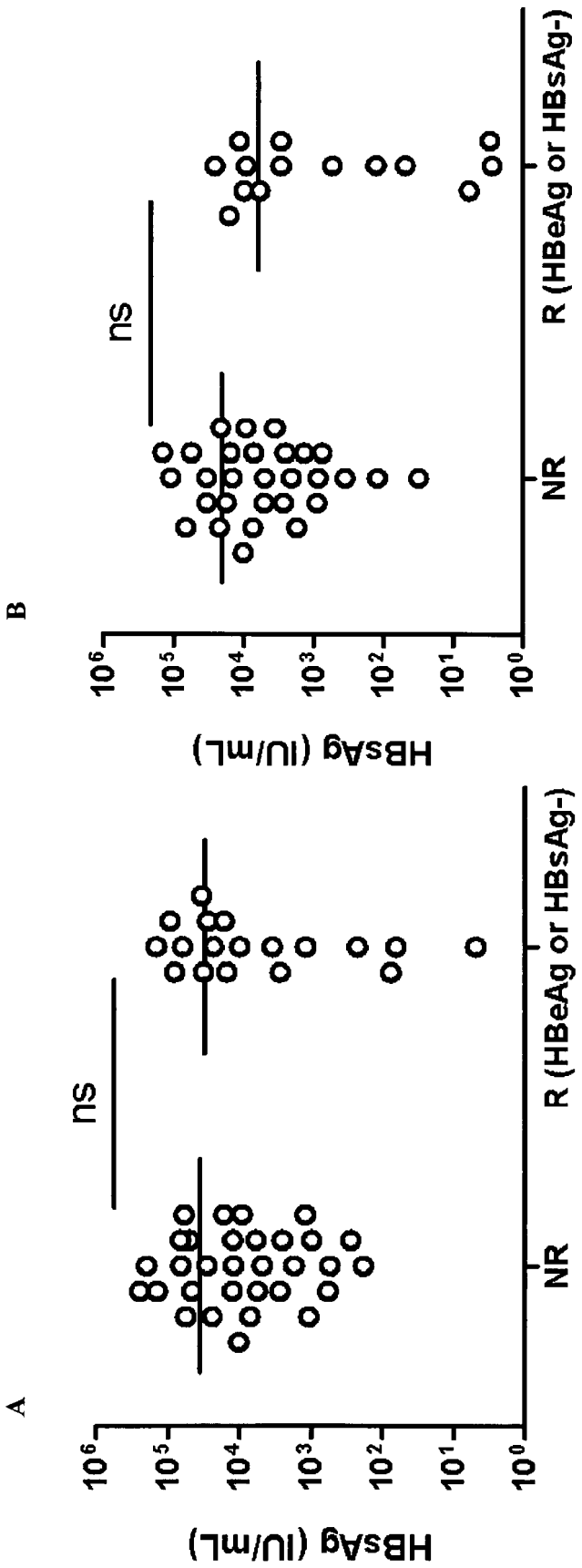

Fig. 3

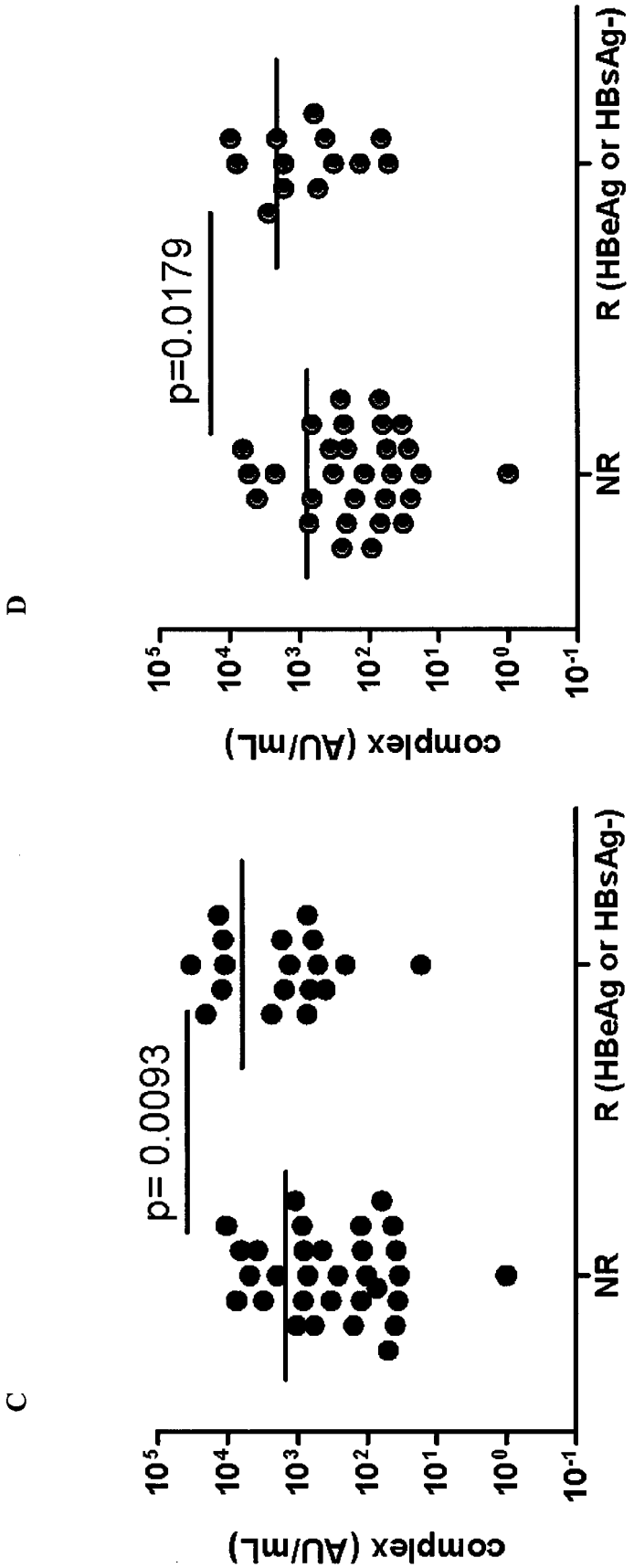
C
p= 0.0093
complex (AU/mL)
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
NR
R (HBeAg or HBsAg-)
D
p=0.0179
complex (AU/mL)
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
NR
R (HBeAg or HBsAg-)

Fig. 3

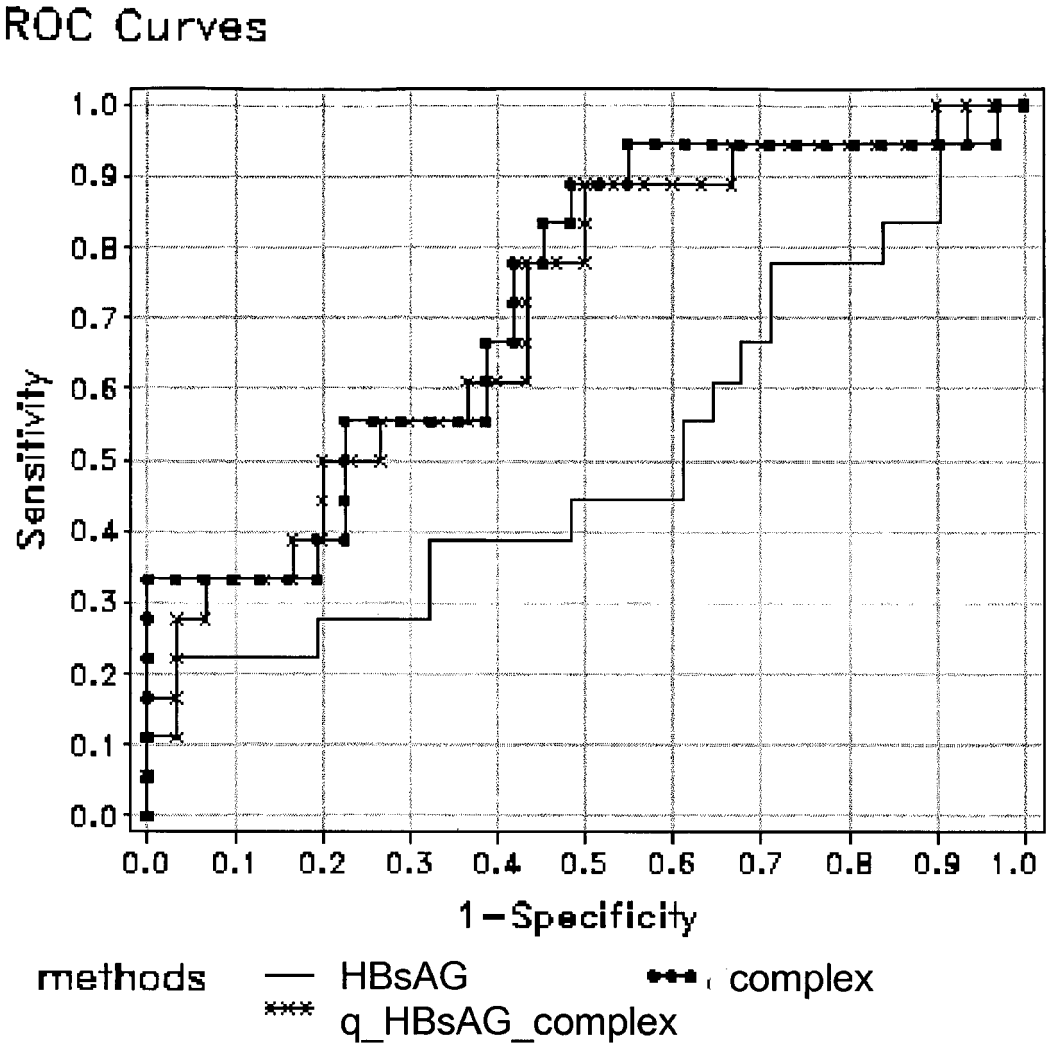
ROC Curves
Sensitivity
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
1-Specificity
methods
HBsAG
complex
q_HBsAG_complex

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/053150

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/576 G01N33/68
ADD.

