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(54) Title: CHITINASE-3-LIKE PROTEIN 1 (CHI3L1) AS AN AUTOANTIGEN IN AUTOIMMUNE DISORDERS OF THE DIGESTIVE SYSTEM

(57) Abstract: The invention relates to an in vitro method for diagnosis, prognosis, risk assessment, monitoring, therapy guidance and/or therapy control of an autoimmune disorder of the digestive system, such as inflammatory bowel disease and autoimmune liver disease, in which autoantibodies that bind Chitinase-3-Like Protein 1 (CHI3L1) protein are detected as a marker of such disease. The invention therefore relate to the use of CHI3L1 as an autoantigen in autoimmune diagnostics if diseases of the digestive system. A kit and system for conducting the method are encompassed by the invention.

# CHITINASE-3-LIKE PROTEIN 1 (CHI3L1) AS AN AUTOANTIGEN IN AUTOIMMUNE DISORDERS OF THE DIGESTIVE SYSTEM

## DESCRIPTION

The invention relates to an in vitro method for diagnosis, prognosis, risk assessment, monitoring, therapy guidance and/or therapy control of an autoimmune disorder of the digestive system, such as inflammatory bowel disease and autoimmune liver disease, in which autoantibodies that bind Chitinase-3-Like Protein 1 (CHI3L1) protein are detected as a marker of such disease. The invention therefore relates to the use of CHI3L1 as an autoantigen in autoimmune diagnostics if diseases of the digestive system. A kit and system for conducting the method are encompassed by the invention.

## BACKGROUND OF THE INVENTION

Autoimmune diseases are commonly considered to be medical conditions arising from an abnormal immune response to a normal body part, for example where so-called autoantibodies react to and damage parts of the patient's body. It is currently estimated that approximately 24 million people in the United States are affected by an autoimmune disease. Of the most common and debilitating autoimmune diseases are those of the digestive system, including for example inflammatory bowel disease and autoimmune liver disease.

Inflammatory bowel disease:

Crohn's disease (CD) and/or ulcerative colitis (UC) represent the two most important inflammatory bowel diseases (IBD) (44)(45). They are characterized by chronic, relapsing tissue-destroying inflammatory processes in the digestive system. To date, etiology and pathogenesis of CD as well as UC are unclear. While inflammation in UC predominantly appears in the mucosa and submucosa of colon and rectum, CD is characterized by wall-penetrating, granulomatous inflammatory processes of the entire gastrointestinal tract.

Highly complex and comparatively cost-intensive histological investigations of mucosa biopsies constitute common means in IBD (CD/UC) diagnostics. To this end, biopsies are collected especially from macroscopically conspicuous as well as inconspicuous areas. To efficiently utilize the potential of histopathological differential diagnostics it is, however, necessary to collect biopsies from at least five different anatomic segments of the entire colon, including the rectum, from the terminal ileum and upper gastrointestinal tract. Such analyses are time intensive and invasive, providing significant discomfort to the patient.

Straightforward diagnostic approaches for diagnosis of IBD, such as CD and UC, remain elusive and despite the various assays available for CD and UC diagnosis, there is still significant uncertainty regarding which approach is ideal.

Autoimmune liver diseases:

Autoimmune liver diseases have been recognised as clinical entities for decades. The three main categories of autoimmune liver disease are autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). All are well-defined entities with diagnosis based upon a constellation of clinical, serologic, and liver pathology findings. Although these diseases are considered autoimmune in nature, the etiology and possible environmental triggers of each remain obscure (42).

The worldwide prevalence of autoimmune disease of the liver is presently unknown, although the incidence of AIH, PBC and PSC has been assessed in various human populations. Reported incidence rates of AIH are approximately 1 per 100,000 population per year. A majority of PBC studies found incidence rates of 1-2 per 100,000 population per year and an increasing time trend. Previous studies of PSC found incidence rates around 1 per 100,000 population per year. The incidence of IAC remains unknown (43).

The characteristic morphologic patterns of autoimmune liver disease are a chronic hepatitis pattern of injury with prominent plasma cells in AIH, destruction of small intrahepatic bile ducts and canals of Hering in PBC, and periductal fibrosis and inflammation involving larger bile ducts with variable small duct damage in PSC. Serological findings include the presence of antimitochondrial antibodies in PBC, antinuclear, anti-smooth muscle, and anti-LKM antibodies in AIH, and pANCA in PSC (42).

Despite serological and morphological patterns being established in the field, straightforward diagnostic approaches for autoimmune diseases of the liver remain elusive.

Chitinase-3-like protein 1 (CHI3L1):

The 40 kDa protein CHI3L1 was first described in 1990 as a protein secreted by synovial cells and was also referred to as HC gp-39 (1). Later, it was also termed YKL-40 because of the one-letter code of its three N-terminal amino acids, Tyrosine (Y)- Lysine (K)- Leucine (L), and its molecular weight of 40 kDa (2). Although the protein was first discovered to be secreted by synovial cells, it was also shown to be produced in chondrocytes (4), colonic epithelial cells (8), smooth muscle cells (9), osteosarcoma cells (10), as well as macrophages (5) and neutrophils (11). Although CHI3L1 has no catalytic activity, it was shown to bind chitin, heparin and collagen.

Bigg et al. reported two different isoforms of CHI3L1. Depending on the source, these isoforms show different effects on collagen. Cartilage derived CHI3L1 had an inhibitory effect on type I collagen fibril formation. In contrast, the chondrocyte derived CHI3L1 promotes fibril formation and prevents type I collagen from cleavage (6).

The interaction with heparin was first described in 1990 when heparin-Sepharose chromatography was used to isolate CHI3L1 (1). Although CHI3L1 has a heparin-binding motif it seems to be more likely that heparan sulfate is a ligand for CHI3L1, since heparan sulfate proteoglycans play a role in cell adhesion or growth factor interaction (3).

Beside this, CHI3L1 was reported to be associated with tissue remodeling in vascular smooth muscle cells (12) and to act as a mitogen or growth factor in chondrocytes and synovial cells stimulating tissue remodeling (13). Here, CHI3L1 was shown to interact with IGF-1 and can also promote growth of skin and fetal lung fibroblasts in a comparable way like IGF-1. AKT-, ERK- and PKB-mediated mechanisms are involved in CHI3L1 mitogenic function (14). It acts as a

chemoattractant in vascular endothelial cell (HUVEC) stimulating cell migration and adhesion (15)(16).

In addition to this, CHI3L1 has also an impact on bacterial adhesion and migration in colonic epithelial cells. Mizoguchi showed that expression of CHI3L1 is upregulated in lamina propria and colonic epithelial cells (CECs) of patients suffering from ulcerative colitis and Crohn's disease as well as in murine colitis models. In accordance to this, this upregulation enhances adhesion and invasion of *Salmonella typhimurium* and *Adherent-invasive E. coli* (AIEC) but not non-pathogenic *E. coli* (DH5α) in CEC and thus may act as a pathogenic mediator in acute colitis (8).

In 1992, CHI3L1 was found to be secreted by the human osteosarcoma cell line MG63 (17) leading to extensive studies on its function in different cancers. In addition, its divers biological function as adhesion, migration and growth factor, may indicate that CHI3L1 plays an important role in cancer development.

CHI3L1 in inflammatory conditions:

Since elevated CHI3L1 levels were first discovered in synovial cells and cartilage of patients with rheumatoid arthritis (RA), a lot of efforts has been made in investigating its role in chronic inflammatory diseases. In RA, serum CHI3L1 levels were significantly increased but when disease becomes inactive, serum levels decrease (18). In addition to disease activity, CHI3L1 levels correlate with two other proinflammatory markers, interleukin 6 (IL-6) and c-reactive protein (CRP) (19) and increased tissue expression was found in chondrocytes of arthritic cartilage as well as in lining and stromal cells (macrophages) in the synovium (20). Although increased CHI3L1 levels were reported in many chronic inflammatory conditions, it was identified as an autoantigen in RA only (21) (Verheijden et al., Arthritis & Rheumatism, vol. 40, no. 6, 1June 1997, 1115-1125). In about approximately 50% of RA patients, CHI3L1 peptide-MHC complexes could be detected, but not in spondyloarthropathy (SpA) and psoriatic arthritis patients (22). The use of antigen microarrays comprising CHI3L1 peptides for profiling of autoantibodies in the diagnosis of RA has been suggested (Hueber et al., Arthritis & Rheuma, vol. 52, no. 9, 1 September 2005, 2645-2655).

An increased tissue and serum level of CHI3L1 was also reported in osteoarthritis (OA) but appears not to be a good marker for monitoring disease progression. Depending on disease severity, CHI3L1 is expressed in different cartilage zones. It can be detected in the deep, mid and superficial zone in advanced OA but only in the superficial zone in mild OA, whereas it could not be detected in chondrocytes from normal articular cartilage (23)(24).

Furthermore, CHI3L1 has been reported to as a marker for autoimmune disorder of the digestive system, such as in Crohn's disease (Yusuf Erzin et al., *Journal of Gastroenterology and Hepatology*, vol. 23, 1August 2008, e357-e362) and inflammatory bowel disease (Koutroubakis et al., *International Journal of Colorectal Disease*, vol. 18, no. 3, 1May 2003, 254-259; A. Buisson et al., *Alimentary Pharmacology & Therapeutics*., vol. 43, no. 10, 8March 2016, 1069-1079). It was shown in patients with Inflammatory Bowel Disease (IBD) that the serum CHI3L1 level correlates with the disease activity. In about half of patients with Ulcerative Colitis (UC) serum levels were increased. In contrast, they decreased when disease becomes inactive and se-

rum levels correlate with the simple clinical colitis activity index (SCCAI). Conversely, half of patient with Crohn's Disease also showed increased CHI3L1 levels but these levels did not drop when disease becomes inactive and did not correlate with the Harvey-Bradshaw score (25)(26).

CHI3L1 serum levels were also found to be increased in liver disorders like alcoholic liver cirrhosis or hepatitis C virus (HCV)-mediated fibrosis, where serum CHI3L1 levels correlate with the degree of fibrosis but its potential to be a good marker depends on the cause of liver fibrosis (27)(28). Regarding hepatitis C virus (HCV)-mediated fibrosis, CHI3L1 is only one of different serum markers of liver fibrosis but it is a better marker in disease progression in liver fibrosis due to schistosomiasis japonica infection (29)(30).

Interestingly, there was no upregulation of CHI3L1 expression in autoimmune hepatitis (AIH)-induced cirrhosis or primary biliary cirrhosis (PBC) (31). Elevated serum CHI3L1 levels were also found in atherosclerosis (32), pulmonary sarcoidosis (33) and systemic sclerosis (34).

In summary, in the context of inflammatory conditions, CHI3L1 has been suggested as a marker for inflammatory and autoimmune disorders of the digestive system and of RA and the generation of auto reactivity against CHI3L1 has been suggested in RA (Coffman F D, Critical Reviews in Clinical Laboratory Science, vol. 45, no. 6, 1 January 2008, 531-562).

Present diagnostic approaches:

For the diagnosis and differentiation of inflammatory bowel disease, invasive measures such as colonoscopy are necessary. In this way inflammations of the intestinal mucosa as well as fissures, lesions and ulcers can be recognized. In addition, biopsies are taken, whereby histological features such as granulomas or accumulations of neutrophil granulocytes can be detected (35)(36).