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

B. FIELDS SEARCHED

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE NIET A ET AL: "A NEW HBSAG/ANTI-HBS IMMUNE COMPLEX ASSAY FOR PREDICTION OF TREATMENT OUTCOME IN CHRONIC HEPATITIS B PATIENTS", HEPATOLOGY, vol. 54, no. Supplement 1, 1530, 1 October 2011 (2011-10-01), page 1087A, XP008151964, abstract ----- -/--	1-14

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 March 2013	05/04/2013

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

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/053150

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BART TAKKENBERG ET AL: "Intrahepatic response markers in chronic hepatitis B patients treated with peginterferon alpha-2a and adefovir", JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY, vol. 26, no. 10, 1 October 2011 (2011-10-01), pages 1527-1535, XP055027237, ISSN: 0815-9319, DOI: 10.1111/j.1440-1746.2011.06766.x abstract; figures 1-5; tables 1-3	1-14
A	US 2007/054264 A1 (HELLSTROM ULLA [SE] ET AL) 8 March 2007 (2007-03-08) paragraph [0086] - paragraph [0174]; claims 1-23; tables 1-3	1-14
A	WO 2010/120759 A1 (SCHERING CORP [US]; YVER ANTOINE JEAN [US]) 21 October 2010 (2010-10-21) page 2 - page 4; claims 1-30	1-14

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/053150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2007054264 A1	08-03-2007	AT 494555 T	15-01-2011
		CN 1906488 A	31-01-2007
		US 2007054264 A1	08-03-2007
WO 2010120759 A1	21-10-2010	AU 2010236606 A1	03-11-2011
		EP 2419727 A1	22-02-2012
		US 2012035347 A1	09-02-2012
		WO 2010120759 A1	21-10-2010

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权利要求书1页 说明书14页
序列表6页 附图5页

(54)发明名称

用于慢性HBV患者的反应预测和疗法监测的HBV免疫复合物

(57)摘要

本发明涉及用于将患有乙型肝炎病毒(HBV)感染的受试者鉴定为对干扰素治疗敏感的方法，所述方法包括步骤:a)确定所述受试者的样品中HBV免疫复合物的量，b)将步骤a)中获得的HBV免疫复合物的量与参考值比较，和c)基于步骤b)中比较的结果，将患有HBV感染的受试者鉴定为对干扰素治疗敏感。本发明进一步涉及确定来自患有HBV感染的受试者的样品中HBV免疫复合物的量的用途和HBV免疫复合物的检测剂用于将患有HBV感染的受试者鉴定为对干扰素治疗敏感的用途。另外，本发明涉及可将患有HBV感染的受试者鉴定为对干扰素治疗敏感的装置和试剂盒。

CN 104126124 A

1. 一种用于将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对干扰素治疗敏感的方法，所述方法包括步骤：

a) 确定所述受试者的样品中 HBV 免疫复合物的量，

b) 将步骤 a) 中获得的 HBV 免疫复合物的量与参考量比较，并且

c) 基于步骤 b) 中比较的结果，将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感，

其中受试者的 HBV e 抗原状态是未知的。

2. 根据权利要求 1 所述的方法，其中所述 HBV 感染是慢性 HBV 感染。

3. 根据权利要求 1 至 2 中任一项所述的方法，其中干扰素治疗包括用 PEG- 干扰素和病毒 DNA 聚合酶抑制剂治疗。

4. 根据权利要求 3 所述的方法，其中所述 PEG- 干扰素治疗是 **PEGasys®** 和所述病毒 DNA 聚合酶抑制剂是 **Adefovir®**。

5. 根据权利要求 1 至 4 中任一项所述的方法，其中 HBV 免疫复合物包含乙型肝炎可溶性抗原 (HBsAg) 和抗 HBsAG IgG。

6. 根据权利要求 1 至 5 中任一项所述的方法，其中增加的 HBV 免疫复合物的量表示受试者对干扰素治疗敏感，减少的 HBV 免疫复合物的量表示受试者对干扰素治疗不敏感。

7. 根据权利要求 1 至 6 中任一项所述的方法，其中样品是血清样品。

8. 根据权利要求 1 至 7 中任一项所述的方法，其中用抗免疫球蛋白抗体检测 HBV 免疫复合物的量。

9. 根据权利要求 1 至 8 中任一项所述的方法，其中 i)HBV 免疫复合物包含抗 HBsAG IgG、抗 HBeAg IgG 或抗 HBcAg IgG，其中 ii) 用抗 (聚集的人 IgG) IgM 检测 HBV 免疫复合物的量。

10. 根据权利要求 1 至 9 中任一项所述的方法，其中用酶联免疫吸附测定法 (ELISA) 确定 HBV 免疫复合物的量。

11. 来自患有 HBV 感染的受试者的样品中 HBV 免疫复合物的量或用于这种样品中的 HBV 免疫复合物的检测剂用于将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感的用途。

12. 一种用于将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对干扰素治疗敏感的装置，包括用于确定 HBV 免疫复合物的量的分析单元，和用于将所述量与参考量比较以及用于将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感的评价装置。

13. 一种试剂盒，其包含实施本发明方法的说明书、用于确定 HBV 免疫复合物的量的检测剂，和优选地允许将患有 HBV 感染的受试者鉴定为对标准干扰素治疗敏感的参考量的标准。

14. 根据权利要求 11 所述的用途，根据权利要求 12 所述的装置，或根据权利要求 13 所述的试剂盒，其中受试者的 HBV e 抗原状态是未知的。

用于慢性 HBV 患者的反应预测和疗法监测的 HBV 免疫复合物

[0001] 本发明涉及诊断学领域。特别地，它涉及用于将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对干扰素治疗敏感的方法，所述方法包括步骤 :a) 确定所述受试者的样品中 HBV 免疫复合物的量，b) 将步骤 a) 中获得的 HBV 免疫复合物的量与参考值比较，和 c) 基于步骤 b) 中比较的结果，将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感。本发明进一步涉及确定来自患有 HBV 感染的受试者的样品中 HBV 免疫复合物的量的用途和 HBV 免疫复合物的检测剂的用途，用于将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感。另外，本发明涉及允许将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感的装置和试剂盒。

[0002] 乙型肝炎是由乙型肝炎病毒 (HBV) 引起的肝脏传染性炎性疾病。该病毒因暴露于体液而传播，通过输血、透析、毒品成瘾者共用针头和围产期感染传播是主要的传播模式。

[0003] 急性乙型肝炎病毒感染以肝脏炎症、呕吐和黄疸为特征。症状一般持续几周并且随后逐渐改善，同时免疫系统清除感染。完全康复和建立保护性免疫的概率随年龄而增加 : 尽管仅 5％成人将患有持续多于数周的持续性感染，即慢性 HBV 感染，但是对于幼儿，这种比率升至 70％、并且对于围产期感染的新生儿，升至 95％。

[0004] 慢性 HBV 与形成肝硬化和肝癌的严重风险相关，这是为何 HBV 已经由 WHO 国际癌症研究机构 (WHO's International Agency for the Research on Cancer, IARC) 划归为 I 类致癌原，即对人致癌的病原体。因此对患者而言接受治疗允许患者的免疫系统清除感染的治疗是极为重要的。常见治疗方案包括抑制病毒 DNA 聚合酶的核苷或核苷酸类似物，连同干扰素 α。一项重要改进是将 PEG 化干扰素 (例如 Pegasys®) 引入疗法，从而提供延长的干扰素半寿期。关于 HBV 感染的流行病学和治疗的综述，见 J. L. Dienstag N Engl J Med 2008, 359 ;1486-1500。

[0005] 慢性 HBV 感染的血清学是复杂的，因为在一方面，抗 HBV 高滴度与疾病的严重性直接相关，但在另一方面，发现抗 HBV e 抗原抗体在自发性血清转换 (即从血清清除 HBV e 抗原) 多年前即存在于患者中 (Maruyama 等人，(1993), J. Clin. Invest. 91, 2586-2595)。尽管治疗改进，但仍仅一部分患者将经过治疗清除感染，而其他患者将不清除。第二组患者 (非反应个体) 的成员常常可以在更激进的治疗方案 (例如剂量增加、治疗延长或额外用药) 下清除感染。因此将需要能够预测患者对标准干扰素治疗的反应，目的在于从一开始就可以调整治疗。在最近的改进中，发现在 12 周治疗后确定患者样品中 HBV 可溶性抗原 (HBsAg) 的变化允许利用临界值预测疗法成效。然而，临界值仅对还存在 HBV e 抗原 (HBeAg) 阳性的患者有效，所致患者主要是亚太地区患者中的病例。在例如欧洲主要为 HBeAg 阴性的患者中，临界值非常难以限定，使用在疗法启动和第 12 周之间至少 10％的下降作为临时替代物。

[0006] 最近，据报道在 HBeAg 阳性患者中，在疗法启动之前高含量的包含 HBsAG 和抗 HBsAg-IgG 的 HBV 复合物与对标准干扰素疗法的良好反应相关。然而，这不允许预测 HBeAg 阴性患者。总之，目前没有这样的方法，所述方法将允许在治疗启动之前对全部患者组预测

疗法成效，无论他们的 HBeAg 状态如何。

[0007] 因此，作为本发明基础的技术问题可以视为提供用于符合前述需要的手段和方法。这个技术问题通过权利要求中及下文表征的实施方案解决。

[0008] 因此，本发明涉及一种用于将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对干扰素治疗敏感的方法，所述方法包括步骤：

[0009] a) 确定所述受试者的样品中 HBV 免疫复合物的量，

[0010] b) 将步骤 a) 中获得的 HBV 免疫复合物的量与参考值比较；并且

[0011] c) 基于步骤 b) 中比较的结果，将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感。

[0012] 本发明的方法优选地是一种体外方法。另外，它可以包括除上文明确提到的那些之外的步骤。例如，其他步骤可以例如涉及预处理步骤 a) 的样品或评价由该方法获得的结果。另外，可以使用内部对照，如样品质量对照或性能对照。该方法可以手工地实施或由自动化辅助。优选地，步骤 (a) 至 (c) 可以总体上或部分地由自动化辅助，例如由用于确定步骤 (a) 中 HBV 免疫复合物的合适机器人设备和感知设备辅助。

[0013] 如本文所用，术语“鉴定”意指将受试者划入对干扰素治疗敏感的受试者组（所谓“反应个体”），或划入对干扰素治疗不敏感的受试者组（所谓“非反应个体”）。如本领域技术人员将理解，前述鉴定通常不旨在对于待分析的受试者达到 100％正确。然而，本术语要求评估将对统计显著部分的待分析的受试者有效。本领域技术人员可容易地使用各种熟知的统计评价工具（例如，置信区间确定、p- 值测定、Student 氏 t- 检验、Mann-Whitney 检验等）确定某个部分是否为统计显著。详见 Dowdy 和 Wearden, Statistics for Research, John Wiley&Sons, New York 1983。优选的置信区间是至少 90％、至少 95％、至少 97％、至少 98％或至少 99％。p- 值优选地是 0.1、0.05、0.01、0.005 或 0.0001。优选地，本发明构思的概率允许这种区分将对给定族群或群体的至少 60％、至少 70％、至少 80％或至少 90％受试者是正确的。

[0014] 优选地，根据本发明方法的鉴定可以在不知晓受试者的 HBV e 抗原状态的情况下实现，即这种鉴定导致将受试者正确划分至对干扰素治疗敏感的受试者组或对干扰素治疗不敏感的受试者组，无论所研究受试者的 HBeAg 状态如何。因而，该方法可以适用于血清样品中具有可检测量的 HBeAg 的受试者以及适用于血清样品中没有可检测量的 HBeAg 的受试者。

[0015] 术语“乙型肝炎病毒 (HBV)”指来自嗜肝 DNA 病毒科的作为乙型肝炎的致病因子的病毒物种。HBV 病毒粒子由外膜（也称作脂质包膜）、二十面体核衣壳和 DNA 基因组组成。HBV 在本领域中熟知并已得到表征。

[0016] 术语“乙型肝炎病毒感染”或“HBV 感染”指 HBV 在受试者中的可检测存在。优选地，通过在来自受试者的样品中检测出至少一种病毒多肽诊断 HBV 存在，更优选地，检测至少一种病毒抗原 HBs(HBsAg, Genbank 登录号 AAL66340.1 GI:18252577, SEQ ID NO:1)，HBc(HBcAg, Genbank 登录号 CAA51257.1 GI:288930, SEQ ID NO:2) 和 HBe(HBeAg, Genbank 登录号 AAM96930.1 GI:22530876, SEQ ID NO:3)。技术人员理解，将 HBV 多肽作为生物标记提及，不作为具体多肽提及，并且术语 HBV 涵盖可能包含前述 HBV 多肽的序列变体的各种 HBV 毒株。因此，具有以 GenBank 登录号保藏的具体序列的前述多肽应理解为代表生物

标记的示例性序列。根据本发明还涵盖变体多肽，所述变体多肽因至少一个氨基酸添加、置换和/或缺失而与具体序列的多肽不同，只要它们也合适作为如上文讨论的 HBV 感染的生物标记。优选地，变体多肽与具体多肽同一至少 70%、至少 75%、至少 80%、至少 85%、至少 90%、至少 95%、至少 98%或至少 99%。如本文所用，术语“同一”指以如下为特征的序列同一性：确定两个核酸序列或氨基酸序列之间相同氨基酸的数目，其中比对所述序列以获得最高等级的匹配。可以使用在计算机程序中代码化的公开技术或方法如，例如 BLASTP、BLASTN 或 FASTA(Altschul 1990, J Mol Biol 215, 403) 来计算。在一个方面，在整个氨基酸序列范围内计算同一性百分数值。对于技术人员，一系列基于多种算法的程序可用于比较不同的序列。在这种情况下中，Needleman 和 Wunsch 算法或 Smith 和 Waterman 算法产生特别可靠的结果。为实施序列比对，可以使用程序 PileUp(Higgins 1989, CABIOS 5, 151) 或程序 Gap 和 BestFit(Needleman 1970, J Mol Biol 48 ;443 ;Smith 1981, Adv Appl Math 2, 482)，所述程序是GCG软件包 (Genetics Computer Group 1991, 575 Science Drive, Madison, Wisconsin, USA 53711) 的部分。在本发明的另一个方面，使用程序 GAP，在整个序列区域范围内采用以下设置确定上文以百分数 (%) 提及的序列同一性值：空位权重 :50，长度权重 :3，平均匹配 :10.000 和平均错配 :0.000，除非另外说明，否则应当总是使用所述设置作为序列比对的标准设置。

[0017] 还优选地，通过在来自受试者的样品中检出至少一种病毒多核苷酸、优选地病毒 DNA，检测 HBV 的存在。病毒多核苷酸或整个 HBV 基因组的核苷酸序列是本领域熟知的。优选地，HBV 核苷酸序列以 GenBank 登录号 NC_003977.1 保藏。技术人员理解，将 HBV 多核苷酸作为生物标记提及，不作为具体多核苷酸提及，并且术语 HBV 涵盖包含变异核苷酸序列的各种 HBV 毒株。因此，具有以 GenBank 登录号保藏的具体序列的前述多核苷酸应理解为代表生物标记的示例性序列。根据本发明还涵盖变体多核苷酸，所述变体多核苷酸因至少一个核苷酸添加、置换和/或缺失而不同于具有具体序列的变体多核苷酸，只要它们也合适作为如上文讨论的 HBV 感染的生物标记。优选地，变体多核苷酸与具体多核苷酸同一至少 70%、至少 75%、至少 80%、至少 85%、至少 90%、至少 95%、至少 98%或至少 99%。同一性可以如本文中他处对氨基酸序列所述那样计算。

[0018] 优选地，本文中提及的 HBV 感染是慢性 HBV 感染，其中所述慢性 HBV 感染优选地以 HBV 在受试者中可检测地存在多于 4 周、多于 5 周、多于 6 周、多于 7 周、多于 8 周、多于 9 周、多于 10 周、多于 11 周或多于 12 周为特征。更优选地，本文中提及的慢性 HBV 感染符合疾病控制中心 (CDC) 公开的定义，根据所述定义，慢性 HBV 感染以如下实验室标准为特征：乙型肝炎核心抗原的 IgM 抗体 (IgM 抗 HBc) 阴性并且以下检验之一为阳性结果：乙型肝炎表面抗原 (HBsAg)、乙型肝炎 e 抗原 (HBeAg) 或乙型肝炎病毒 (HBV)DNA，或者间隔至少 6 个月 HBsAg 阳性或 HBV DNA 阳性 2 次 (间隔 6 个月进行的这些检验的任何组合都是可接受的)。

[0019] 术语“干扰素治疗”和“标准治疗”优选地指用干扰素和优选地病毒 DNA 聚合酶抑制剂治疗 HBV 感染。然而，本发明还构思干扰素治疗优选地是用干扰素单一治疗并且标准治疗优选地是用病毒 DNA 聚合酶抑制剂单一治疗。优选地，如这个情境下提到的干扰素是与聚乙二醇共价结合的干扰素 (PEG- 干扰素)，更优选地，干扰素是干扰素 2α，最优选地，干扰素是与聚乙二醇共价结合的干扰素 2α (PEG- 干扰素 2α，市售为**PEGasys®**)。优选地，病毒 DNA 聚合酶抑制剂是核苷酸或核苷类似物，更优选地是 {[2-(6- 氨基 -9H- 嘌

呤 -9- 基）乙氧基] 甲基 } 膦酸（例如**adefovir®**）。因而，标准或干扰素治疗优选地是用干扰素 2α 和核苷酸类似物治疗。更优选地，标准或干扰素治疗是用 PEG- 干扰素 2α 和核苷酸类似物治疗，并且甚至更优选地标准干扰素治疗是用 PEG- 干扰素 2α（例如**PEGasys®**）和**adefovir®**治疗持续少于 1 年。最优选地，标准干扰素治疗是根据以下治疗计划用 PEG- 干扰素 2α（例如**PEGasys®**）和**adefovir®**治疗：通常，通过皮下施用（每周皮下注射）实施**PEGasys®**治疗持续 48 周时间，而将病毒 DNA 聚合酶抑制剂（核苷 / 核苷酸类似物）经口服施用更长时间，即在 80％患者中持续超过 1 年。关于详细内容，还见 J. L. Dienstag N Engl J Med 2008, 359 ;1486-1500。

[0020] 术语“对干扰素治疗敏感”意指与成功治疗的机率相比，可以成功地治疗受试者或可以至少以显著增加的可能性成功地治疗受试者。可以通过如本文提到的干扰素治疗成功治疗的受试者也称作反应个体。如果将 HBV 感染、与之相关的至少一种症状或与之相伴的至少一种并发症改善到显著程度和 / 或治愈，则如本文提到的治疗是成功的。优选地，成功的治疗还伴随在如本文以上所述来自受试者的样品中可检测的 HBV 多肽和 / 或 HBV 多核苷酸减少。更优选地，成功的治疗特征如下 ：在治疗最多 1 年、最多 50 周、最多 49 周或最多 48 周后来自受试者的样品中不存在可检测量的 HBsAG 和 / 或 HBeAG，如治疗启动后 60 至 80 周、优选地 65 至 75 周，或最优选地 72 周所测定。

[0021] 不能通过干扰素治疗成功治疗的受试者也称作非反应个体。在这类受试者中，不出现 HBV 感染、与之相关的至少一种症状或至少一个并发症的改善或治愈。优选地，这种非反应个体受试者患有严重形式的慢性肝炎 B 病毒感染，其中在干扰素治疗之前和之后可检出 HBsAG，但是不可检出 HBeAG，或其中在标准干扰素治疗之前和之后可检出 HBsAG 和 HBeAG。应当考虑被鉴定为对干扰素治疗不敏感的受试者施用改良治疗措施，例如增加干扰素和 / 或聚合酶抑制剂的剂量和 / 或延长治疗和 / 或额外用药。

[0022] 如本文所用，术语“HBV 免疫复合物”指在至少一种 HBV 多肽和如下文所述的针对所述至少一种 HBV 多肽的至少一种免疫球蛋白（即抗 HBV 免疫球蛋白）之间形成的复合物。优选地，该复合物在至少一种 HBV 多肽和多于 1 种，多于 2 种、多于 3 种、多于 4 种、多于 5 种、多于 10 种、多于 20 种、多于 50 种或多于 100 种针对所述至少一种 HBV 多肽的抗 HBV 免疫球蛋白分子之间形成。优选地，HBV 多肽选自与上文相关的一系列 HBV 抗原，更优选地，HBV 多肽是 HBsAg。抗 HBV 免疫球蛋白优选地是在受试者的至少一种体液、优选地血液或血清中存在的可溶性免疫球蛋白。优选地，抗 HBV 免疫球蛋白是 IgG，更优选地是血清 IgG。最优选地，包含于 HBV 免疫复合物中的抗 HBV 免疫球蛋白是针对 HBsAg 的 IgG，即抗 HBsAG IgG。应当理解 HBV 免疫复合物可以包含在本说明书中明确详述之外的其他分子，然而，优选本发明的抗 HBV 免疫球蛋白和 HBV 多肽形成主要部分的 HBV 免疫复合物。因此，抗 HBV 免疫球蛋白和 HBV 多肽优选地构成 HBV 免疫复合物质量的至少 25％、至少 30％、至少 40％、至少 50％、至少 60％、至少 70％或至少 75％。更优选地，HBV 免疫复合物基本上由抗 HBV 免疫球蛋白和 HBV 多肽组成，即抗 HBV 免疫球蛋白和 HBV 多肽构成 HBV 免疫复合物质量的至少 80％、至少 90％或至少 95％。甚至更优选地，HBsAg 和抗 HBsAG IgG 构成主要部分的 HBV 免疫复合物。最优选地，HBV 免疫复合物基本上由 HBsAg 和抗 HBsAG IgG 组成。

[0023] 技术人员理解术语“免疫球蛋白”或“Ig”。它们指免疫球蛋白家族的成员分子，特征在于它们是由响应于与外来抗原接触的个体的免疫系统产生的可溶性多肽。优选地，免疫球蛋白是来自哺乳动物的免疫球蛋白，并且更优选地，免疫球蛋白是人免疫球蛋白。还优选地，免疫球蛋白选自 IgA、IgD、IgE、IgG 和 IgM。更优选地，免疫球蛋白是 IgG，最优选地是人 IgG。人 IgG 以大约 150kDa 的分子质量和由两条约 50kDa 的相同重链和两条约 25kDa 的相同轻链组成的结构为特征。这两条重链经由二硫键彼此连接并与轻链连接以形成常见的 Y 形分子。优选地，IgG 是糖基化的，更优选地 IgG 通过 N- 糖基化过程糖基化。优选地，IgG 属于亚组 IgG1、IgG2、IgG3 或 IgG4 之一。