In addition, serological tests may be performed and various (auto) antibodies examined, as described in several review articles (Conrad et al., *Autoimmunity Reviews*, vol. 13, no. 4-5, 1 April 2014, 463-466; Laass et al., *Autoimmunity Reviews*, vol. 13, no. 4-5, 1 April 2014, 467-471; Hennes et al., *Hepatology*, vol. 48, no. 1, 1 July 2008, 169-176) and the patent application EP2913675A2. These include anti-Saccharomyces cerevisiae antibodies (ASCA) directed against mannan in the cell wall of S. cerevisiae (37), as well as autoantibodies against exocrine pancreas (PAH), which are directed against GP2 (zymogen granule membrane Glycoprotein 2) and CUZD1 (CUB / zona pellucida-like domain-containing protein 1) (38)(39). These antibodies are specific for Crohn's disease, but show a prevalence of only about 50%. In addition, in about 50% of patients with ulcerative colitis, so-called perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) can be detected (37)(39)(46)(47)(48).

For the diagnosis of autoimmune hepatitis (AIH), a so-called panel was created by the International Autoimmune Hepatitis Group (IAIHG) in 1992 after the diseases were defined. This includes the gender, the IgG titer, the titer of various liver enzymes and the antibody spectrum in the blood, but also factors such as alcohol consumption, response to treatments and genetic factors (40). This version was already revised in 1998 and replaced in 2008 by a simplified form. Here, there are four criteria for the diagnosis of AIH: IgG levels, antibody spectrum and antibody titers, virological markers and histological features (41).

According to established approaches in the art, the concentration of the CHI3L1 protein in patient material (preferably blood serum) is examined with the aid of commercial sandwich ELISA

products. CHI3L1 protein levels are used in the diagnosis of various diseases, and conclusions are drawn about the intensity and course of the disease. Due to the incidence of elevated CHI3L1 protein levels in multiple diseases, the simple detection of the protein is not suitable for determining specific diseases.

In the case of AIH-mediated hepatic cirrhosis, no increased serum concentrations and expression of the CHI3L1 protein could be shown (31). Among the diseases mentioned above, rheumatoid arthritis is the only one in which CHI3L1 has been described as an autoantigen (21).

Despite advances in the diagnosis of autoimmune disorders of the digestive system, such as inflammatory bowel disease and autoimmune liver disease, more straightforward and effective means for diagnosis are required, in particular means for disease diagnosis that are closely based on the underlying pathology of the respective diseases.

**SUMMARY OF THE INVENTION**

In light of the prior art the technical problem underlying the present invention is the provision of means for diagnosing autoimmune diseases of the digestive system, such as inflammatory bowel disease and/or autoimmune liver disease, that do not exhibit the disadvantages of the prior art. A further problem to be solved may be considered the provision of means for differentiating between CD and UC, for example in patients with similar symptoms of disease. The invention is intended to improve the serological (differential) diagnosis of inflammatory bowel diseases and autoimmune liver diseases.

The problem is solved by the invention according to the independent claims. Preferred embodiments are provided in the dependent claims.

The invention relates to Chitinase-3-Like Protein 1 (CHI3L1) protein as an autoantigen in immune disorders of the digestive system, and corresponding methods and kits for diagnosis and associated embodiments.

The invention therefore relates to an in vitro method for diagnosis, prognosis, risk assessment, monitoring, therapy guidance and/or therapy control of an autoimmune disorder of the digestive system, comprising:

- providing a sample of a subject exhibiting symptoms of and/or suspected of having said disorder,
- providing Chitinase-3-Like Protein 1 (CHI3L1) protein,
- contacting said sample or parts thereof with said CHI3L1 protein, and
- detecting autoantibodies from said sample that bind to said CHI3L1 protein,
- wherein the presence of said autoantibodies and/or increased levels of said autoantibodies compared to an appropriate control, such as a sample from a healthy subject, indicate the presence an autoimmune disorder of the digestive system.

The invention therefore relates to the surprising and unexpected finding that CHI3L1 protein is a target (autoantigen) for autoantibodies that are associated with different autoimmune diseases of the digestive system. This surprising finding makes it possible to diagnose autoimmune disorder of the digestive system. Furthermore, on the basis of the method of diagnosis it is also

possible to provide a prognosis and a risk assessment for the subject exhibiting symptoms of an autoimmune disorder of the digestive system depending on the result of a method. In case of presence of the autoantibodies against CHI3L1 protein it may be concluded that said subject the symptoms are related to an autoimmune reaction, which allows it to categorize the subject as a patient suffering from an autoimmune disorder of the digestive system. Such categorization of the patients allows conclusions about the prognosis and risk of the patient with respect to disease progression and duration of the symptoms.

Furthermore, the diagnosis of an autoimmune disease of the digestive system enables conclusions with respect to suitable therapeutic measures that should be initiated or that are already ongoing and may be modified. Such suitable therapeutic measures may differ on the basis of the result of the method of the invention, since autoimmune disorders of the digestive system may require different therapeutic approaches than other disorders of the GI tract that cause similar symptoms. Accordingly, the method of the invention can be used for therapy guidance and therapy control. Additionally, the method of the present invention enables quantification of the amount of autoantibodies against CHI3L1, which may be used as a measure for the current severity of the autoimmune disease in a patient and therefore is an indicator of the disease status of the patient. Accordingly, the method of the present invention can be used for monitoring the status of a subject or patient.

In one embodiment of the invention the in vitro method described herein is characterised in that the autoimmune disorder of the digestive system to be diagnosed is an inflammatory intestinal disease in which autoantibodies bind components of the gastrointestinal tract of said subject.

In one embodiment of the invention the in vitro method described herein is characterised in that the inflammatory intestinal disease to be diagnosed is Crohn's disease (CD) and/or ulcerative colitis (UC).

In one embodiment of the invention the in vitro method described herein is characterised in that the autoimmune disorder of the digestive system to be diagnosed is an inflammatory liver disease in which autoantibodies bind components of the liver of said subject.

In one embodiment of the invention the in vitro method described herein is characterised in that the inflammatory liver disease to be diagnosed is autoimmune hepatitis and/or primary sclerosing cholangitis.

According to the present invention the components of the gastrointestinal tract, to which autoantibodies may bind, include, but are not limited to, the mucosa of the small intestine or other small-bowel tissue, the villous extracellular matrix, intestinal epithelial cells, in particular villous epithelial cells, the endomysium or other tissues or cells of the stomach, small intestine, and colon, in particular the cells lining of the stomach, small intestine, and colon.

According to the present invention the components of the liver, to which autoantibodies may bind, include, but are not limited to hepatocytes and/or biliary epithelial cells.

It was at the time of the invention entirely unknown that CHI3L1 protein could be used as an epitope detect the presence or absence of different autoimmune diseases, preferably those characterised by autoantibodies that bind components of the gastrointestinal tract or liver of a subject.

The method thereby allows diagnosis and in some cases differentiation between such diseases on the basis of their distinct autoantibody profiles. The use of CHI3L1 protein as an autoantigen in diagnosis of autoimmune diseases of the digestive tract thereby represents a novel and inventive concept in light of the prior art with respect to the diagnosis of autoimmune diseases.

In embodiments of the invention the CHI3L1 protein comprises or consists of the amino acid sequence according to SEQ ID NO. 1 (see below). Potential isoforms of CHI3L1 are also intended for employment in embodiments of the present invention.

Isoforms of CHI3L1, such as the cartilage derived CHI3L1 and/or chondrocyte derived CHI3L1, relate to proteins comprising or consisting of substantially the same or similar amino acid sequences as SEQ ID NO 1. Potential isoforms may also refer to one or more amino acid sequences that is similar, but not identical to, the amino acid sequences provided explicitly herein.

Sequences according to SEQ ID NO 2, 3 and/or 4 may also be employed in the invention.

In preferred embodiments the CHI3L1-similar sequences employed are functionally equivalent to CHI3L1 itself, in other words, such functional equivalence is define by the ability to detect/bind autoantibodies indicative of the herein mentioned diseases.

Variation in length of the amino acid sequences as described herein is also encompassed by the present invention. A skilled person is capable of providing artificial amino acid sequence variants that are longer or shorter than the specific sequence of SEQ ID NO 1, 2, 3 and/or 4, which will still exhibit sufficient similarity to the natural form in order to provide the diagnostic outcomes described herein. For example, shorter variants of SEQ ID NO 1, 2 and/or 3 comprising 10, 20, 30, 40, 50 or up to 100 amino acids less than the full length form may also enable effective diagnostic outcomes, as described herein. Fragments of CHI3L1 are therefore also considered. Additionally, longer variants of SEQ ID NO 1, 2, 3 and/or 4 comprising 10, 20, 30, 40, 50 or up to 100 amino acids any given additional sequence more than the natural CHI3L1 sequence may also enable effective diagnostic outcomes, as described herein.

In other embodiments of the invention, the CHI3L1 protein employed may comprise or consist of an amino acid sequence with at least 50%, 60%, 70%, 80%, 90% or 95% sequence identity to one or more of SEQ ID NO 1, 2, 3 and/or 4. Preferably the sequence variant comprises at least 80%, 90% or 95% sequence identity to one of SEQ ID NO 1, 2, 3 and/or 4 and preferably exhibits functional identity to SEQ ID NO 1, 2, 3 and/or 4 with respect to the binding and subsequent identification of autoantibodies directed against a natural form of CHI3L1.

The provision of the sample to be analysed may relate to either obtaining a sample from a patient, or providing a pre-prepared sample already having been obtained, preferably from a patient exhibiting symptoms and/or suspecting of having an autoimmune disorder, preferably an autoimmune disorder associated with autoantibodies that bind components of the digestive or intestinal tract or liver of said subject.

Examples of the symptoms of said disorders are provided herein and are not intended to limit the scope of the invention. Such symptoms are well-known to skilled practitioners in the field.

The sample of the present invention relates preferably to a sample obtained from a patient, such as a bodily fluid, preferably a blood, plasma or serum sample, but may also relate to stool

sample. Tissue samples may also be used in the method of the invention. Any particular processing of the sample is not intended to be limiting to the scope of the invention, essentially any given sample obtained from the patient may be used, with or without additional processing steps before administration in the method described herein.

In one embodiment of the invention the in vitro method described herein is characterised in that the sample of a subject is as a bodily fluid selected from the group consisting of blood, a sample derived from blood, such as a plasma or serum sample, a stool sample or sample derived from stool, mucous secretions, such as tears, saliva, sweat or colostrum, and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium.

The contacting of a sample to CHI3L1 protein may take place in any given setting. In one embodiment, a solid phase, to which the isoforms are attached, is used. The sample is preferably provided as a liquid sample and is brought into contact with the CHI3L1 protein, thereby allowing the autoantibodies of the sample to interact with the CHI3L1 protein under conditions that allow binding of said antibodies to the CHI3L1 protein. Such conditions are known to a skilled person and may represent biological conditions, in which the relevant proteins are capable of forming their native or near-native structures, in order to allow the binding properties of the antibodies to enable interaction with said isoforms.

The contacting and detection steps may in further embodiments be carried out as follows: allowing the antibody to bind CHI3L1, thereby forming a complex (CHI3L1-autoantibody complex), contacting the complex with a label, such as a labeled indicator antibody, preferably an antibody that binds human immunoglobulin, to form a labeled complex; and detecting the presence or absence of the labeled complex, and preferably associating the presence of the detected antibodies in the sample with the autoimmune disease.

The detection of bound antibodies may be carried out in any given suitable manner, including but not limited to the use of a spectrophotometer to detect colour from a chromogenic substrate, a radiation counter to detect radiation such as a gamma counter for detection of 125I, or a fluorometer to detect fluorescence in the presence of light of a certain wavelength.

Washing of the bound antibodies may be carried out as is commonly known in the art, for example as is carried out in a standard immunoassay, such as an ELISA assay. Additional detection means are described herein.