[0024] 术语“抗免疫球蛋白抗体”优选地涉及来自抗体的蛋白家族中的可溶性分子，所述抗体识别免疫球蛋白，优选地识别人免疫球蛋白，即本发明的抗免疫球蛋白抗体优选地是抗人免疫球蛋白抗体，更优选地识别人 IgG，即抗人 IgG 抗体。本发明的抗免疫球蛋白抗体优选地是具有低到中等亲和力 (affinity) 的抗体和具有高亲合力 (avidity) 的抗体，如下文详述。抗体对表位的亲和力定义为在所述抗体上的抗原识别位点和该表位之间全部非共价相互作用的强度。具有高亲和力的抗体通过许多和 / 或强烈非共价相互作用与抗原牢固结合，并且因此保持与抗原结合持续相对长的时间段。在另一方面，具有低亲和力的抗体抗体以少和 / 或弱的非共价相互作用与抗原进行相互作用，并且因此与抗原快速解离。本领域技术人员已知的抗体的亲和力可以由它的解离常数 (K_d) 描述。优选地，10^{-10}mol/l 至 10^{-9}mol/l 的解离常数表示极高亲和力，10^{-8}mol/l 的解离常数表示高亲和力，10^{-7}mol/l 的解离常数表示低亲和力，并且 10^{-6}mol/l 及更高的解离常数表示很低的亲和力。因而，抗免疫球蛋白抗体的解离常数优选地是 10^{-6}mol/l 至 10^{-8}mol/l，更优选地，解离常数是 10^{-7} 至 10^{-8}mol/l。在包含多于一个结合位点的分子（例如抗体）中，第一结合位点的相互作用增加其他结合位点相互作用的概率。技术人员将包含多于一个结合位点的分子和待结合的分子之间这类多重相互作用的强度称作亲合力。高亲合力因此可以补偿单个结合位点的相对低的亲和力。因而，抗免疫球蛋白抗体，优选地，具有多于 2 个抗原识别位点，更优选地多于 4 个抗原识别位点。最优选地，抗免疫球蛋白抗体具有 10 个或更多个抗原识别位点。优选地，抗免疫球蛋白抗体是 IgG、IgD、IgE，更优选地抗免疫球蛋白抗体是包含多于 2 个抗原识别位点的抗体，例如 IgA，最优选地，抗免疫球蛋白抗体是在一个分子中包含多于 4 个抗原识别位点的抗体，例如 IgM。抗免疫球蛋白抗体是多克隆或单克隆的，并且在在适用于抗体生产的哺乳动物中或哺乳动物细胞中产生。优选地，抗免疫球蛋白抗体与本发明免疫球蛋白的表位特异性结合。更优选地，抗免疫球蛋白抗体是抗人免疫球蛋白抗体，即与本发明的人免疫球蛋白结合，甚至更优选地，该结合是对人免疫球蛋白特异的。最优选地，抗免疫球蛋白抗体是抗人 IgG 抗体，即与人 IgG 特异性结合。然而，本发明还构思，抗免疫球蛋白抗体与由免疫球蛋白与 HBV 免疫复合物中的 HBV 多肽结合所形成的新表位 (neoepitope) 特异性结合。怎样产生抗体的方法是本领域熟知的并且例如包括，根据已知的方案免疫活动物或在细胞培养系统中产生单克隆抗体或多克隆抗体 (Sambrook 等人，1989，1989，Molecular Cloning:A Laboratory Manual)。优选地，抗免疫球蛋白抗体在小鼠、大鼠、兔、猪、牛、驴、山羊、绵羊等中或在卵中产生。然而，本发明还考虑在转基因植物中产生抗免疫球蛋白抗体。优选地，抗免疫球蛋白抗体是 IgM，最优选地是单克隆 IgM。更优选地，抗免疫球蛋白抗体是抗（聚集的人 IgG)IgM，即对人 IgG 具有如本文以上所述的低亲和力和高亲合力

的 IgM，例如，如 WO 2008/135274 中所述 <h-agg. IgG>IgM，最优选地，抗人免疫球蛋白抗体 是 MAb<h-Agg. -IgG>M-3.022.5-IgM(DSM ACC2873)、MAb<h-Agg. -IgG>M-1.010.2-IgM 和 MAb<h-Agg. -IgG>M-1.1.7-IgM(表 1 中 显 示)，MAb<h-Agg. -IgG>M-3.022.5-IgM(DSM ACC2873) 是最优选的。

[0025] 优选地，抗免疫球蛋白抗体携带标签。如本文所用的术语“标签”指能够产生可检测信号的任何物质。优选地，标签是色原体、荧光、化学发光或电化学发光化合物、催化剂、酶、酶底物、染料、胶态金属或非金属粒子或有机聚合物颗粒等。

[0026] 如本文所用，术语“受试者”指哺乳动物，并且优选地指人。受试者优选地患有 HBV 感染。更优选地，受试者患有慢性 HBV 感染。另外，受试者优选地具有未知的 HBV e 抗原状态。

[0027] 术语“样品”指体液样品，指来自组织或器官的样品，或指从外体表或内体表获得的洗液 / 淋洗 (wash/rinse) 液样品。样品优选地包含多肽，更优选地是 HBV 抗原，最优选地是 HBsAg。本发明的方法涵盖血、血浆、血清、尿、唾液或泪液的样品。这类样品可以通过使用刷、(棉) 药签、刮铲 (spatula)、淋洗液 / 洗液、钻取活组织检查装置、用针和外科器械穿刺腔体 (cavity) 获得。然而，作为本发明的样品，还包括通过熟知技术获得的样品，所述样品优选地包括来自泌尿生殖道、肛周区、肛管、口腔、上呼吸消化道和表皮的刮擦、药签或活组织检查样品。无细胞流体可以通过分离技术如过滤或离心从体液或组织或器官获得。优选地，样品从已知在被 HBV 感染的受试者中包含 HBV 多肽的体液 (即，优选地，血、血清、唾液等) 获得。应当理解可以进一步加工样品以实施本发明的方法。特别地，可能通过本领域已知的方法和工具从获得的样品移除细胞。另外，可通过本领域已知的方法和工具从获得的样品提取和 / 或纯化 HBV 免疫复合物。因而，术语样品还可以指从如上文提到的任何样品纯化和 / 或提取的 HBV 免疫复合物。

[0028] 术语“确定”指对样品中存在的 HBV 免疫复合物的量定量，即测量所述 HBV 免疫复合物的量或浓度，优选地半定量或定量地测量。可以直接或间接地进行测量。确定免疫复合物的量可以按照技术人员已知的多种方式，例如凝胶过滤层析，随后蛋白质印迹法、免疫共沉淀法等完成。优选地，通过在下文实施例中描述的方法确定 HBV 免疫复合物的量。有利地，发现在实施例中使用的夹心 -ELISA 允许可靠地对 HBV 免疫复合物定量。另外，发现使用聚集的抗体，例如聚集的 IgG，作为抗人 Ig 抗体，导致高度改善的信噪比。

[0029] 根据本发明，可以通过用于确定样品中多肽或肽的量的全部已知手段实现确定 HBV 免疫复合物的量，只要其适用于特异性检测本发明的 HBV 免疫复合物。优选地，将使用与 HBV 免疫复合物特异性结合、从而可对其进行检测的检测剂。检测剂优选地涵盖与所述复合物特异性结合的抗体或其片段、与所述复合物特异性结合的适配体 (aptamer)、抗运载蛋白 (Anticalin) 或设计的锚蛋白重复序列蛋白 (DARPin)。优选地，适用双特异性免疫测定法，即如下测定法，其中信号的存在或强度将取决于包含于 HBV 免疫复合物中的两种分子 (即 HBV 多肽和抗 HBV 免疫球蛋白) 的存在。所述工具包括可以按各种夹心、竞争或其他分析模式利用标记分子的免疫测定法装置和方法。所述测定法将形成指示 HBV 免疫复合物存在或不存在的信号。另外，信号强度可以优选地直接或间接地相关于 (例如反比于) 样品中存在的 HBV 免疫复合物的量。所述方法优选地包括生物传感器、与免疫测定法偶联的光学装置、生物芯片、分析装置如质谱仪、NMR 分析仪或色谱装置。另外，方法包括基于微量平

板 ELISA 的方法、全自动化或机器人免疫测定法（例如在多参数生物芯片平台或 Elecsys™ 分析仪上可用）、CBA（酶促钴结合测定法，例如在 Roche-Hitachi™ 分析仪上可用）和乳胶凝集测定法（例如在 Roche-Hitachi™ 分析仪上可用）。

[0030] 如本文所用的术语“量”涵盖本文中提及的 HBV 免疫复合物的绝对量、本文中提及的 HBV 免疫复合物的相对量或浓度，以及与之相关的任何值或参数。这些值或参数包括通过测量从本文中提及的 HBV 免疫复合物获得的来自所有特定物理或化学特性的强度信号值，例如，响应于本文中提及的多肽从生物读出系统确定的表达水平，或从特异性结合的配体获得的强度信号。应当理解也可以通过所有标准数学操作获得与前述量或参数相关的值。

[0031] 如本文所用的“比较”涵盖将待分析样品所包含的本文提及的 HBV 免疫复合物的量与本说明书中他处所述的合适参比样品中的所述 HBV 免疫复合物的量比较。还涵盖将样品中 HBV 免疫复合物的量与 HBV 抗原、优选地 HBsAg 的量的比率与合适的参考比率比较。应当理解如本文所用的比较，指比较相应的参数或值，例如，将如本文提到的 HBV 免疫复合物绝对量与所述 HBV 免疫复合物的绝对参比量比较；将如本文提到的 HBV 免疫复合物的浓度与所述 HBV 免疫复合物的参比浓度比较；将测试样品中从如本文提到的 HBV 免疫复合物获得的强度信号与参比样品中所述 HBV 免疫复合物的相同类型的强度信号比较；或将 HBV 免疫复合物的量对如本文提到的 HBV 抗原的量的比率与相应的参考比率比较。在本发明的方法中提及的比较可以人工地或在计算机辅助下实施。对于计算机辅助比较，测定的量或比率的值可以与对应于由计算机程序在数据库中存储的合适参比的值比较。该计算机程序还可以借助专家系统评价该比较的结果。因此，本文中提及的鉴定的结果可以按合适的输出格式自动提供。

[0032] 如本文所用，术语“参考值”指 HBV 免疫复合物的量，所述量允许评估样品所来源的受试者应对干扰素治疗敏感或对干扰素治疗不敏感。合适的参考值可以从待分析的参比样品连同样品一起（即同时或相继）确定。

[0033] 原则上，可以基于给定的 HBV 免疫复合物的平均值或均值 (average or mean)，通过采用标准统计学方法，对如本文所述的受试者组或群组计算参考量。具体而言，一种检验法验如旨在诊断某事件存在与否的方法的准确度由其接受者操作特征 (ROC) 最佳描述（特别见 Zweig 1993, Clin. Chem. 39:561-577）。ROC 曲线是因在观察到的整个数据范围内连续变动决策阈值所产生的全部灵敏度与特异性成对值的曲线。诊断方法的临床表现取决于其准确度，即其将受试者正确划至某种预后或诊断的能力。通过在适于进行区分的完整阈值区间将灵敏度对 1- 特异性作图，ROC 曲线指示两种分布之间的重叠。在 y- 轴上是灵敏度或真阳性部分，其定义为真阳性试验结果数对真阳性数和假阴性试验结果数的乘积的比率。在疾病或病症存在的情况下，这又称作阳性 (positivity)。它完全从患病的亚组计算。在 x- 轴上是假阳性部分或 1- 特异性，其定义为假阳性试结果数对真阴性数和假阳性结果数的乘积的比率。它是特异性的指标并且完全从未患病的亚组计算。因为完全通过使用来自两个不同亚组的试验结果分别计算真阳性分数和假阳性分数，所以 ROC 曲线与群组中事件的患病率无关。ROC 曲线上的每个点表示与特定决策阈值相对应的灵敏度 /- 特异性配对物。完美区分（结果的两个分布中无重叠）的检验具有穿过左上角的 ROC 曲线，其中真阳性分数是 1.0 或 100%（完美灵敏度），假阳性分数是 0（完美特异性）。无区分（两个组的