In one embodiment of the invention the in vitro method as described herein comprises:

- Bringing the sample of the subject into contact with a solid phase-immobilised CHI3L1 protein, allowing autoantibodies in said sample to bind to said immobilised CHI3L1 protein, thereby forming a solid phase-CHI3L1-autoantibody complex,
- Bringing the solid phase-CHI3L1-autoantibody complex into contact with a signal-producing affinity reagent that binds said complex, such as a labelled human anti-immunoglobulin antibody, and
- Detecting bound autoantibodies via the signal from said affinity reagent.

A further aspect of the invention relates to a solid phase-immobilised CHI3L1 protein, and its use in the assay described herein.

In one embodiment of the invention the in vitro method is characterized in that the autoantibody detected is an IgG, IgA, IgM and/or secretory IgA autoantibody. The detection of different subclasses of autoantibody is possible according to the knowledge of a skilled person, for example IgG-, IgA-, IgM- and/or secretory IgA autoantibody-specific secondary antibodies may be employed in an ELISA assay or similar.

In one embodiment of the invention the in vitro method is characterized in that the autoimmune disorder of the digestive system is Crohn's disease (CD), ulcerative colitis (UC) or primary sclerosing cholangitis, and wherein the autoantibody detected is an IgA and/or secretory IgA autoantibody that binds CHI3L1 protein.

As is demonstrated in the examples below, the presence of IgA and/or secretory IgA autoantibodies that bind CHI3L1 protein are detected, preferably in elevated levels, in subjects with Crohn's disease (CD), ulcerative colitis (UC) or primary sclerosing cholangitis. This finding is novel and enables a preferred embodiment of the invention based on entirely unexpected experimental findings.

In one embodiment of the invention the in vitro method is characterized in that the autoimmune disorder of the digestive system is autoimmune hepatitis, and wherein the autoantibody detected is an IgG autoantibody that binds CHI3L1 protein.

As is demonstrated in the examples below, the presence of IgG autoantibodies that bind CHI3L1 protein are detected, preferably in elevated levels, in subjects with autoimmune hepatitis. This finding is novel and enables a preferred embodiment of the invention based on entirely unexpected experimental findings.

In one embodiment of the invention the in vitro method is used in the differential diagnosis between Crohn's disease (CD) and ulcerative colitis (UC), wherein the amount of IgA and/or secretory IgA autoantibodies from said sample that bind to said CHI3L1 protein indicates the presence of Crohn's disease (CD), when said amount is above a reference level, wherein said reference level corresponds to amounts of autoantibodies that bind to said CHI3L1 protein in patients with ulcerative colitis (UC).

As is demonstrated in the examples below, the presence of IgA and/or secretory IgA autoantibodies that bind CHI3L1 protein are detected in elevated levels in subjects with CD, compared to the levels in samples from patients with UC. This finding is novel and enables a preferred embodiment of the invention based on entirely unexpected experimental findings. This embodiment enables the use of reference values or reference samples representing amounts of autoantibodies that bind to said CHI3L1 protein in patients with ulcerative colitis (UC), and comparison to these levels, such that when levels are above a particular reference (potential a cut-off value, or population average), the presence of CD may be diagnosed.

The method thereby allows the differentiation of autoimmune diseases, which may show very similar disease symptoms with respect to digestive problems, stomach cramps and pain, diarrhea, amongst others, via a simple and cost effective immunoassay, such as an ELISA, thereby avoiding more complicated diagnostic procedures such as endoscopies or biopsy analysis.

A further aspect of the invention relates to a kit for diagnosing an autoimmune disorder of the digestive system by detecting autoantibodies from a sample that bind to CHI3L1 protein, comprising:

- CHI3L1 protein immobilized on a solid phase, and optionally
- one or more human anti-immunoglobulin antibodies, wherein said human anti-immunoglobulin antibodies bind autoantibodies of Ig-subtypes IgG, IgA and/or IgM,
- a label for detection of said one or more human anti-immunoglobulin antibodies, either capable of binding said human anti-Immunoglobulin antibody or linked to said anti-Immunoglobulin antibody, and means for detecting said label.

In one embodiment of the invention the kit further comprises reference data, such as a reference level, cut-off values, populations averages, or similar, corresponding to autoantibody amounts in healthy subjects, in subjects with Crohn's disease (CD), with ulcerative colitis (UC), autoimmune hepatitis and/or primary sclerosing cholangitis. These reference levels may be employed in order to make diagnostic and/or differential diagnostic statements, preferably those as described above, on the basis of the level of anti-CHI3L1 autoantibodies detected in the method of the invention.

In one embodiment of the invention said reference data is stored on a computer readable medium and/or is present in the form of computer executable code configured for comparing the determined amounts of autoantibodies that bind to said CHI3L1 protein in said sample to said reference data.

In further embodiments of the invention, the kit also comprises a computer program. Preferably, the computer program is adapted to perform the method of the present invention.

A skilled person is capable of developing reference levels for either healthy or diseased subjects using methods established in the art.

The embodiments described herein with reference to the kit of the present invention are intended to also relate to structural features of the components of the method as described herein. The features of the kit as described herein may therefore also be used to characterize the method, and vice versa.

The invention therefore also relates to the use of a kit as described herein for the diagnosis of an autoimmune disorder as described herein.

The invention further relates to a system for diagnosing an autoimmune disorder of the digestive system by detecting autoantibodies from a sample that bind to CHI3L1 protein.

In one embodiment the system comprises:

- CHI3L1 protein immobilized on a solid phase, and

At least one of:

- A microscopic device configured to determine the presence and/or intensity of signal produced by a label bound to an autoantibody, and/or
- An optionally networked computer processing device configured to perform executable instructions; and a computer program, the computer program comprising a software module executed by the computer processing device to apply a model or algorithm for analyzing said autoantibodies,

And optionally:

- one or more human anti-immunoglobulin antibodies, wherein said human anti-immunoglobulin antibodies bind autoantibodies of Ig-subtypes IgG, IgA and/or IgM, and a label for detection of said one or more human anti-immunoglobulin antibodies, either capable of binding said human anti-Immunoglobulin antibody or linked to said anti-Immunoglobulin antibody, and means for detecting said label.

In one embodiment the system and/or kit described herein comprises an optionally networked computer processing device configured to perform executable instructions; and a computer program, the computer program comprising a software module executed by the computer processing device to apply a model or algorithm for analyzing said autoantibodies.

In one embodiment the system is characterised in that the computer program further comprises a software module executed by the computer processing device to designate a treatment regimen for the individual.

In embodiments of the system of the invention, the computer program is adapted to perform the method of the invention. Preferably, the computer program of the system of the invention is adapted to perform the method of the invention and comprises a software module executed by the computer processing device to apply a model or algorithm for analyzing said autoantibodies,

In one embodiment the system is characterised in that a patient from which said sample has been taken is identified as providing said sample and is optionally treated for the autoimmune disease. The invention therefore relates to a method for treating an autoimmune disease of the digestive system, comprising carrying out the method as described herein and subsequently treating said subject.

Treatment for an autoimmune disease of the gastrointestinal tract may relate to any appropriate treatment known to a skilled medical practitioner. Medical treatment of IBD may be individualized to each patient. The choice of which drugs to use and by which route to administer them (oral, rectal, injection, infusion) depends on factors including the type, distribution, and severity of the patient's disease, as well as other historical and biochemical prognostic factors, and patient preferences. For example, mesalazine may be administered. Generally, depending on the level of severity, autoimmune IBD may require immunosuppression to control the symptoms, such as prednisone, TNF inhibition, azathioprine (Imuran), methotrexate, or 6-mercaptopurine administration. Often, anti-inflammatory steroids are used to control disease flares. Crohn's disease and ulcerative colitis patients may receive TNF inhibitors. Severe cases may require surgery, such as bowel resection or a temporary or permanent colostomy or ileostomy. Surgery can cure ulcerative colitis if the large intestine is removed. A pouch can be created from the small intestine when required, this serves as the rectum and prevents the need for a permanent ileostomy.

Treatment for an autoimmune disease of the liver may relate to any appropriate treatment known to a skilled medical practitioner. Medical treatment of autoimmune liver disease may be individualized to each patient. The choice of which drugs to use and by which route to administer them (oral, rectal, injection, infusion) depends on factors including the type, distribution, and

severity of the patient's disease, as well as other historical and biochemical prognostic factors, and patient preferences. For example, drugs and medication may be administered. Immunosuppressant drugs can be used to stop the immune system's attack. Such drugs include, for example, 6-mercaptopurine and azathioprine. Corticosteroids may be administered, usually in the form of prednisone, and can directly treat liver inflammation. Such medications typically serve as immunosuppressants. Surgery is also one option for treatment. A liver transplant (replacing your liver with a donor organ) can treat autoimmune liver disease. According to the National Digestive Diseases Information Clearinghouse (NDDIC), there is a 90 percent one-year survival rate for patients who have a transplant.

The invention also relates to a method for the treatment of the autoimmune disease mentioned herein, comprising the following steps:

- providing a column having CHI3L1 protein coupled thereto;
- passing the plasma of a patient over the column under conditions allowing effective binding of CHI3L1 protein to antibodies in the patient's plasma, thereby removing a significant amount of antibodies from the plasma of the patient; and
- returning the plasma thus obtained into the patient.

In a preferred embodiment of the method according to the invention, CHI3L1 protein in accordance with SEQ ID NO 1 recognizes autoantibodies directed against intestinal and/or liver tissue. This treatment embodiment has the advantageous effect that diagnosis of autoimmune diseases by identifying autoantibodies, underlying the pathology of the disease, can be simply and effectively removed from body fluids without drug treatment interventions frequently involving stress to the patient.

**DETAILED DESCRIPTION OF THE INVENTION**

Chitinase-3-Like Protein 1 (CHI3L1) is a 40 kDa protein and was first described in 1990 as a protein secreted by synovial cells and was also referred to as HC gp-39 (1). Later, it was also termed YKL-40 because of the one-letter code of its three N-terminal amino acids, Tyrosine (Y)-Lysine (K)- Leucine (L), and its molecular weight of 40 kDa (2).

It comprises 383 amino acids and is a member of the 18-glycosylhydrolase family, like other chitinases and chitinase-related proteins, which can bind to heparin, chitin and collagen. A conserved region DXXDXDXE is necessary for chitinase activity, but due to a change of glutamate 140 into leucine and aspartate 142 into alanine, CHI3L1 lacks chitinase activity (1)(3)(4)(5)(6). The gene for CHI3L1 consists of 10 exons, spanning 8kb genomic DNA and is located on chromosome 1q31-q32 (7).

CHI3L1 may also be known as GP39; ASRT7; GP-39; YKL40; CGP-39; YKL-40; YYL-40; HC-gp39; HCGP-3P; or hCGP-39.

In one embodiment the CHI3L1 protein comprises or consists of the amino acid sequence of SEQ ID NO 1 (Accession No. AAH08568, EAW91467.1, EAW91468.1 BAG35757, AAP35887.1, P36222.2, NP_001267.2).

| Protein Name | Chitinase 3-like 1 (cartilage glycoprotein-39) [Homo sapiens] |
|---|---|

| GenBank Number | GenBank: AAH08568.1 |
|---|---|
| Amino Acid Sequence (SEQ ID NO. 1) | MGVKASQTGFVVLVLLQCCSAYKLVCYYTSWSQYREGDGSCF PDALDRFLCTHIIYSFANISNDHIDTWEWNDVTLYGMLNTLKNR NPNLKTLLSVGGWNFGSQRFSKIASNTQSRRTFIKSVPPFLRTH GFDGLDLAWLYPGRRDKQHFTTLIKEMKAEFIKEAQPGKKQLLL SAALSAGKVTIDSSYDIAKISQHLDFISIMTYDFHGAWRGTTGHH SPLFRGQEDASPDRFSNTDYAVGYMLRLGAPASKLVMGIPTFG RSFTLASSETGVGAPISGPGIPGRFTKEAGTLAYYEICDFLRGAT VHRILGQQVPYATKGNQWVGYDDQESVKSKVQYLKDRQLAGA MVWALDLDDFQGSFCGQDLRFPLTNAIKDALAAT |

Natural variants of CHI3L1 are also known, which may be employed in the invention, such as:

Natural variant VAR_019838, aa 145, R → G2, Corresponds dbSNP:rs880633 Ensembl.

| Protein Name | Chitinase 3-like 1 (cartilage glycoprotein-39) [Homo sapiens] |
|---|---|
| GenBank Number | GenBank: AAH38354.1; AAH39132.1 |
| Amino Acid Sequence (SEQ ID NO. 2) | MGVKASQTGFVVLVLLQCCSAYKLVCYYTSWSQYREGDGSCF PDALDRFLCTHIIYSFANISNDHIDTWEWNDVTLYGMLNTLKNR NPNLKTLLSVGGWNFGSQRFSKIASNTQSRRTFIKSVPPFLRTH GFDGLDLAWLYPGR**G**DKQHFTTLIKEMKAEFIKEAQPGKKQLLL SAALSAGKVTIDSSYDIAKISQHLDFISIMTYDFHGAWRGTTGHH SPLFRGQEDASPDRFSNTDYAVGYMLRLGAPASKLVMGIPTFG RSFTLASSETGVGAPISGPGIPGRFTKEAGTLAYYEICDFLRGAT VHRILGQQVPYATKGNQWVGYDDQESVKSKVQYLKDRQLAGA MVWALDLDDFQGSFCGQDLRFPLTNAIKDALAAT |

Natural variant VAR_019839, aa 311, I → T2, Corresponds dbSNP:rs1049407 Ensembl.

| Protein Name | GP-39 cartilage protein [Homo sapiens] / glycoprotein [Homo sapiens] |
|---|---|
| GenBank Number | GenBank: CAA69661.1; AAA16074.1 |
| Amino Acid Sequence (SEQ ID NO. 3) | MGVKASQTGFVVLVLLQCCSAYKLVCYYTSWSQYREGDGSCF PDALDRFLCTHIIYSFANISNDHIDTWEWNDVTLYGMLNTLKNR NPNLKTLLSVGGWNFGSQRFSKIASNTQSRRTFIKSVPPFLRTH GFDGLDLAWLYPGRRDKQHFTTLIKEMKAEFIKEAQPGKKQLLL SAALSAGKVTIDSSYDIAKISQHLDFISIMTYDFHGAWRGTTGHH SPLFRGQEDASPDRFSNTDYAVGYMLRLGAPASKLVMGIPTFG RSFTLASSETGVGAPISGPGIPGRFTKEAGTLAYYEICDFLRGAT VHR**T**LGQQVPYATKGNQWVGYDDQESVKSKVQYLKDRQLAG AMVWALDLDDFQGSFCGQDLRFPLTNAIKDALAAT |

| Protein Name | cartilage glycoprotein-39, partial [Homo sapiens] |
|---|---|
| GenBank Number | GenBank: CAB76472.1 |
| Amino Acid Sequence (SEQ ID NO. 4) | VQYLKDRQLAGAMVWALDLDDFQGSFCGQDLRFPLTNAIKDAL AAT |

In some embodiments of the invention the CHI3L1 protein, preferably according to sequences disclosed herein, may comprise a 0 to 10 amino acid addition or deletion at the N and/or C terminus of a sequence.

As used herein the term “a 0 to 10 amino acid addition or deletion at the N and/or C terminus of a sequence” means that the polypeptide may have a) 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at its N terminus and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids deleted at its C terminus or b) 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at its C terminus and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides deleted at its N terminus, c) 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at its N terminus and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids at its N terminus or d) 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids deleted at its N terminus and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids deleted at its C terminus.

Furthermore, in addition to the polypeptides described herein, peptidomimetics are also contemplated. Peptide analogs are commonly used in the pharmaceutical and diagnostic industry as non-peptide analogues with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics” (Fauchere (1986) Adv. Drug Res. 15: 29; Veber and Freidinger (1985) TINS p. 392; and Evans et al. (1987) J. Med. Chem. 30: 1229) and are usually developed with the aid of computerized molecular modelling.

Preferably, the amino acid sequence of the CHI3L1 protein is as described according to one or more of SEQ ID NO 1 to 4. In some embodiments sequence variants of these peptides are also contemplated, in which protein sequences with at least 60%, 65%, 70%, 75%, 80%, 85%, 90% or preferably 95% sequence identity to any one or more of SEQ ID NO 1-4 are employed, preferably those with functionally analogy, in particular when the CHI3L1 variant protein is capable of binding autoantibodies indicative of disease.

Protein modifications to the polypeptides of the present invention, which may occur through substitutions in amino acid sequence, and nucleic acid sequences encoding such molecules, are also included within the scope of the invention. Substitutions as defined herein are modifications made to the amino acid sequence of the protein, whereby one or more amino acids are replaced with the same number of (different) amino acids, producing a protein which contains a different amino acid sequence than the primary protein. In some embodiments this amendment will not significantly alter the function of the protein. Like additions, substitutions may be natural or artificial. It is well known in the art that amino acid substitutions may be made without significantly altering the protein's function.

This is particularly true when the modification relates to a "conservative" amino acid substitution, which is the substitution of one amino acid for another of similar properties. Such "conserved" amino acids can be natural or synthetic amino acids which because of size, charge, polarity and conformation can be substituted without significantly affecting the structure and function of the protein. Frequently, many amino acids may be substituted by conservative amino acids without deleteriously affecting the protein's function.

In general, the non-polar amino acids Gly, Ala, Val, lie and Leu; the non-polar aromatic amino acids Phe, Trp and Tyr; the neutral polar amino acids Ser, Thr, Cys, Gin, Asn and Met; the positively charged amino acids Lys, Arg and His; the negatively charged amino acids Asp and Glu,

represent groups of conservative amino acids. This list is not exhaustive. For example, it is well known that Ala, Gly, Ser and sometimes Cys can substitute for each other even though they belong to different groups.

An autoantibody is an antibody (a type of protein) manufactured by the immune system that is directed against one or more of the individual's own proteins. Many autoimmune diseases are associated with and/or caused by such autoantibodies.

The term “autoimmune disease” refers to any given disease associated with and/or caused by the presence of autoantibodies. Autoimmune diseases arise from an abnormal immune response of the body against substances and tissues normally present in the body (autoimmunity). This may be restricted to certain organs or involve a particular tissue.

The term “autoimmune disorder of the digestive system” relates to an immune disease in which parts of the digestive system are attacked by autoreactive antibodies. The digestive system consists of the gastrointestinal tract plus accessory organs of digestion, such as the liver. Autoimmune disorders of the digestive system comprise systemic autoimmune disorders with gastrointestinal manifestation or autoimmune disorders that manifest in particular in the GI tract (Andrew Campbell. *Autoimmunity and the Gut*. Autoimmune Diseases Volume 2014, Article ID 152428, 12 pages; Cojocaru et al. Maedica (Buchar). 2011 Jan; 6(1): 45–51).

As used herein the terms “inflammatory bowel disease”, “inflammatory autoimmune gastrointestinal disorder”, and “inflammatory intestinal disease in which autoantibodies bind components of the gastrointestinal tract”, and the like, may be used interchangeably.

A preferred autoimmune disease of the invention is inflammatory bowel disease. The term “inflammatory bowel disease” or “IBD” refers to gastrointestinal disorders including, without limitation, Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis (IC).

A preferred autoimmune disease of the invention is therefore an autoimmune disease of the digestive or intestinal tract of said subject. Such diseases are characterised in that the autoimmune disorder exhibits autoantibodies that bind components of the digestive or intestinal tract of said subject. Such components of the digestive or intestinal tract may be any organ, tissue, cell or protein found in said area of the subject. The digestive or intestinal tract may be understood as the gastrointestinal tract (GI tract), which refers to the stomach and intestine, and is divided into the upper and lower gastrointestinal tracts, and may include all the structures from the mouth to the anus. The tract may also be divided into foregut, midgut, and hindgut, reflecting the embryological origin of each segment of the tract.

Gastrointestinal (GI)-related autoantibodies can be evaluated in autoimmune diseases such as inflammatory bowel disease, autoimmune hepatitis and celiac disease. Such antibodies may relate to ANCA (anti-neutrophil cytoplasmic antibodies) and/or autoantibodies to glycoprotein 2. Anti-GP2 IgA and IgG can be detected in sera from patients with Crohn’s disease and may be used in order to differentiate Crohn’s disease from UC.

Crohn's disease (CD) is a disease of chronic inflammation that can involve any part of the gastrointestinal tract. Commonly, the distal portion of the small intestine, i.e., the ileum, and the cecum are affected. In other cases, the disease is confined to the small intestine, colon, or anorectal region. CD occasionally involves the duodenum and stomach, and more rarely the esoph-

agus and oral cavity. The variable clinical manifestations of CD are, in part, a result of the varying anatomic localization of the disease. The most frequent symptoms of CD are abdominal pain, diarrhea, and recurrent fever. CD is commonly associated with intestinal obstruction or fistula, an abnormal passage between diseased loops of bowel.

Ulcerative colitis (UC) is a disease of the large intestine characterized by chronic diarrhea with cramping, abdominal pain, rectal bleeding, loose discharges of blood, pus, and mucus. The manifestations of UC vary widely. A pattern of exacerbations and remissions typifies the clinical course for about 70% of UC patients, although continuous symptoms without remission are present in some patients with UC. Local and systemic complications of UC include arthritis, eye inflammation such as uveitis, skin ulcers, and liver disease. In addition, UC, and especially the long-standing, extensive form of the disease is associated with an increased risk of colon carcinoma.

As used herein, the terms “autoimmune liver disease” and “inflammatory liver disease in which autoantibodies bind components of the liver” may be used interchangeably.

A preferred autoimmune disease of the invention is therefore an autoimmune disease of the liver of a subject. Such diseases are characterised in that the autoimmune disorder exhibits autoantibodies that bind components of the liver of said subject.

The three main categories of autoimmune liver disease are autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC). AIH is typically characterized as an unresolving hepatitis usually associated with hypergammaglobulinemia and tissue-directed autoantibodies and responding in most cases to immunosuppressive therapy. PBC is typically characterized as a chronic cholestatic liver disease in which the intrahepatic bile ducts are progressively destroyed by a nonsuppurative inflammatory process. PSC is typically characterized as a chronic cholestatic disorder characterized by fibrosis and inflammation of the extra- and intrahepatic biliary tree.

The term “sample” includes any biological specimen obtained from an individual. Suitable samples for use in the present invention include, without limitation, whole blood, plasma, serum, saliva, urine, stool, tears, any other bodily fluid, pure pancreatic juices or duodenal juices, tissue samples (e.g., biopsy) and cellular extracts thereof (e.g., red blood cellular extract). In a preferred embodiment, the sample is a serum sample. The use of samples such as serum, saliva, and urine is well known in the art (see, e.g., Hashida et al., J. Clin. Lab. Anal., 11:267-86 (1997)). One skilled in the art will appreciate that samples such as serum samples can be diluted prior to analysis.

The term “individual,” “subject,” or “patient” typically refers to humans, but also to other animals including, e.g., other primates, rodents, canines, felines, equines, ovines, porcines, and the like.