结果的相同分布）的检验的理论曲线是从左下角至右上角的 45° 对角线。大部分曲线落在这两种极端情况之间。如果 ROC 曲线完全落在 45° 对角线以下，则通过将“阳性”标准从“大于”逆转至“小于”或相反操作容易地纠正这种情况。定性地，曲线越靠近左上角，则检验的总准确度越高。取决于所需的置信区间，可以从 ROC 曲线导出阈值，所述阈值允许以灵敏度和特异性的适宜平衡分别诊断或预测给定事件。因此，可以优选地通过如上文所述那样建立所述群组的 ROC 并从中导出阈值量，产生针对本发明方法所用的参考。取决于诊断方法的所需灵敏度和特异性，ROC 曲线允许导出合适的阈值。

[0034] 优选地，如本文所用的参考量从治疗之前的受试者样品获得，但是对所述样品，已知它们的供体是否响应治疗。该参考量水平可以是离散的数值或可以是数值范围。显然，参考水平或量可以在 HBV 免疫复合物的个体种类之间变动。因此，测量系统优选地用一份样品或用一系列样品校准，所述样品包含已知量的 HBV 免疫复合物或多种 HBV 免疫复合物。更优选地，该系统用一系列混合物校准，所述混合物包含限定体积的仅含 HBsAg 的血清，即，包含大量的 HBsAg 但不含抗 HBV 免疫球蛋白或 HBV 免疫复合物的血清，和仅含抗 HBsAg 免疫球蛋白的血清，例如，包含高滴度抗 HBsAG IgG 但不包含 HBsAg 或 HBV 免疫复合物的血清。技术人员理解在这种情况下 HBV 免疫复合物的量将优选地表述为任意单位 (AU)。因此，优选地通过将从样品获得的信号与包含于校正曲线中的信号比较，确定 HBV 免疫复合物或多种 HBV 免疫复合物的量。

[0035] 适用于个体受试者的参考量可以根据各种生理学参数如年龄、性别或亚群变动。因此，合适的参考值可以通过本发明的方法，从待分析的参考样品连同测试样品一起（即同时或相继）确定。另外，可以优选地使用阈值量作为参考量。优选地，高于阈值量的 HBV 免疫复合物的量表示轻度的 HBV 感染；并且等于或低于阈值量的 HBV 免疫复合物的量表示重度的 HBV 感染。应当理解前述的量可以因统计学和测量误差变动。

[0036] 在本发明方法的一个实施方案中，已经发现增加的 HBV 免疫复合物的量优选地表示受试者对干扰素治疗敏感，而减少的 HBV 免疫复合物的量表示受试者对干扰素治疗不敏感。在这种情况下，参考量优选地是这些量，所述量是在治疗给定受试者群体或群组之前存在于患有 HBV 的受试者中的平均量或均数量 (average or mean amount)。本文中提及的 HBV 免疫复合物量的减少或增加优选地是统计显著的减少或增加。

[0037] 参考量可以优选地从已知对干扰素治疗敏感的患有 HBV 的受试者或受试者组的样品导出。在这种情况下，确定与该参考量相比实质上相同或增加的 HBV 免疫复合物的量应当表示受试者对干扰素治疗敏感。减少的量应当表示对干扰素治疗不敏感的受试者。

[0038] 参考量还可以优选地从已知对干扰素治疗不敏感的患有 HBV 的受试者或受试者组的样品导出。在这种情况下，确定与该参考量相比实质上相同或减少的 HBV 免疫复合物的量应当表示受试者对干扰素治疗不敏感。增加的量应当表示对干扰素治疗敏感的受试者。

[0039] 有利地，发现确定来自受试者的样品中 HBV 免疫复合物的量允许鉴定将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对标准干扰素治疗敏感。如本文在实施例中详述的，优选地，增加的 HBV 免疫复合物的量表示受试者对干扰素治疗敏感，而减少的 HBV 免疫复合物的量表示受试者对干扰素治疗不敏感。优选地，这意味具有大量 HBV 免疫复合物的受试者具有响应于标准干扰素治疗的高概率，并且意味具有少量 HBV 免疫复合物的受试者具有

响应于标准干扰素治疗的低概率。因此可以在疗法启动之前使用本发明的方法决定受试者是否应当用标准干扰素疗法治疗或该受试者是否应当优选地接受改良疗法。还有利地，发现知晓受试者的 HBeAg 状态并且因此确定其 HBeAg 状态与本发明的用于正确地鉴定对干扰素治疗敏感的受试者的方法无关。

[0040] 上文作出的定义在已作必要修正时适用于以下情况：

[0041] 本发明还构思一种通过干扰素疗法治疗患有 HBV 感染的受试者的方法，所述方法包括将受试者鉴定为对干扰素治疗敏感，优选地，通过本发明的前述方法鉴定，并且向被鉴定为干扰素治疗对敏感的受试者施用治疗有效量的如本文中他处所述的干扰素治疗。

[0042] 本发明还涉及一种用于在患有 HBV 感染的受试者中区分轻度 HBV 感染和重度 HBV 感染的方法，所述方法包括步骤：

[0043] a) 确定所述受试者的样品中 HBV 免疫复合物的量，

[0044] b) 将步骤 a) 中获得的 HBV 免疫复合物的量与参考值比较；并且

[0045] c) 基于步骤 b) 中比较的结果区分轻度 HBV 感染和严重 HBV 感染。

[0046] 在这种情况下，重度的 HBV 感染以如本文中他处所述的减少的 HBV 免疫复合物的量为特征，而轻度的 HBV 感染以增加的 HBV 免疫复合物的量为特征。在这种情况下，参考量优选地是这些量，所述量是在治疗给定受试者群体或群组之前存在于患有 HBV 的受试者中的平均量或均数量。

[0047] “轻度的 HBV 感染”优选地是一种可以通过如本文中他处所述的干扰素疗法治疗的形式，而“重度”优选地是不能通过干扰素疗法治疗的慢性 HBV 感染。

[0048] 本发明也涉及来自患有 HBV 感染的受试者的样品中 HBV 免疫复合物的量或用于这种样品中 HBV 免疫复合物的检测剂用于将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感的用途。

[0049] 本文中他处详细描述了可以用于确定样品中存在的 HBV 复合物的合适检测剂。

[0050] 另外，本发明还涉及一种用于将患有乙型肝炎病毒 (HBV) 感染的受试者鉴定为对干扰素治疗敏感的装置，包括用于确定 HBV 免疫复合物的量的分析单元，和用于将所述量与参考量比较以及用于将患有 HBV 感染的受试者鉴定为对干扰素治疗敏感的评价装置。

[0051] 如本文所用的术语“装置”指一种工具系统，所述系统至少包括彼此有效关联以允许区分的前述工具。上文与本发明方法相关地公开了用于确定所述 HBV 免疫复合物的量的优选工具和用于实施比较的工具。怎样以运行方式连接工具将取决于纳入装置中的工具的类型。例如，在使用自动确定 HBV 免疫复合物的量的工具情况下，由所述自动运行工具获得的数据可以由例如计算机程序处理，以建立诊断（即鉴定对敏感干扰素治疗的受试者）。在这种情况下，优选地，所述工具包含在单一装置中。所述装置可以因此包括用于测量样品中 HBV 免疫复合物的量的分析单元和用于处理所得数据以便诊断的评价装置。备选地，在工具如测试条 (test stripe) 用于确定 HBV 免疫复合物的量情况下，用于诊断的工具可以包括对照条或对照表，所述对照纸条或对照表将测定的量划至已知伴随响应于标准干扰素治疗或伴随不响应于干扰素治疗的量。用于检测的优选工具与涉及本发明以上方法的实施方案相联系地公开。在这种情况下，工具是有效关联的，在于该系统的用户由手册中给出的说明和阐释将确定该量的结果和其诊断值联系到一起。该工具在这种实施方案中可以作为独立的装置出现并且优选地包装在一起作为试剂盒。本领域技术人员将无需进行其他创造

性工作即可了解如何关联该工具。优选的设备是可以在不具备专业临床医生的特定知识的情况下应用的那些，例如，仅需要加载样品的测试条或电子装置。结果可以作为参量诊断原始数据的输出，优选地，作为绝对量或相对量给出。应当理解这些数据将需要由临床医生解释。但是，还构思了专家系统装置，其中输出包含不需要专业临床医生解释的处理过的诊断原始数据。其他优选的装置包括上文根据本发明方法所提及的分析单元 / 装置（例如，生物传感器、阵列、与特异性识别多肽的配体连接的固相支持物、等离子体表面共振装置、NMR 波谱仪、质谱仪等）或评价单元 / 装置。

[0052] 本发明构思一种试剂盒，其包含实施本发明方法的说明书、用于确定 HBV 免疫复合物的量的检测剂，和优选地允许将患有 HBV 感染的受试者鉴定为对标准干扰素治疗敏感的参考量的标准。

[0053] 如本文所用，术语“试剂盒”指前述组分的综合，所述组分优选地分别或在单一容器内部提供。该容器还优选地包括用于实施本发明方法的说明书。已经在本说明书中给出试剂盒的这类组分的例子以及它们的使用方法。试剂盒优选地含有即用型制剂形式的前述组分。优选地，试剂盒可以额外地包括说明书，例如，相对于本发明方法提供的诊断，解读任何测定结果的用户手册。特别地，这类手册可以包括将所确定 HBV 免疫复合物的量划分至诊断类型的信息。在本说明书中他处将找到详细内容。另外，这类用户手册可以提供关于正确使用试剂盒组分以确定相应生物标记的量的说明。用户手册可以按纸质形式或电子形式提供，例如存储在 CD 或 CDROM 上。本发明还涉及所述试剂盒在本发明的任何方法中的用途。

[0054] 将本说明书中援引的全部参考文献在此就其完整公开内容和本说明书中具体提到的公开内容而言均通过引用的方式并入。

[0055] 附图

[0056] 图 1 :HBV 免疫复合物检测原理 ;抗 HBsAg 捕获抗体（生物素 aHBS1，生物素 aHBS2）与固体表面结合。将 HBV 免疫复合物（包含 HBsAg 和抗 HBsAG IgG）捕获至该表面并用单克隆 IgM 检测，所述单克隆 IgM 具有低亲和力，但对与地高辛配基 (Digoxigenin) 缀合的人 IgG 具有高亲合力（抗 < 聚集的人 IgG>IgM- 地高辛配基）。

[0057] 图 2 :研究设计的示意图。

[0058] 图 3 :HBV 免疫复合物的量与不根据 HBeAg 状态区分的群组中针对标准治疗的反应 ;A) 和 B) :在第 0 周（即在启动治疗之前，A) 和在治疗第 12 周 (B)HBsAg 在来自响应于 (R) 或不响应于 (NR) 标准治疗的患者的血清样品中的浓度。反应个体和非反应个体之间的差异不是统计显著的。C) 和 D) :在第 0 周 (C) 和在治疗第 12 周 (D)HBsAg/ 抗 HBsAG IgG 复合物在来自响应于 (R) 或不响应于 (NR) 标准治疗的患者的血清样品中的浓度。在第 0 周 ($p = 0.0093$) 和在第 12 周 ($P = 0.0179$)，反应个体和非反应个体之间的差异是统计显著的。

[0059] 图 4 :图 3 中获得的数据的 ROC 曲线。

实施例

[0060] 以下实施例应当仅说明本发明。它们应当不被解释为限制本发明的范围。

[0061] 实施例 1

[0062] 产生具有类风湿性因子样特异性的单克隆小鼠 IgM 抗体

[0063] 免疫原 :H-IgG 聚合物 :