As used herein, the term “substantially the same or similar amino acid sequence” includes an amino acid sequence that is similar, but not identical to, the naturally-occurring amino acid sequence. For example, an amino acid sequence, i.e., polypeptide, that has substantially the same amino acid sequence as CHI3L1 in SEQ ID NO 1, 2, 3 and/or 4 and can have one or more modifications such as amino acid additions, deletions, or substitutions relative to the amino acid sequence of CHI3L1, provided that the modified polypeptide retains substantially at

least one biological activity of CHI3L1 such as immunoreactivity, in particular the immune reactivity specific to the diseases capable of being diagnosed according to the present invention.

A particularly useful modification of a polypeptide of the present invention, or a fragment thereof, is a modification that confers, for example, increased stability or reactivity. Incorporation of one or more D-amino acids is a modification useful in increasing stability of a polypeptide or polypeptide fragment. Similarly, deletion or substitution of lysine residues can increase stability by protecting the polypeptide or polypeptide fragment against degradation.

The amino acid sequences may also comprise 0 to 100, 2 to 50, 5 to 20, or for example 8 to 15, or any value from 0 to 20, amino acid additions or deletions at either the N- and/or C-terminus of the proteins. The termini may also be modified with additional linker sequences, or removal of sequences, as long as the autoantibody binding properties and immunoreactivity of the protein is essentially maintained and the autoantibodies as described herein bind in an analogous manner to the specific sequence provided.

Various ways of preparing functionally analogous peptides have been disclosed in the prior art. Peptides designed starting from the peptides of the invention using such methods are included in the teaching according to the invention. For example, one way of generating functionally analogous peptides has been described in PNAS USA 1998, Oct. 13, 9521, 12179-84; WO 99/6293 and/or WO 02/38592; the above teachings are hereby incorporated in the disclosure of the invention. That is, all peptides, peptide fragments or structures comprising peptides generated using the methods mentioned above - starting from the peptides of the invention - are peptides according to the invention, provided they accomplish the object of the invention and, in particular, interact with the pathogenic autoantibodies. For example, these autoantibodies can be agonistic autoantibodies activating receptors.

The CHI3L1 protein may also be described as an antigen, as it reacts with an antibody targeted to said CHI3L1 protein. CHI3L1 may also be referred to as a protein or target. For use in the methods of the invention, a CHI3L1 antigen can be partially purified. A CHI3L1 antigen also can be prepared recombinantly by expressing an encoding nucleic acid sequence as described herein using methods well known in the art (see, for example, Ausubel et al., Current Protocols in Molecular Biology John Wiley & Sons, Inc. New York (1999)).

The term “diagnosing” includes the use of the devices, methods, kits, and systems, of the present invention to determine the presence or absence of a medically relevant disorder in an individual. The term also includes devices, methods, kits, and systems for assessing the level of disease activity in an individual. In some embodiments, statistical algorithms are used to diagnose a mild, moderate, severe, or fulminant form of the disorder based upon the criteria developed by Truelove et al., Br. Med. J., 12:1041-1048 (1955). In other embodiments, statistical algorithms are used to diagnose a mild to moderate, moderate to severe, or severe to fulminant form of the IBD based upon the criteria developed by Hanauer et al., Am. J. Gastroenterol., 92:559-566 (1997).

The term “prognosis” relates to the prediction of an outcome or a specific risk for a subject to suffer from an autoimmune disease as described herein. This may also include an estimation of the chance of recovery or the chance of an adverse outcome for said subject.

In the present invention, the terms “risk assessment” and “risk stratification”, may be used interchangeably, and relate to the grouping of subjects into different risk groups according to their further prognosis. Risk assessment also relates to stratification for applying preventive and/or therapeutic measures.

The term “therapy control” in the context of the present invention refers to the monitoring and/or adjustment of a therapeutic treatment of said subject. As used herein, the term “therapy guidance” refers to application of certain therapies or medical interventions based on the value of the outcome of the methods described herein.

The invention also encompasses use of the method for “disease monitoring”, also known as monitoring the progression or regression of the autoimmune disease. The term “monitoring the progression or regression of the autoimmune disease” includes the use of the devices, methods, kits and systems of the present invention to determine the disease state (e.g., presence or severity of the autoimmune disease) of an individual. In certain instances, the results of a statistical algorithm (e.g., a learning statistical classifier system) are compared to those results obtained for the same individual at an earlier time. In some aspects, the devices, methods, kits and systems of the present invention can also be used to predict the progression of the autoimmune disease, e.g., by determining a likelihood for the autoimmune disease to progress either rapidly or slowly in an individual based on the presence or level of at least one marker in a sample. In other aspects, the devices, methods, and systems of the present invention can also be used to predict the regression of the autoimmune disease, e.g., by determining a likelihood for the autoimmune disease to regress either rapidly or slowly in an individual based on the presence or level of at least one marker in a sample. Therapy monitoring may also be conducted, whereby a subject is monitored for disease progression during the course of any given therapy.

The comparative analysis described herein between autoantibody binding to CHI3L1 in different patient populations, ie CD vs UC, is a preferred method of the present invention. Direct comparison based on autoantibody binding as measured in the same experiment may be used. For this embodiment the amount of CHI3L1 provided for the experiment should preferably be controlled carefully to enable direct comparative analysis. Alternatively, or in combination, control values or standards may be used that provide samples with autoantibodies or represent control amounts thereof, as have already been obtained from previous analytical tests. It is possible to use control values having been generated by the testing of cohorts or other large numbers of subjects suffering from any given disease or control group. Appropriate statistical means are known to those skilled in the art for analysis and comparison of such data sets. Control samples for positive controls (such as disease sufferers) or negative controls (such as from healthy subjects) may be used for reference values in either simultaneous of non-simultaneous comparison.

In certain instances, the presence or level of anti-CHI3L1 antibodies or at least one marker is determined using an immunoassay or an immunohistochemical assay. A non-limiting example of an immunoassay suitable for use in the method of the present invention includes an ELISA. Examples of immunohistochemical assays suitable for use in the method of the present invention include, but are not limited to, immunofluorescence assays such as direct fluorescent antibody assays, IFA assays, anticomplement immunofluorescence assays, and avidin-biotin immunofluorescence assays. Other types of immunohistochemical assays include immunoperoxidase assays.

In another advantageous embodiment the immunoassay is used in the detection of antibodies, to which end binding of CHI3L1 antigen to a solid phase is envisaged. Following addition of sample solution, the patient's antibody included therein binds to the CHI3L1 antigen. The antibody which is obtained e.g. from the serum or stool of a patient and bound to CHI3L1 is subsequently detected using a label, or labelled reagent and optionally quantified. Thus, according to the invention, detection of the antibodies in this method is effected using labelled reagents according to the well-known ELISA (Enzyme-Linked Immunosorbent Assay) technology. Labels according to the invention therefore comprise enzymes catalyzing a chemical reaction which can be determined by optical means, especially by means of chromogenic substrates, chemiluminescent methods or fluorescent dyes. In another preferred embodiment the autoantibodies are detected by labelling with weakly radioactive substances in radioimmunoassays (RIA) wherein the resulting radioactivity is measured.

As examples of means for detecting the label in the method of the present invention, a variety of immunoassay techniques, including competitive and non-competitive immunoassays, can be used to determine the presence or level of one or more markers in a sample (see, e.g., Self et al., Curr. Opin. Biotechnol., 7:60-65 (1996)). The term immunoassay encompasses techniques including, without limitation, enzyme immunoassays (EIA) such as enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), antigen capture ELISA, sandwich ELISA, IgM antibody capture ELISA (MAC ELISA), and microparticle enzyme immunoassay (MEIA); capillary electrophoresis immunoassays (CEIA); radioimmunoassays (RIA); immunoradiometric assays (IRMA); fluorescence polarization immunoassays (FPIA); and chemiluminescence assays (CL). If desired, such immunoassays can be automated. Immunoassays can also be used in conjunction with laser induced fluorescence (see, e.g., Schmalzing et al., Electrophoresis, 18:2184-2193 (1997); Bao, J. Chromatogr. B. Biomed. Sci., 699:463-480 (1997)). Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, are also suitable for use in the present invention (see, e.g., Rongen et al., J. Immunol. Methods, 204:105-133 (1997)). In addition, nephelometry assays, in which the formation of protein/antibody complexes results in increased light scatter that is converted to a peak rate signal as a function of the marker concentration, are suitable for use in the present invention. Nephelometry assays are commercially available from Beckman Coulter (Brea, Calif.; Kit #449430) and can be performed using a Behring Nephelometer Analyzer (Fink et al., J. Clin. Chem. Clin. Biol. Chem., 27:261-276 (1989)).

The immunoassays described above are particularly useful for determining the presence or level of one or more markers in a sample (and may be considered examples of means for detecting a label), wherein a marker may be understood as an autoantibody targeted to CHI3L1. As a non-limiting example, a fixed neutrophil ELISA is useful for determining whether a sample is positive for ANCA or for determining ANCA levels in a sample. Similarly, an ELISA using yeast cell wall phosphopeptidomannan is useful for determining whether a sample is positive for ASCA-IgA and/or ASCA-IgG, or for determining ASCA-IgA and/or ASCA-IgG levels in a sample. An ELISA using CHI3L1 protein or a fragment thereof is useful for determining whether a sample is positive for anti-CHI3L1 antibodies, or for determining anti-CHI3L1 antibody levels in a sample.

In another preferred embodiment of the method according to the invention the autoantibodies are detected in an immunoassay, preferably with direct or indirect coupling of one reactant to a

labelling substance. This enables flexible adaptation of the method to the potentials and requirements of different laboratories and their laboratory diagnostic equipment. In one advantageous embodiment the autoimmune disease-specific antibodies are detected in an immunoassay wherein the antibodies are present dissolved in a liquid phase, preferably diluted in a conventional buffer solution well-known to those skilled in the art or in an undiluted body fluid. According to the invention, detection can also be effected using stool samples.

In another preferred embodiment of the invention, soluble or solid phase-bound CHI3L1 molecules are used to bind the antibodies. In a second reaction step, anti-human immunoglobulins are employed, preferably selected from the group comprising anti-human IgA, anti-human IgM and/or anti-human IgG antibodies, said anti-human immunoglobulins being detectably labelled conjugates of two components which can be conjugated with any conventional labelling enzymes, especially chromogenic and/or chemiluminescent substrates, preferably with horseradish peroxidase, alkaline phosphatase. The advantage of this embodiment lies in the use of ELISA technology usually available in laboratory facilities so that detection according to the invention can be established in a cost-effective manner. In another preferred embodiment of the invention the antibody bound to CHI3L1 reacts with anti-human immunoglobulins, preferably selected from the group comprising anti-human IgA, anti-human IgM and/or anti-human IgG antibodies, detectably coupled to fluorescein isothiocyanate (FITC). Much like the above-mentioned ELISA, the FITC technology represents a system that is available in many places and therefore allows smooth and low-cost establishment of the inventive detection in laboratory routine.

Specific immunological binding of the antibody to the marker of interest can be detected directly or indirectly via a label. Any given means for detecting these labels may be considered means for detecting the label according to the method of the invention. Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. An antibody labeled with iodine-125 (125I) can be used for determining the levels of one or more markers in a sample. A chemiluminescence assay using a chemiluminescent antibody specific for the marker is suitable for sensitive, non-radioactive detection of marker levels. An antibody labeled with fluorochrome is also suitable for determining the levels of one or more markers in a sample. Examples of fluorochromes include, without limitation, DAPI, fluorescein, Hoechst 33258, R-phycocyanin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red, and lissamine. Secondary antibodies linked to fluorochromes can be obtained commercially, e.g., goat F(ab′)2 anti-human IgG-FITC is available from Tago Immunologicals (Burlingame, Calif.).