[0064] 将 10mg 人 IgG1(Sigma 公司) 溶解于 0.6ml 25mM 碳酸氢盐缓冲液 pH 9.5 中。在添加 3.5μl 12.5% 戊二醛溶液后,将它在室温温育 2 小时。随后,将它在冰浴中冷却,用 50mM 三乙醇胺溶液 pH 8.0 调节至 pH 8.3 并且添加 0.15ml 新鲜制备的硼氢化钠溶液 (8mg 硼氢化物 /ml 水)。在 0℃ 2.5 小时后,将制备物在 4℃针对 10mM 磷酸钾缓冲液 /0.2M NaCl, pH 7.5 透析 16 小时。将含有 IgG 聚合物的透析液以等分试样贮存在 80℃或用于免疫和用于杂交瘤细胞的培养上清液中的特异性检验。从人 IgG3(Sigma Company) 开始,以相似方式产生 H-IgG3 聚合物。

[0065] 小鼠的免疫 :

[0066] 将 12 周龄雌性 BALB/c 小鼠用 100μg H-IgG1 或 IgG3 聚合物连同佐剂 CFA(弗氏完全佐剂) 一起腹膜内首次免疫。在 8 日后,用 CFA 中 100μg 相应的 IgG 聚合物实施进一步免疫。在初始免疫后 13 日,在没有佐剂的情况下腹膜内施用 200μg 相应的聚合物,在初始免疫后 14 日和 15 日,在每种情况下腹膜内和静脉内施用 100μg。在 16 日后实施融合。

[0067] 杂交瘤克隆的产生 :

[0068] 融合和克隆 :

[0069] 遵循 Galfré, G., Methods in Enzymology 73(1981)3-46 中的方法,使免疫小鼠的脾细胞与骨髓瘤细胞融合。免疫小鼠的大约 $1x10^8$ 个脾细胞与 $2x10^7$ 个骨髓瘤细胞 (P3X63-Ag8-653, ATCC CRL 1580) 混合并离心 (以 300g 和 4℃持续 10 分钟)。

[0070] 随后将细胞用不含胎牛血清 (FCS) 的 RPMI-1640 培养基洗涤 1 次并在 50ml 锥形管中以 400g 再次离心。添加 1ml PEG(聚乙二醇)(分子量 4000, MERCK, Darmstadt) 并通过抽吸混合。在 37℃于水浴中 1 分钟后,逐滴添加不含 FCS 的 5ml RPMI 1640,混合,用培养基 (RPMI1640+10% FCS) 补充直至 50ml 并随后离心。使沉降的细胞悬于含有 10% FCS 的 RPMI 1640 培养基中并且接种在次黄嘌呤 - 偶氮丝氨酸选择培养基中 (RPMI1640+10% FCS 中的 100mmol/l 次黄嘌呤,1μg/ml 偶氮丝氨酸)。添加白介素 6(100U/mL) 至培养基作为生长因子。在约 10 日后,对原代培养物测试特异性抗体合成。在 96 孔细胞培养平板中借助荧光激活的细胞分选仪克隆了显示与聚集的人 IgG1 的阳性反应,但与单体 IgG 无交叉反应的原代培养物。添加白介素 6(100U/mL) 至培养基作为生长添加物。

[0071] 以这种方式获得以下杂交瘤克隆 :

[0072] 表 1 :

[0073]

单克隆抗体名称	免疫原	亚类特异性聚合物
MAb<h-Agg.-IgG>M-3.022.5-IgM	h-IgG1 聚合物	IgG1>IgG3>IgG4>IgG2
MAb<h-Agg.-IgG>M-1.010.2-IgM	h-IgG1 聚合物	IgG1>IgG3>IgG4>IgG2
MAb<h-Agg.-IgG>M-1.1.7-IgM	h-IgG3 聚合物	IgG1>IgG3>IgG2>IgG4

[0074] 对聚集的人 IgG 具有特异性的单克隆抗体的筛选试验。

[0075] 链霉亲和素包被的微量滴定平板 (MTPs) 用生物素化的人 IgG1 或 IgG3 包被。此

后将它们与细胞培养上清液中的单克隆抗体温育。随后，使用抗＜小鼠-IgM＞-过氧化物酶(POD)通过与POD底物反应，以惯常方式检测结合的抗体。

[0076] 使用与固相结合的人IgG确定亚类特异性：

[0077] 为了确定杂交瘤细胞的培养上清液中抗体的特异性，用重组链霉亲和素包被的MTP(MicroCoat Company，订购号12-K96N)用温育缓冲液中1μg/ml亚类1或2或3或4的生物素化h-IgG(＝h-IgG-Bi)包被。由于通过生物素与固相结合的IgG的行为类似聚集的聚合物IgG，所以这个实验方案可以用来确定亚类特异性。为此，将每孔100μl h-IgG-Bi溶液在室温伴随振摇温育60分钟，并且随后用0.9% NaCl/0.05%**Tween®20**洗涤3次。在下一个步骤中，将待检验的100μl抗体溶液(培养上清液)添加至包被的板孔并且在室温伴以振摇温育1小时。用0.9%氯化钠/0.05%**Tween®20**洗涤3次后，在每种情况下添加100μl POD-标记的来自山羊抗小鼠IgM的多克隆抗体Fab片段(Dianova Company，订购号115-036-075，使用的浓度为0.16μg/ml温育缓冲液)以检测来自样品的结合抗体，在室温伴以振摇温育1小时，并且随后用0.9%氯化钠/0.05%**Tween®20**洗涤3次。最后，添加100μl/孔**ABTS®**底物(Roche Diagnostics GmbH，订购号1684302)，并且在室温30分钟后在来自Dynatech公司的MR700微量平板读数仪中测量405/492nm处的吸光度。

[0078] 温育缓冲液：40mM磷酸钠，pH 7.4，200mM酒石酸钠，0.1%**Tween®20**，0.2%牛血清白蛋白。

[0079] 确定与单体人IgG1的反应性/交叉反应：

[0080] 为了确定与未聚集的单体H-IgG1的反应性/交叉反应，将待检验的单克隆抗体在上文描述的试验中与渐增浓度或过量的非聚集的单体IgG1预温育。如果测量的信号保持不变处于高水平，则不存在交叉反应。如果测量的信号降低，则交叉反应已经出现。

[0081] 为此，将用重组链霉亲和素包被的微量滴定平板(MTP)(MicroCoat Company，订购号12-K96N)以温育缓冲液中的1μg/ml生物素化HIgG1(＝H-IgG1-Bi)包被。每孔使用100μl H-IgG1-Bi溶液并且将其在室温伴随振摇温育60分钟，并随后用0.9% NaCl/0.05%**Tween®20**洗涤3次。将待测试交叉反应的单克隆抗体与系列浓度直至1μg/ml的未聚集的单聚IgG1预温育。预温育在未包被的96孔MTP中在室温1小时进行，同时振摇。

[0082] 在下一个步骤中，将100μl这种溶液(抗体+过量的未聚集的单聚IgG1)添加至包被的板孔并且在室温伴以振摇温育1小时。用0.9%氯化钠/0.05%**Tween®20**洗涤3次后，在每种情况下添加100μl POD-标记的来自山羊抗小鼠IgM的多克隆抗体Fab片段(Dianova Company，订购号115-036-075，使用的浓度为0.16μg/ml温育缓冲液)以检测来自样品的结合抗体，在室温伴以振摇温育1小时，并且随后用0.9%氯化钠/0.05%**Tween®20**洗涤3次。

[0083] 最后，添加100μl/孔**ABTS®**底物(Roche Diagnostics GmbH，订购号1684302)，并且在室温30分钟后在来自Dynatech公司的MR700微量平板读数仪中测量405/492nm处

的吸光度。在本发明意义下适合的具有类风湿性因子样结合单克隆抗体识别全部人 IgG 亚类并且在竞争试验中显示出与单聚 h-IgG 交叉反应小于 10%。如果 HIgG1 聚合物用来确定反应性，则测量的信号大幅度降低。表 1 显示单克隆抗体的主要特性。

[0084] 发酵杂交瘤克隆以分离单克隆抗体：

[0085] 将获得的杂交瘤细胞以 $1x10^5$ 个细胞 /ml 的密度接种在含有 10% FCS 的 RPMI 1640 培养基中并在发酵器 (Thermodux Company,Wertheim/_Main,型号 MCS-104XL,订购号 144-050) 中增殖 7 日。培养上清液中达到 100μg 单克隆抗体 /ml 的平均浓度。

[0086] 单克隆 MAb<h-Agg.-IgG>M-3.022.5-IgM 的分离：

[0087] 将 5mg MAb<h-Agg.-IgG>M-3.022.5-IgM(DSM ACC2873) 用 0.1M 磷酸钠缓冲液，pH 8.6 调节至总体积 2ml。将 50μl 1.11mM 地高辛配基 -3-O- 甲基 - 羰基 - ε - 氨基己酸 -N- 羟基琥珀酰亚胺酯在二甲基亚砜中的溶液添加至这种溶液并随后在 25℃搅拌 60 分钟。IgM 对活化型地高辛配基的比率是 1:10。将形成的 IgM- 地高辛配基针对 20mM 磷酸钾缓冲液 /0.1M NaCl/3% 蔗糖，pH 7.5 透析。透析的 IgM-Dig 以等分试样贮藏在 -80℃。

[0088] 实施例 2

[0089] 多参数生物芯片平台上的全自动免疫测定法 (IMPACT)

[0090] 在 Hornauer,H 等人，BIOspectrum,Special Proteomics 10(2004)564-565 和 Hornauer,H. 等人，Laborwelt 4(2004)38-39 中描述了多参数生物芯片平台。为了确定复合物水平，使用基于阵列的测定法 (IMPACT - 免疫学多参数芯片技术 (Immunological Multi-Parameter Chip Technology),Roche Diagnostics)。

[0091] 将链霉亲和素涂层施加在黑色着染的聚苯乙烯支持物（固相）上约 2.5x6mm 的测试区的整个区域范围内。将成列的由治疗性抗体的生物素化片段组成的每列大约 10 至 20 个的相同点以喷墨方式施加至测试区域，每个点的直径约 150μm。

[0092] 使用以下试验特异性试剂引物：

[0093] 样品稀释缓冲液和检测抗体缓冲液：

[0094] 50mM Tris，pH 6.6；30mM MES；50mM NaCl；0.1 % 去垢剂（聚多卡醇 (polydocanol)）；5mM EDTA；0.5% 酪蛋白；0.2% 防腐剂（醋氧甲苯酸 (oxypyrion) 和盐酸甲基异噻唑啉酮 (methylisothiazolone hydrochloride,MIT)）

[0095] 如果样品显示极高浓度的 HBV- 免疫复合物并且结果不在测量范围内，则使用 Elecsys Diluent MultiAssay（标识号 03609987）进行样品的额外手工稀释。样品以 10 倍进阶（例如 1:10、1:100、1:1000）进一步稀释。

[0096] 洗涤缓冲液：10mM Tris,0.01% 聚多卡醇,0.001% 醋氧甲苯酸,0.001% MIT

[0097] 样品：来自用聚乙二醇化干扰素 α-2(Peginterferon alpha-2a,PEGASYS) 和 Adefovir（图 2,表 2）治疗之前和期间的慢性 HBV 患者的人血清。