Indirect labels include various enzymes well-known in the art, such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, urease, and the like. A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate p-nitrophenyl phosphate, for example, which yields a soluble product readily detectable at 405 nm. Similarly, a β-galactosidase detection system can be used with the chromogenic substrate o-nitrophenyl-β-D-galactopyranoside (ONPG), which yields a soluble product detectable at 410 nm.

A signal from the direct or indirect label can be analyzed, for example, using a spectrophotometer to detect colour from a chromogenic substrate; a radiation counter to detect radiation such

as a gamma counter for detection of 125I; or a fluorometer to detect fluorescence in the presence of light of a certain wavelength. For detection of enzyme-linked antibodies, a quantitative analysis of the amount of marker levels can be made using a spectrophotometer such as an EMAX Microplate Reader (Molecular Devices; Menlo Park, Calif.) in accordance with the manufacturer's instructions. If desired, the assays of the present invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

In certain embodiments, the present invention provides methods of diagnosing the autoimmune disease or clinical subtypes thereof using CHI3L1. A variety of inflammatory bowel disease (the autoimmune disease) markers, such as biochemical markers, serological markers, genetic markers, or other clinical or echographic characteristics, are suitable for use and can be combined with statistical algorithms to classify a sample from an individual as an the autoimmune disease sample. Examples of markers of the diseases (an autoantibody directed against the CHI3L1 as described herein) suitable for use in the present invention include, but are not limited to, anti-neutrophil antibodies (e.g., ANCA, pANCA, cANCA, NSNA, SAPPA, etc.) or anti-Saccharomyces cerevisiae antibodies (e.g., ASCA-IgA, ASCA-IgG, ASCA-IgM, etc.).One skilled in the art will know of additional markers suitable for use in the statistical algorithms of the present invention.

The determination of ANCA levels and/or the presence or absence of pANCA in a sample is useful in the present invention. As used herein, the term “anti-neutrophil cytoplasmic antibody” or “ANCA” includes antibodies directed to cytoplasmic and/or nuclear components of neutrophils. ANCA activity can be divided into several broad categories based upon the ANCA staining pattern in neutrophils: (1) cytoplasmic neutrophil staining without perinuclear highlighting (cANCA); (2) perinuclear staining around the outside edge of the nucleus (pANCA); (3) perinuclear staining around the inside edge of the nucleus (NSNA); and (4) diffuse staining with speckling across the entire neutrophil (SAPPA). ANCA levels in a sample from an individual can be determined, for example, using an immunoassay such as an enzyme-linked immunosorbent assay (ELISA) with alcohol-fixed neutrophils.

The determination of ASCA (e.g., ASCA-IgA and/or ASCA-IgG) levels in a sample is also useful in the present invention. As used herein, the term “anti-Saccharomyces cerevisiae immunoglobulin A” or “ASCA-IgA” includes antibodies of the immunoglobulin A isotype that react specifically with S. cerevisiae. Similarly, the term “anti-Saccharomyces cerevisiae immunoglobulin G” or “ASCA-IgG” includes antibodies of the immunoglobulin G isotype that react specifically with S. cerevisiae. The determination of whether a sample is positive for ASCA-IgA or ASCA-IgG is made using an antigen specific for ASCA. Such an antigen can be any antigen or mixture of antigens that is bound specifically by ASCA-IgA and/or ASCA-IgG. Although ASCA antibodies were initially characterized by their ability to bind S. cerevisiae, those of skill in the art will understand that an antigen that is bound specifically by ASCA can be obtained from S. cerevisiae or from a variety of other sources as long as the antigen is capable of binding specifically to ASCA antibodies.

The invention also relates to protein and nucleic acid molecules corresponding to the sequences described herein, for example proteins or nucleic acid molecules comprising or consisting of said sequences.

The determination of autoantibodies to the CHI3L1 or use thereof in an ELISA as a solid-phase antigen in serological diagnostics of the diseases described herein has neither been considered nor mentioned in the prior art.

In another aspect, the invention relates to a method wherein human IgA, IgM and/or IgG antibody autoimmune diseases are detected.

As used herein, the term “CHI3L1”, “CHI3L1-isoform”, “CHI3L1-antigen”, “CHI3L1-molecule”, “CHI3L1-protein”, “CHI3L1-peptide” or “CHI3L1-autoantigen”, or other CHI3L1-referencing phrase relates to the CHI3L1 protein or substantially similar or functionally analogous versions of the sequence SEQ ID NO 1 (and/or preferably SEQ ID NO 2, 3 and/or 4) as disclosed herein. In a preferred embodiment of the method according to the invention the CHI3L1 is of human, animal, recombinant or synthetic origin.

In another preferred embodiment of the invention the CHI3L1 in accordance with one or more of the sequences disclosed herein is bound to a solid phase. Binding of CHI3L1 in accordance with one or more of the sequences disclosed herein to the solid phase can be effected via a spacer. All those chemical compounds having suitable structural and functional preconditions for spacer function can be used as spacers as long as they do not modify the binding behavior in such a way that binding of the CHI3L1 autoantibody in accordance with one or more of the sequences disclosed herein is adversely affected.

In another preferred embodiment of the invention the molecule comprises a linker or spacer selected from the group of $\alpha$-aminocarboxylic acids as well as homo- and heterooligomers thereof, $\alpha,\omega$-aminocarboxylic acids and branched homo- or heterooligomers thereof, other amino acids, as well as linear and branched homo- or heterooligomers; amino-oligoalkoxyalkylamines; maleinimidocarboxylic acid derivatives; oligomers of alkylamines; 4-alkylphenyl derivatives; 4-oligoalkoxyphenyl or 4-oligoalkoxyphenoxy derivatives; 4-oligoalkylmercaptophenyl or 4-oligoalkylmercaptophenoxy derivatives; 4-oligoalkylaminophenyl or 4-oligoalkylaminophenoxy derivatives; (oligoalkylbenzyl)phenyl or 4-(oligoalkylbenzyl)phenoxy derivatives, as well as 4-(oligoalkoxybenzyl)phenyl or 4-(oligoalkoxybenzyl)phenoxy derivatives; trityl derivatives; benzyloxyaryl or benzyloxyalkyl derivatives; xanthen-3-yloxyalkyl derivatives; (4-alkylphenyl)- or $\omega$-(4-alkylphenoxy)alkanoic acid derivatives; oligoalkylphenoxyalkyl or oligoalkoxyphenoxyalkyl derivatives; carbamate derivatives; amines; trialkylsilyl or dialkylalkoxysilyl derivatives; alkyl or aryl derivatives or combinations thereof.

According to the invention it is also preferred to perform the above-described detection method on a solid phase, for example by connection of the CHI3L1 molecule to the solid phase via a linker, in which case the storability of the peptide is advantageously increased as a result of the surprisingly stable linkage of the CHI3L1 antigen to the solid phase.

In another preferred embodiment of the invention the CHI3L1 molecule is used as a soluble or solid phase-bound autoantigen for direct or indirect autoantibody detection or removal in stool and/or body fluids, especially blood and/or serum, in which case the use of the CHI3L1 molecule in accordance with one or more of the sequences as disclosed herein was found particularly advantageous.

In another preferred embodiment of the invention the sequences according to the present application, or the peptides which can be generated therefrom, are immobilized. More specifically,

the solid phase-bound CHI3L1 molecule in accordance with one or more of the sequences as disclosed herein is bound to organic, inorganic, synthetic and/or mixed polymers, preferably agarose, cellulose, silica gel, polyamides and/or polyvinyl alcohols. In the meaning of the invention, immobilization is understood to involve various methods and techniques to fix the peptides on specific carriers, e.g. according to WO 99/56126 or WO 02/26292. For example, immobilization can serve to stabilize the peptides so that their activity would not be reduced or adversely modified by biological, chemical or physical exposure, especially during storage or in single-batch use. Immobilization of the peptides allows repeated use under technical or clinical routine conditions; furthermore, a sample - preferably blood components - can be reacted with at least one of the peptides according to the invention in a continuous fashion. In particular, this can be achieved by means of various immobilization techniques, with binding of the peptides to other peptides or molecules or to a carrier proceeding in such a way that the three-dimensional structure - particularly in the active center mediating the interaction with the autoantibodies - of the corresponding molecules, especially of said peptides, would not be changed. Advantageously, there is no loss in specificity to the autoantibodies of patients as a result of such immobilization. In the meaning of the invention, three basic methods can be used for immobilization:

(i) Crosslinking: in crosslinking, the peptides are fixed to one another without adversely affecting their activity. Advantageously, they are no longer soluble as a result of such crosslinking.

(ii) Binding to a carrier: binding to a carrier proceeds via adsorption, ionic binding or covalent binding, for example. Such binding may also take place inside microbial cells or liposomes or other membranous, closed or open structures. Advantageously, the peptides are not adversely affected by such fixing. For example, multiple or continuous use of carrier-bound peptides is possible with advantage in clinical diagnosis or therapy.

(iii) Inclusion: inclusion in the meaning of the invention especially proceeds in a semipermeable membrane in the form of gels, fibrils or fibers. Advantageously, encapsulated peptides are separated from the surrounding sample solution by a semipermeable membrane in such a way that interaction with the autoantibodies or fragments thereof still is possible. Various methods are available for immobilization, such as adsorption on an inert or electrically charged inorganic or organic carrier. For example, such carriers can be porous gels, aluminum oxide, bentonite, agarose, starch, nylon or polyacrylamide. Immobilization proceeds via physical binding forces, frequently involving hydrophobic interactions and ionic binding. Advantageously, such methods are easy to handle and have little influence on the conformation of the peptides. Advantageously, binding can be improved as a result of electrostatic binding forces between the charged groups of the peptides and the carrier, e.g. by using ion exchangers, particularly Sephadex.

Another method is covalent binding to carrier materials. In addition, the carriers may have reactive groups forming homopolar bonds with amino acid side chains. Suitable groups in peptides are carboxy, hydroxy and sulfide groups and especially the terminal amino groups of lysines. Aromatic groups offer the possibility of diazo coupling. The surface of microscopic porous glass particles can be activated by treatment with silanes and subsequently reacted with peptides. For example, hydroxy groups of natural polymers can be activated with bromocyanogen and subsequently coupled with peptides. Advantageously, a large number of peptides can undergo direct covalent binding with polyacrylamide resins. Inclusion in three-dimensional networks involves inclusion of the peptides in ionotropic gels or other structures well-known to those skilled

in the art. More specifically, the pores of the matrix are such in nature that the peptides are retained, allowing interaction with the target molecules. In crosslinking, the peptides are converted into polymer aggregates by crosslinking with bifunctional agents. Such structures are gelatinous, easily deformable and, in particular, suitable for use in various reactors. By adding other inactive components such as gelatin in crosslinking, advantageous improvement of mechanical and binding properties is possible. In microencapsulation, the reaction volume of the peptides is restricted by means of membranes. For example, microencapsulation can be carried out in the form of an interfacial polymerization. Owing to the immobilization during microencapsulation, the peptides are made insoluble and thus reusable. In the meaning of the invention, immobilized peptides are all those peptides being in a condition that allows reuse thereof. Restricting the mobility and solubility of the peptides by chemical, biological or physical means advantageously results in lower process cost, particularly when eliminating autoantibodies from blood components.

The invention also relates to a diagnostic kit for the determination of autoimmune diseases, comprising a CHI3L1 molecule in accordance with one or more of the sequences as disclosed herein. The diagnostic kit optionally includes instructions concerning combining the contents of the kit and/or providing a formulation for the detection of inflammatory bowel diseases, particularly Crohn's disease, autoimmune disease of the liver and/or ulcerative colitis. For example, the instruction can be in the form of an instruction leaflet or other medium providing the user with information as to the type of method wherein the substances mentioned are to be used. Obviously, the information need not necessarily be in the form of an instruction leaflet, and the information may also be imparted via the Internet, for example. To a patient, one advantageous effect of such a kit is, for instance, that he or she, without directly addressing a physician, can determine the actual state of a disease even during a journey and optionally adapt diet and activities accordingly.

**FIGURES**

Without intending to be limiting, the invention will be explained in more detail with reference to the figures.

**Figure 1:**

Enyzme-Linked Immunosorbent Assay (ELISA) for the detection of CHI3L1 antibodies.

First, the surface of the reaction vessel is coated with the CHI3L1 antigen (1). In the next step, unspecific binding sites are blocked with suitable components (here BSA) (2). This is followed by incubation with (diluted) patient material. In addition to the antibodies against CHI3L1, the patient material (or sample) also contains many different other antibodies and proteins, but only CHI3L1-specific antibodies can interact with the bound protein (3). In the next step, a detection antibody is used which can bind the antigen-specific antibodies (4). The detection antibody is conjugated with the horseradish peroxidase (HRPO), which is responsible for the reaction of an added substrate in the last step. The originally colourless substrate is rendered blue by the enzyme. After the reaction with sulfuric acid is stopped, a yellow colour develops in the reaction vessels (5). The optical density (OD) of the solution in the reaction vessels is then measured at a suitable wavelength and varies depending on the amount of the antigen-specific antibody initially bound.

**Figure 2:**

Purification of CHI3L1 expressed in SF9 insect cells by the Baculovirus system

Expression of 6xHis tagged CHI3L1 in Sf9 insect cells was induced by addition of Baculovirus. Since the protein is secreted to the cell growth media, the suspension was harvested by centrifugation and clarified by filtration. The supernatant was applied to Ni-NTA agarose, agarose was washed and the 6xHis tagged CHI3L1 was eluted with 100 mM imidazole elution buffer. All purification steps were analysed by SDS Page following Coomassie staining. Elution fractions two to four (E2- E4) contained the desired protein. Proteins were analyzed in each fraction, fractions were pooled (Em) and subjected to dialysis against the elution buffer without imidazole. M= Page Ruler unstained protein marker (kDa); CM= Sf9 cell culture medium with secreted CHI3L1; F= supernatant after application to Ni-NTA agarose; W= washing of Ni-NTA agarose; E2, E3, E4= different elution fraction containing CHI3L1; Em= mix of three elution fractions; Ed= dialysed protein.

**EXAMPLES**

Without intending to be limiting, the invention will be explained in more detail with reference to an example.

The experiments provided herein demonstrate that autoantibodies targeted to CHI3L1 can be used for the diagnosis of autoimmune disease.

<u>Development and employment of an anti-CHI3L1-ELISA:</u>

An embodiment of the invention is the Enyzme-Linked Immunosorbent Assay (ELISA) as shown in Fig. 1. This assay is performed essentially in 5 steps:

1. First, the antigen (CHI3L1) is applied to the surface of the reaction vessels and binds to it. Excess and unbound antigen is removed after the reaction by washing the reaction vessels.

2. Unspecific binding sites, e.g. Free areas of the vessel floor are blocked with suitable substances (e.g., bovine serum albumin (BSA), skimmed milk) so that in the next step only the antigen is available as a potential binding partner. Excess and unbound substances are removed by washing.

3. Subsequently, the patient material to be examined (e.g., blood serum) is added. This contains countless components, such as antibodies, antigens, proteins and various other blood components. However, only antibodies specifically directed against CHI3L1 can interact with it so that all other components can be removed. Excess and unbound components are removed by washing.

4. To detect antigen-specific antibodies, use is made of a secondary antibody, which in turn is specific to certain antibody structures (e.g., heavy chain of IgG or IgA or secretory component of secretory IgA). In addition, this antibody is conjugated with an enzyme, Horseradish Peroxidase (HRPO). Excess and unbound secondary antibodies are removed by washing.

5. In the last step, the detection is performed. A substrate is placed in the reaction vessel. This is typically 3, 3',5, 5'-tetramethylbenzidine (TMB), which assumes a blue colour by activation with HRPO. After a defined time, this reaction is stopped with sulfuric acid and a colour change

from blue to yellow takes place. The solution has an absorption maximum of 450 nm. At this wavelength, the optical density of the solution is determined. This varies depending on the amount of the antigen-specific antibody initially bound and is used for the subsequent evaluation of the results.

Detection of the presence of anti-CHI3L1 autoantibodies in patients with Crohn's disease (CD), ulcerative colitis (UC), autoimmune hepatitis (AIH) and/or primary sclerosing cholangitis (PSC):

Serum samples were obtained from 183 patients previously diagnosed with CD (n= 82), UC (n= 59), AIH (n= 19) and PSC (n= 23) as well as from 72 healthy donors. Samples were assessed for anti-CHI3L1 autoantibodies employing the ELISA technology described above.

In brief, reaction vessels were coated with CHI3L1 protein, unspecific binding was blocked, following incubation with diluted serum samples and HRPO-conjugated secondary detection antibody. After addition of TMB, the optical density (OD) was determined. Samples were measured in duplicates.

Data were evaluated as follows:

First, $OD_{450nm}$ values were corrected in relation to the blank value using the equation below.

$$OD\ 450nm\ = \frac{(OD\ 450nm\ 1 - Blank\ value) + (OD\ 450nm\ 2 - Blank\ value)\ \ldots}{N}$$

Further, the *cut-off* was defined by employing the mean (x) and standard deviation (s) of $OD_{450nm}$ values obtained for healthy donor sera:

$$cut - off = \bar{x} + 3s$$

**Table 1:** Prevalence of positive and negative sera of patients with inflammatory bowel and autoimmune liver disorders as well as controls in the anti-CHI3L1 (aCHI3L1) ELISA.

| | aCHI3L1 positive | | | aCHI3L1 negative | | | |
|---|---|---|---|---|---|---|---|
| | IgG | IgA | sIgA | IgG | IgA | sIgA | N |
| MC | 10 | 19 | 36 | 72 | 63 | 46 | 82 |
| CU | 0 | 5 | 10 | 59 | 54 | 49 | 59 |
| PSC | 2 | 1 | 7 | 21 | 22 | 16 | 23 |
| AIH | 10 | 3 | 6 | 9 | 16 | 13 | 19 |
| control | 2 | 2 | 3 | 70 | 70 | 69 | 72 |

N= total number of diseases-specific or control serum samples; MC= Crohn's disease; CU= colitis ulcerosa; PSC= primary sclerosing cholangitis; AIH= autoimmune hepatitis; sIgA= secre-tory IgA

**Table 2:** Statistical analysis of anti-CHI3L1 (aCHI3L1) ELISA data.

| | aCHI3L1 IgG | | | aCHI3L1 IgA | | | aCHI3L1 sIgA | | |
|---|---|---|---|---|---|---|---|---|---|
| | **positive** | **P- value** | **significance** | **positive** | **P- value** | **significance** | **positive** | **P- value** | **significance** |
| MC | 10 | 0,0359 | * | 19 | 0,0002 | *** | 36 | < 0,0001 | **** |
| CU | 0 | 0,5011 | ns | 5 | 0,2426 | ns | 10 | 0,019 | * |
| PSC | 2 | 0,246 | ns | 1 | 0,5691 | ns | 7 | 0,0016 | ** |
| AIH | 10 | < 0,0001 | **** | 3 | 0,0595 | ns | 6 | 0,0022 | ** |
| control | 2 | | | 2 | | | 3 | | |

MC= Crohn's Disease; CU= colitis ulcerosa; PSC= primary sclerosing cholangitis; AIH= auto-immune hepatitis;sIgA = secretory IgA
ns= not significant; *= p< 0.1; **= p< 0.01; ***= p< 0.001; ****= p< 0.0001.

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**CLAIMS:**

1. An in vitro method for diagnosis, prognosis, risk assessment, monitoring, therapy guidance and/or therapy control of an autoimmune disorder of the digestive system, comprising:

   - providing a sample of a subject exhibiting symptoms of and/or suspected of having said disorder,
   - providing Chitinase-3-Like Protein 1 (CHI3L1) protein,
   - contacting said sample with said CHI3L1 protein, and
   - detecting autoantibodies from said sample that bind to said CHI3L1 protein,
   - wherein the presence of said autoantibodies and/or increased levels of said autoantibodies compared to an appropriate control, such as a sample from a healthy subject, indicate the presence an autoimmune disorder of the digestive system.

2. The in vitro method according to claim 1, wherein the autoimmune disorder of the digestive system is an inflammatory intestinal disease in which autoantibodies bind components of the gastrointestinal tract of said subject.

3. The in vitro method according to the preceding claim, wherein the inflammatory intestinal disease is Crohn's disease (CD) and/or ulcerative colitis (UC).

4. The in vitro method according to claim 1, wherein the autoimmune disorder of the digestive system is an inflammatory liver disease in which autoantibodies bind components of the liver of said subject.

5. The in vitro method according to the preceding claim, wherein the inflammatory liver disease is autoimmune hepatitis and/or primary sclerosing cholangitis.

6. The in vitro method according to any one of the preceding claims, wherein the sample of a subject is as a bodily fluid selected from the group consisting of blood, a sample derived from blood, such as a plasma or serum sample, a stool sample or sample derived from stool, mucous secretions, such as tears, saliva, sweat or colostrum, and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium.

7. The in vitro method according to any one of the preceding claims, wherein the autoantibody detected is an IgG, IgA, IgM and/or secretory IgA autoantibody.

8. The in vitro method according to claim 1, wherein the autoimmune disorder of the digestive system is Crohn's disease (CD), ulcerative colitis (UC) or primary sclerosing cholangitis, and wherein the autoantibody detected is an IgA and/or secretory IgA autoantibody that binds CHI3L1 protein.

9. The in vitro method according to claim 1, wherein the autoimmune disorder of the digestive system is autoimmune hepatitis, and wherein the autoantibody detected is an IgG autoantibody that binds CHI3L1 protein.

10. The in vitro method according to claim 1 for use in the differential diagnosis between Crohn's disease (CD) and ulcerative colitis (UC), wherein the amount of IgA and/or secretory IgA autoantibodies from said sample that bind to said CHI3L1 protein indicates the presence of Crohn's disease (CD), when said amount is above a reference level, wherein said reference level corresponds to amounts of autoantibodies that bind to said CHI3L1 protein in patients with ulcerative colitis (UC).

11. The in vitro method according to any one of the preceding claims, wherein said method comprises:

- Bringing the sample of the subject into contact with a solid phase-immobilised CHI3L1 protein, allowing autoantibodies in said sample to bind to said immobilised CHI3L1 protein, thereby forming a solid phase-CHI3L1-autoantibody complex,
- Bringing the solid phase-CHI3L1-autoantibody complex into contact with a signal-producing affinity reagent that binds said complex, such as a labelled human anti-immunoglobulin antibody, and
- Detecting bound autoantibodies via the signal from said affinity reagent.