[0098] 使用与 HBs 抗原特异性结合的两种不同的生物素化抗体作为生物素化的捕获抗体。HBs 抗原和抗 HBs 抗体的免疫复合物与固相结合的捕获抗体结合。通过对人聚集的 IgG 特异的单克隆 IgM 抗体 (MAb<h-Agg.-IgG>M-3.022.5-IgM) 检测免疫复合物（图 1）。

[0099] 将样品用样品稀释缓冲液 1:5 稀释用于测量。将稀释的样品在 37℃温育 12 分钟。在抽出样品后并用洗涤缓冲液洗涤测试区域后，与 MAb<h-Agg.-IgG>M-3.022.5-IgM(DSM ACC2873)（一种用地高辛标记的抗体 (Dig 标记的单克隆抗体 <h-Agg.-IgG>) 在 37℃温

育 6 分钟，接着是一个洗涤步骤。与荧光标记的 <Dig> 抗体在 37℃温育 3 分钟并随后洗涤及吸干测试区域后，信号由 CCD 照相机检测（图 3）。可以用合适的复合物 - 校准物计算浓度。

[0100] 表 2 :研究群体

[0101]

HBeAg 阳性 (n=29)			HBeAG 阴性 (n=21)	
HBsAg 血清转换	HBeAg 血清转换	非反应个体	HBsAg 血清转换	非反应个体
n=4	n=10	n=15	n=5	n=16

[0102] 表 3 :ROC 分析的统计值（图 4）

[0103]

检验	auc	std	LCL	UCL
复合物 (HBsAg/ 抗 HBsAG IgG)	0.7258	0.0768	0.5753	0.8764
HBsAg	0.5054	0.0920	0.3250	0.6858
HBsAg/ 复合物比率	0.7093	0.0776	0.5572	0.8613

[0001]

序列表

<110> Roche Diagnostics GmbH

<120> 用于慢性 HBV 患者的反应预测和疗法监测的 HBV 免疫复合物

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<160> 3

<170> PatentIn version 3.5

<210> 1
<211> 400
<212> PRT
<213> 乙型肝炎病毒

<400> 1

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1 5 10 15

Ser Val Pro Asn Pro Leu Gly Phe Phe Pro Asp His Gln Leu Asp Pro
20 25 30

Ala Phe Gly Ala Asn Ser Asn Asn Pro Asp Trp Asp Phe Asn Pro Asn
35 40 45

Lys Asp His Trp Pro Glu Ala Asn Gln Val Gly Ala Gly Ala Phe Gly
50 55 60

Pro Gly Phe Thr Pro Pro His Gly Gly Leu Leu Gly Trp Ser Pro Gln
65 70 75 80

Ala Gln Gly Ile Leu Thr Thr Val Pro Val Ala Pro Pro Pro Ala Ser
85 90 95

[0002]

Thr Asn Arg Gln Ser Gly Arg Gln Pro Thr Pro Ile Ser Pro Pro Leu
100 105 110

Arg Asp Ser His Pro Gln Ala Met Gln Trp Asn Ser Thr Thr Phe His
115 120 125

Gln Ala Leu Leu Asp Pro Arg Val Arg Gly Leu Tyr Phe Pro Ala Gly
130 135 140

Gly Ser Ser Ser Gly Thr Val Asn Pro Val Pro Thr Thr Ala Ser Pro
145 150 155 160

Ile Ser Ser Ile Phe Ser Arg Thr Gly Asp Pro Ala Pro Asn Met Glu
165 170 175

Ser Thr Thr Ser Gly Phe Leu Gly Pro Leu Leu Val Leu Gln Ala Gly
180 185 190

Phe Phe Leu Leu Thr Arg Ile Leu Thr Ile Pro Gln Ser Leu Asp Ser
195 200 205

Trp Trp Thr Ser Leu Asn Phe Leu Gly Gly Ala Pro Thr Cys Pro Gly
210 215 220

Gln Asn Ser Gln Ser Pro Thr Ser Asn His Ser Pro Thr Ser Cys Pro
225 230 235 240

Pro Ile Cys Pro Gly Tyr Arg Trp Met Cys Leu Arg Arg Phe Ile Ile
245 250 255

Phe Leu Phe Ile Leu Leu Leu Cys Leu Ile Phe Leu Leu Val Leu Leu
260 265 270

[0003]

Asp Tyr Gln Gly Met Leu Pro Val Cys Pro Leu Leu Pro Gly Thr Ser
275 280 285

Thr Thr Ser Thr Gly Pro Cys Lys Thr Cys Thr Ser Pro Ala Gln Gly
290 295 300

Thr Ser Met Phe Pro Ser Cys Cys Cys Thr Lys Pro Ser Asp Gly Asn
305 310 315 320

Cys Thr Cys Ile Pro Ile Pro Ser Ser Trp Ala Phe Ala Arg Phe Leu
325 330 335

Trp Glu Trp Ala Ser Val Arg Phe Ser Trp Leu Ser Leu Leu Val Pro
340 345 350

Phe Val Gln Trp Phe Val Gly Leu Ser Pro Thr Val Trp Leu Ser Val
355 360 365

Ile Trp Met Met Trp Tyr Trp Gly Pro Cys Leu Tyr Asn Ile Leu Ser
370 375 380

Pro Phe Leu Pro Leu Leu Pro Ile Phe Phe Cys Leu Trp Val Tyr Ile
385 390 395 400

<210> 2
<211> 212
<212> PRT
<213> 乙型肝炎病毒

<400> 2

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Ser Cys Pro Thr
1 5 10 15

Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile

[0004]

20 25 30

Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu Ser Phe Leu
35 40 45

Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp Thr Ala Ser
50 55 60

Ala Leu Tyr Arg Asp Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65 70 75 80

His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu Met Thr
85 90 95

Leu Ala Thr Trp Val Gly Val Asn Leu Glu Asp Pro Ala Ser Arg Asp
100 105 110

Leu Val Val Ser Tyr Val Asn Thr Asn Met Gly Leu Lys Phe Arg Gln
115 120 125

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
130 135 140

Ile Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145 150 155 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
165 170 175

Val Val Arg Arg Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro Ser Pro
180 185 190

Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln Ser Arg

[0005]

195 200 205

Glu Ser Gln Cys
210

<210> 3
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<212> PRT
<213> 乙型肝炎病毒

<400> 3

Met Gln Leu Phe His Leu Cys Leu Ile Ile Ser Cys Thr Cys Pro Thr
1 5 10 15

Val Gln Ala Ser Lys Leu Cys Leu Gly Trp Leu Trp Gly Met Asp Ile
20 25 30

Asp Pro Tyr Lys Glu Phe Gly Ala Thr Val Glu Leu Leu Ser Phe Leu
35 40 45

Pro Ser Asp Phe Phe Pro Ser Val Arg Asp Leu Leu Asp Thr Ala Ser
50 55 60

Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys Ser Pro His
65 70 75 80

His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu Leu Met Thr
85 90 95

Leu Ala Thr Trp Val Gly Asn Asn Leu Glu Asp Pro Ala Ser Arg Asp
100 105 110

Leu Val Val Asn Tyr Val Asn Thr Asn Met Gly Leu Lys Ile Arg Gln
115 120 125

[0006]

Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg Glu Thr Val
130 135 140

Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr Pro Pro Ala
145 150 155 160

Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro Glu Thr Thr
165 170 175

Val Val Arg Arg Arg Asp Arg Gly Arg Ser Pro Arg Arg Arg Thr Pro
180 185 190

Ser Pro Arg Arg Arg Arg Ser Gln Ser Pro Arg Arg Arg Arg Ser Gln
195 200 205

Ser Arg Glu Ser Gln Cys
210

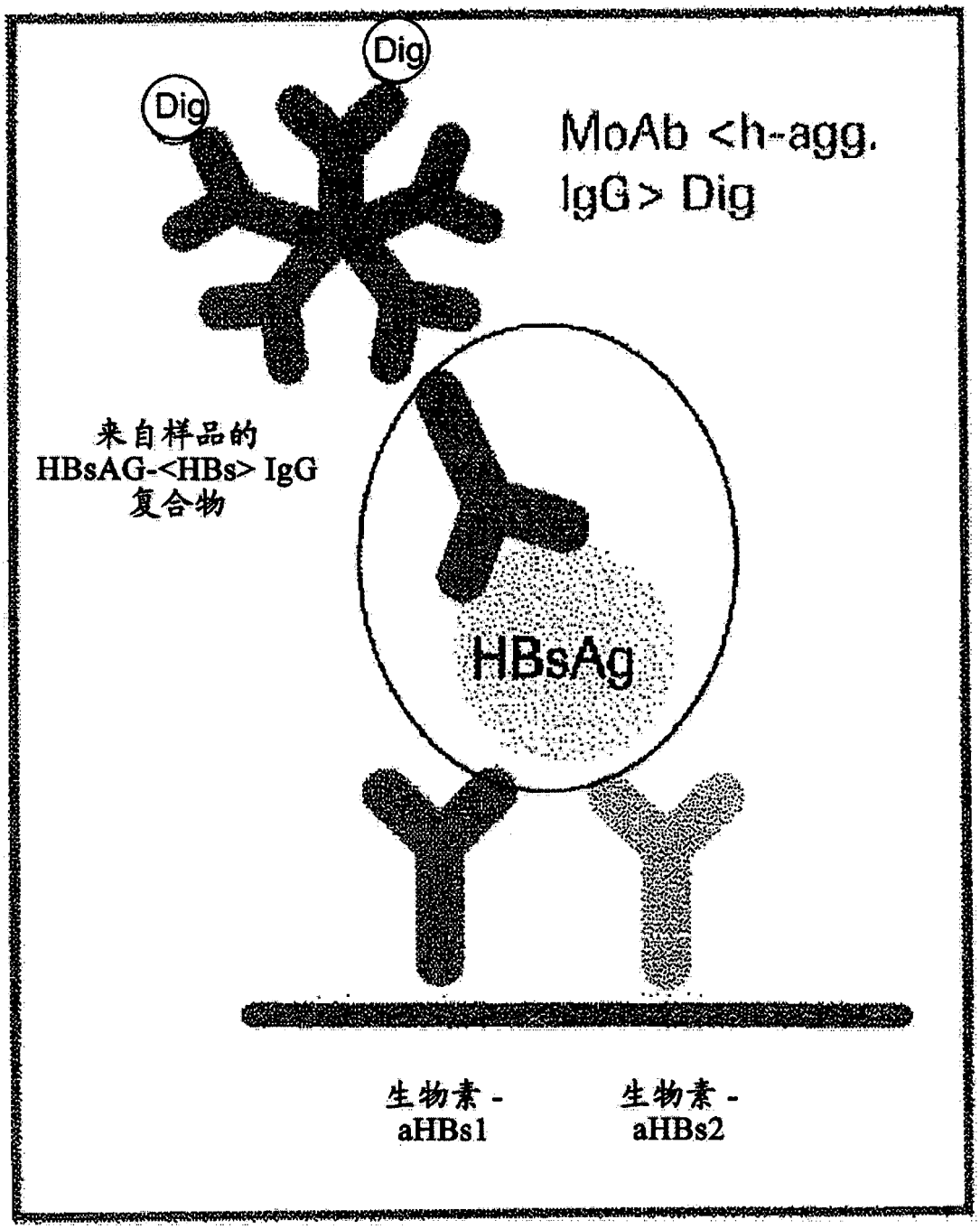
Dig
Dig
MoAb <h-agg. IgG> Dig
来自样品的
HBsAG-<HBs> IgG
复合物
HBsAg
生物素 -
aHBs1
生物素 -
aHBs2