12. A kit for diagnosing an autoimmune disorder of the digestive system by detecting autoantibodies from a sample that bind to CHI3L1 protein, comprising:

- CHI3L1 protein immobilized on a solid phase, and
- reference data, such as a reference level, corresponding to anti-CHI3L1 autoantibody amounts in one or more of subjects with Crohn's disease (CD), ulcerative colitis (UC), autoimmune hepatitis and/or primary sclerosing cholangitis, and optionally additionally reference data corresponding to autoantibody amounts in healthy subjects, wherein said reference data is stored on a computer readable medium and/or is present in the form of computer executable code configured for comparing the determined amounts of autoantibodies that bind to said CHI3L1 protein in said sample to said reference data,
- a computer program adapted to perform the method of any of claims 1-11,

  and optionally

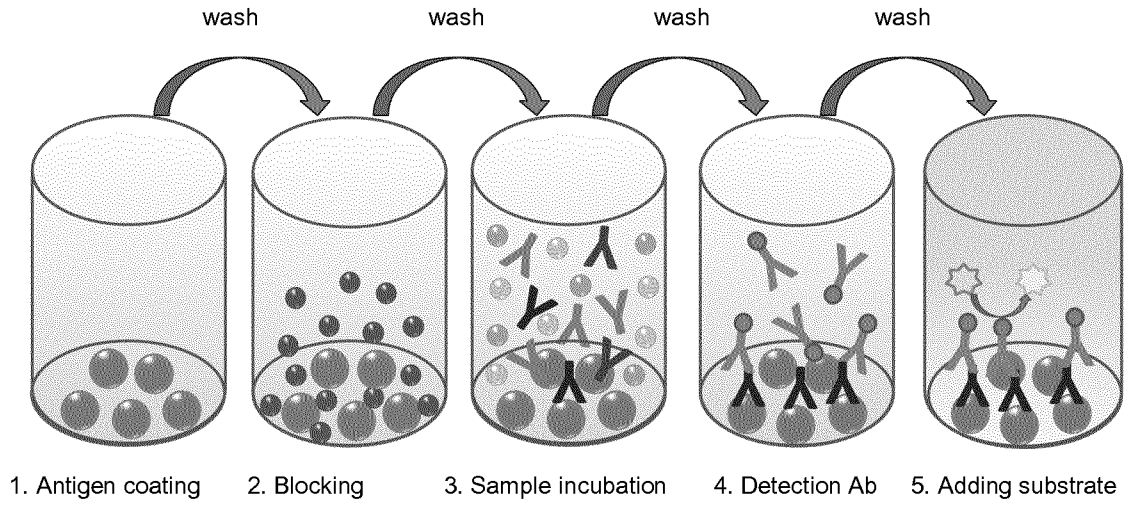
- one or more human anti-immunoglobulin antibodies, wherein said human anti-immunoglobulin antibodies bind autoantibodies of Ig-subtypes IgG, IgA and/or IgM, and/or

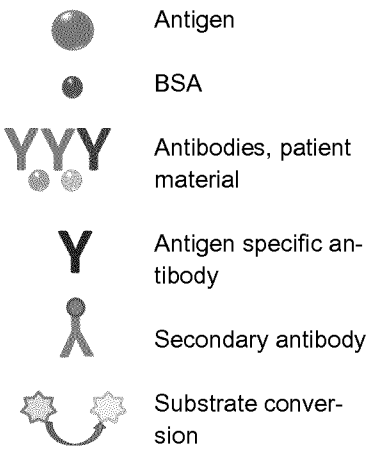
- a label for detection of said one or more human anti-immunoglobulin antibodies, either capable of binding said human anti-Immunoglobulin antibody or linked to said anti-Immunoglobulin antibody, and means for detecting said label.

13. System for diagnosing an autoimmune disorder of the digestive system by detecting autoantibodies from a sample that bind to CHI3L1 protein, comprising:

- CHI3L1 protein immobilized on a solid phase,
- A microscopic device configured to determine the presence and/or intensity of signal produced by a label bound to an autoantibody, and
- An optionally networked computer processing device configured to perform executable instructions; and a computer program, wherein the computer program is adapted to perform the method of any of claims 1-11 and comprises a software module executed by the computer processing device to apply a model or algorithm for analyzing said autoantibodies, wherein reference data is used, such as a reference level, corresponding to anti-CHI3L1 autoantibody amounts in one or more of subjects with Crohn's disease (CD), ulcerative colitis (UC), autoimmune hepatitis and/or primary sclerosing cholangitis, and optionally additionally reference data corresponding to autoantibody amounts in healthy subjects.

Fig. 1


wash
wash
wash
wash
1. Antigen coating
2. Blocking
3. Sample incubation
4. Detection Ab
5. Adding substrate


Antigen
BSA
Antibodies, patient material
Antigen specific antibody
Secondary antibody
Substrate conversion

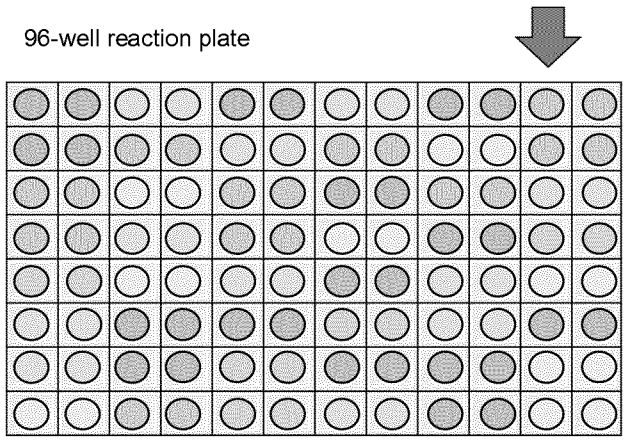
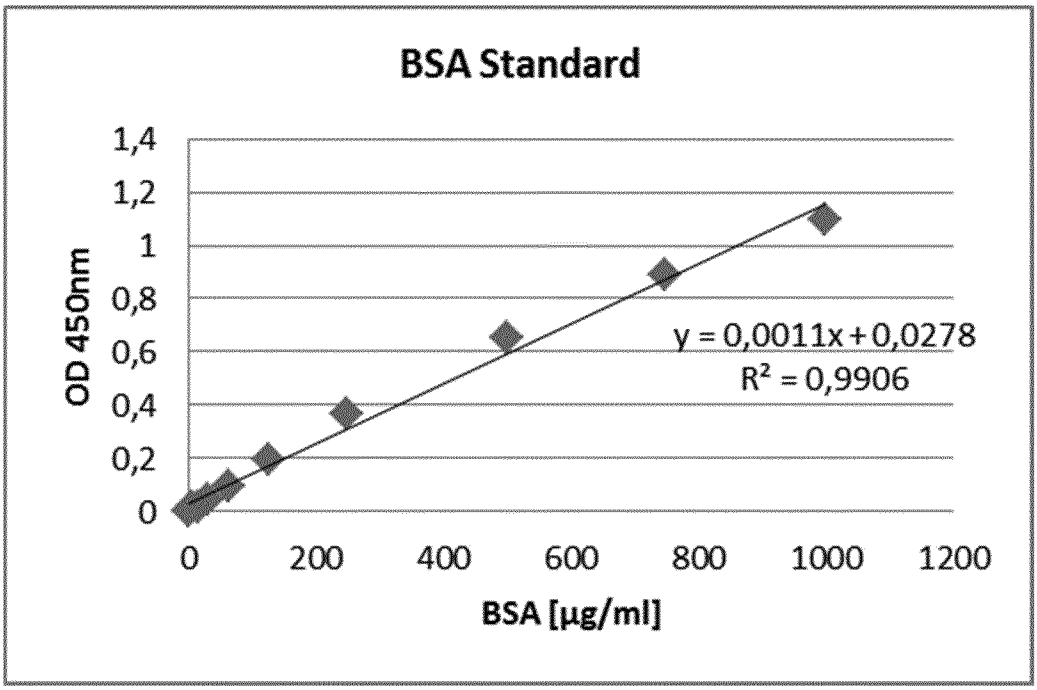

96-well reaction plate

Fig. 2

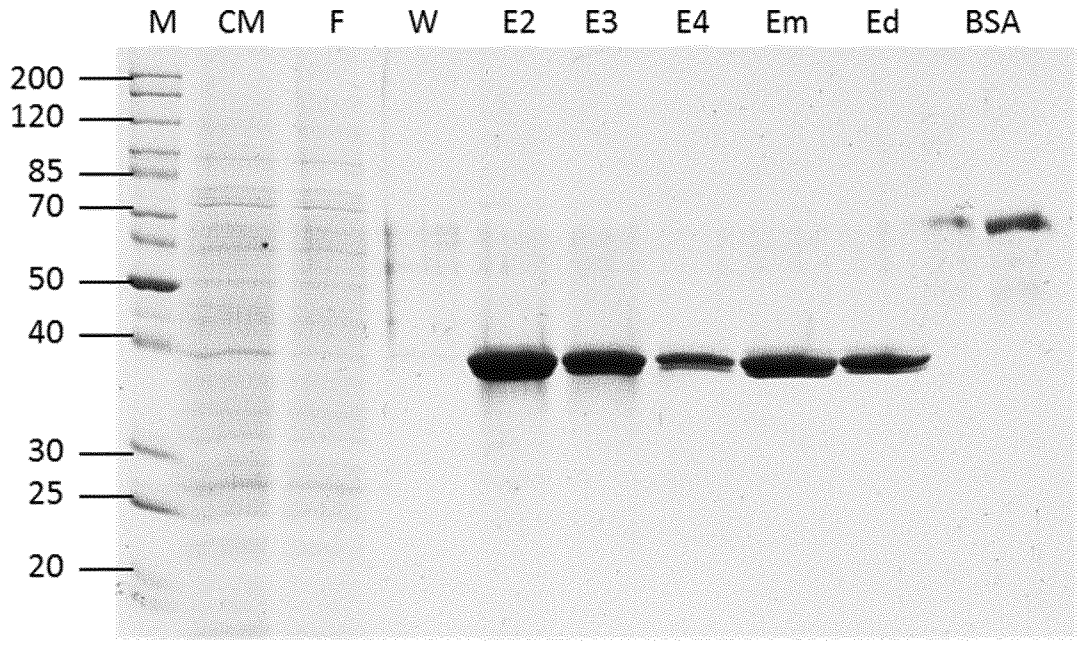
A




| | Mean | blank | Mean-Blank | dilution | µg/ml |
|---|---|---|---|---|---|
| OD1 | 0,1405 | 0,107 | 0,0335 | 10 | **279,27** |
| OD2 | 0,1235 | 0,107 | 0,0165 | 20 | **274,73** |

Fig. 2 (cont.)

**B**




M= Page Ruler unstained Protein Marker
CM= Sf9 Cell culture medium
F= Flowthrough
W= Wash
E2-E4= Elution steps
Em= mix of E2-4
Ed= dialysed sample

E2= ~ 500 µg/ml
E3= ~ 250 µg/ml
E4= ~ 100 µg/ml
Em= ~ 260 µg/ml
Ed= ~ 260 µg/ml

# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/058219

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/564
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, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | YUSUF ERZIN ET AL: "Serum YKL-40 as a marker of disease activity and stricture formation in patients with Crohn's disease", JOURNAL OF GASTROENTEROLOGY AND HEPATOLOGY, vol. 23, no. 8pt2, 1 August 2008 (2008-08-01), pages e357-e362, XP055403215, AU ISSN: 0815-9319, DOI: 10.1111/j.1440-1746.2007.05121.x whole document, in particular abstract<br>-----<br>-/-- | 1-13 |

[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

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"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 |
|---|---|
| 17 April 2018 | 02/05/2018 |

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

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

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | MARTIN W. LAASS ET AL: "Diagnosis and classification of Crohn's disease", AUTOIMMUNITY REVIEWS, vol. 13, no. 4-5, 1 April 2014 (2014-04-01), pages 467-471, XP055403529, NL ISSN: 1568-9972, DOI: 10.1016/j.autrev.2014.01.029 cited in the application whole document, in particular table 1 and paragraph 4.3 | 1-13 |
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Information on patent family members

International application No
PCT/EP2018/058219

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | US 2015247850 A1 | 03-09-2015 |