图 1

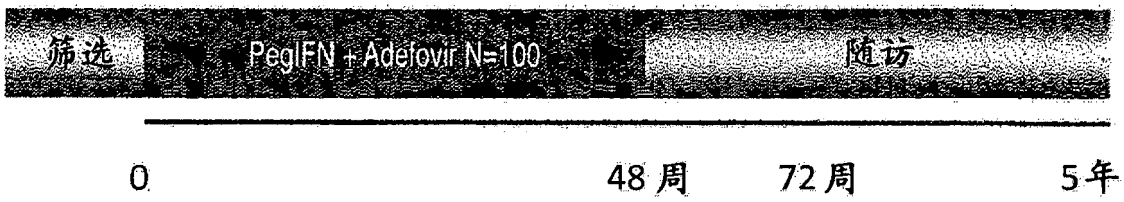

图 2

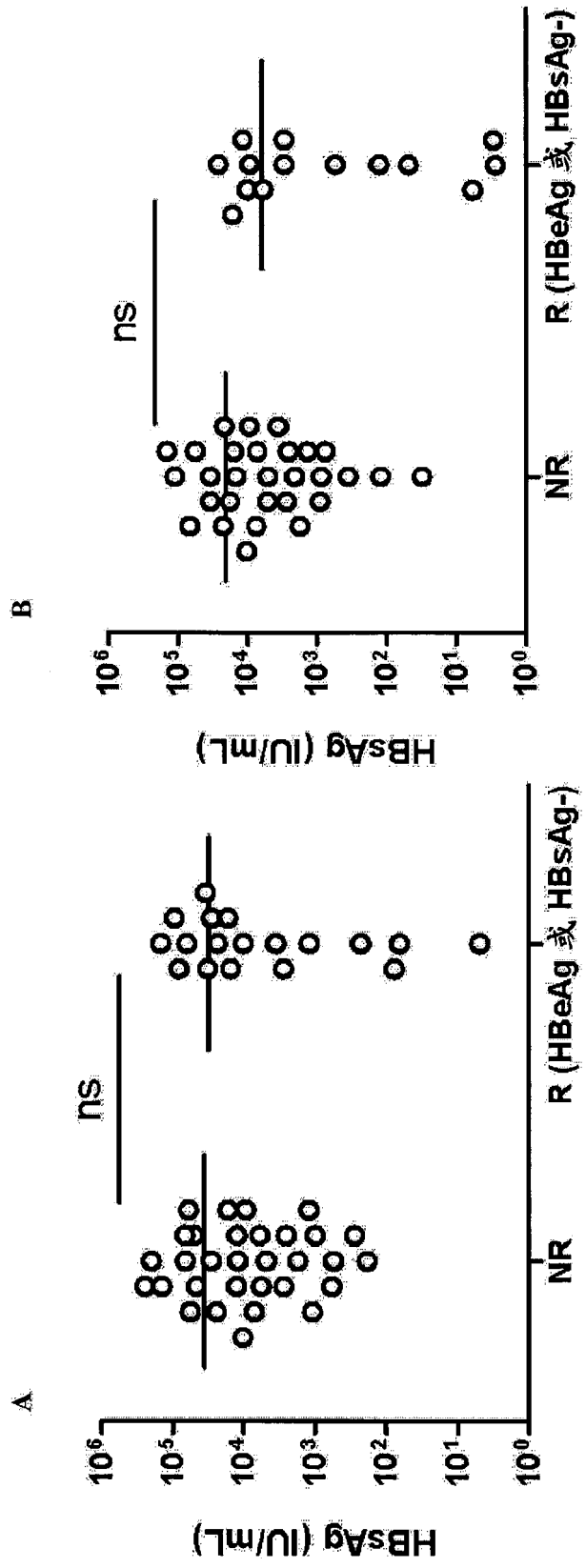

图 3

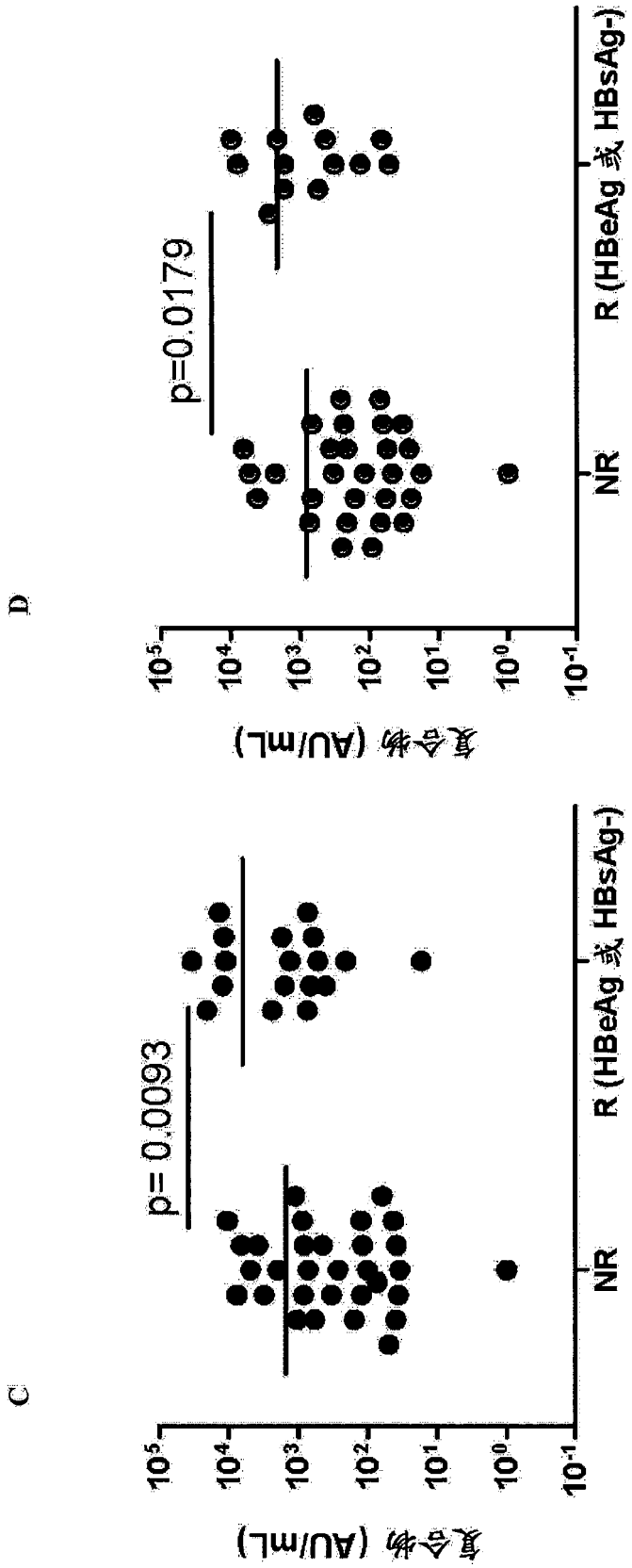
C
D
p= 0.0093
p=0.0179
复合物 (AU/mL)
NR
R (HBeAg 或 HBsAg-)
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}

图 3（续）

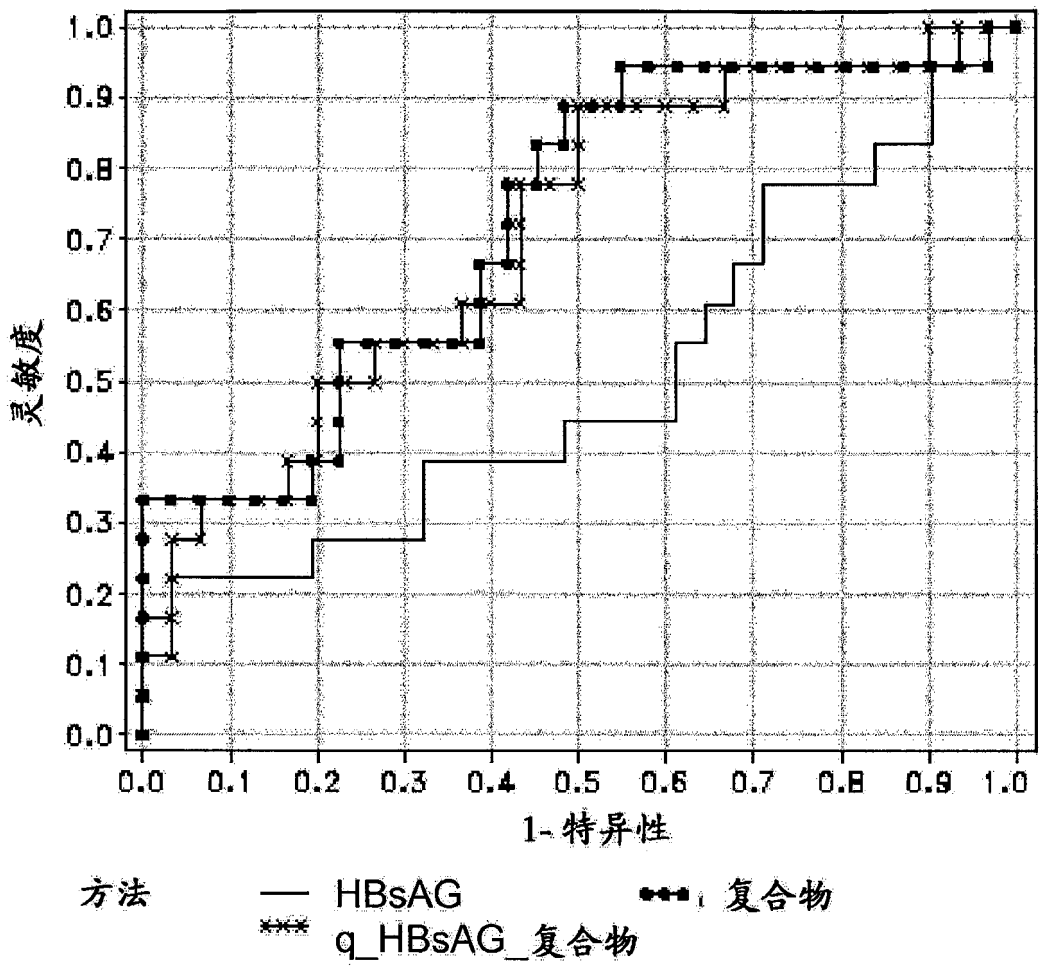
ROC 曲线
灵敏度
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
1-特异性
方法
HBsAG
复合物
q_HBsAG_复合物

图 4

Abstract

The present invention relates to a method for identifying a subject suffering from hepatitis B virus (HBV) infection as being susceptible to interferon treatment, said method comprising the steps of a) determining, in a sample of said subject, the amount of HBV immune complexes, b)comparing the amount of HBV immune complexes obtained in step a) to a reference value, and c) identifying a subject suffering from HBV infection as being susceptible to interferon treatment based on the result of the comparison made in step b). The present invention further relates to the use of the determination of the amount of HBV immune complexes in a sample from a subject suffering from HBV infection and of a detection agent for HBV immune complexes for identifying a subject suffering from HBV infection as being susceptible to interferon treatment. Furthermore, the present invention relates to a device and a kit allowing identifying a subject suffering from HBV infection as being susceptible to interferon treatment.