

Enhanced Liver Fibrosis (ELF™)

Current Revision and Date^a	Rev. 02, 2023-01	
Product Name	Atellica IM Enhanced Liver Fibrosis (ELF)	REF 11208118
Abbreviated Product Name	Atellica IM ELF	
Test Name/ID	ELF	
Systems	Atellica IM Analyzer	
Materials Provided	Atellica IM Hyaluronic Acid (HA)	50 tests
	Atellica IM Amino-Terminal Propeptide of Type III Procollagen (PIIINP)	50 tests
	Atellica IM Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP-1)	50 tests
	Atellica IM ELF CAL	
Materials Required but Not Provided	Atellica IM ELF QC	REF 10995566
	Atellica IM PW3 (for PIIINP)	REF 10995666
Optional Materials	Atellica IM ELF MCM	REF 11208708
	Atellica IM Multi-Diluent 10 (diluent) (for TIMP-1)	REF 10995640
	Atellica IM Multi-Diluent 13 (diluent) (for HA and PIIINP)	REF 10995643
Specimen Types	Serum	
Sample Volume	Atellica IM HA: 20 µL Atellica IM PIIINP: 20 µL Atellica IM TIMP-1: 25 µL	
Measuring Interval	Atellica IM HA: 3.00–1000.00 ng/mL Atellica IM PIIINP: 1.00–150.00 ng/mL Atellica IM TIMP-1: 5.0–1300.0 ng/mL	

^a A vertical bar in the page margin indicates technical content that differs from the previous version.



Intended Use

Atellica® IM Enhanced Liver Fibrosis (ELF™) is for *in vitro* diagnostic use in the determination of an ELF score based on the combined quantitative measurements of hyaluronic acid, amino-terminal propeptide of type III procollagen, and tissue inhibitor of matrix metalloproteinase-1 in human serum using the Atellica® IM Analyzer.

Atellica IM ELF is indicated, in conjunction with other laboratory findings and clinical assessments, for the assessment of severity of liver fibrosis in patients with signs, symptoms, or risk factors of chronic liver disease. Atellica IM ELF may be used diagnostically for fibrosis staging or prognostically to assess the likelihood of progression to cirrhosis and liver-related clinical events.

Summary and Explanation

Chronic liver disease is a major cause of morbidity and mortality throughout the world.¹⁻³ The clinical course of chronic liver disease is highly variable, but the typical progression involves the advancement of fibrosis, leading to cirrhosis, followed by either decompensation or hepatocellular carcinoma (HCC), and ultimately to liver transplantation or death.⁴

Typical disease etiologies that lead to chronic liver disease include non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and viral hepatitis due to chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV).

NAFLD and NASH

NAFLD is now the most common underlying cause of chronic liver disease.^{5,6} In 1994, NAFLD accounted for < 50% of chronic liver disease cases, but due to the increasing rate of obesity, the proportion of NAFLD of chronic liver disease cases jumped to 75% by 2008.⁵ The global prevalence of NAFLD is estimated to be 25% in the general population and 56% in individuals with type-2 diabetes mellitus.^{7,8} Furthermore, it is anticipated that the prevalence of NAFLD will continue to rise in parallel with the increasing incidence of obesity and type-2 diabetes mellitus.⁹

At least 20%–30% of NAFLD patients will develop the more severe form of the disease termed nonalcoholic steatohepatitis (NASH), which is characterized by hepatic steatosis (fatty infiltration of the liver), inflammation, and hepatocyte injury (ballooning).^{10,11}

The prevalence of NASH in obese individuals is up to 56%, with a worldwide prevalence of NASH among biopsied NAFLD patients of 59%.^{8,12} In the United States, waitlist registrations for transplants due to NASH have increased 170% from 2004 to 2013.¹³ Over the next 20 years, it is estimated that the 6.4 million individuals living with NASH and type-2 diabetes mellitus in the U.S. will account for 65,000 transplants, 1.37 million cardiovascular-related deaths, and 812,000 liver-related deaths.¹⁴

ALD

ALD has higher liver-related mortality than any other etiology of chronic liver disease.^{15,16} Alcohol-attributable liver cirrhosis is the cause of 14 million years of life lost every year.¹⁷ Compared to NAFLD, ALD patients progress faster and are more likely to have cirrhosis at the time of diagnosis.¹⁸

Chronic Viral Hepatitis

HBV and HCV can cause chronic infections leading to viral hepatitis.¹⁹ Viral hepatitis remains the most common cause of liver deaths worldwide, with an estimated 55% of cirrhosis-related deaths in 2017 due to HBV and HCV.²⁰ Chronic HBV infection (CHB) progresses to cirrhosis in up to 40% of cases if left untreated.²¹ The risk of cirrhosis and HCC are positively correlated with increases in serum HBV DNA copies/mL.²² Although current treatments can slow the progression of liver disease, cure rates are low and most patients require therapy indefinitely.²¹

Chronic HCV infection (CHC) is defined as the persistence of HCV RNA in the blood for more than 6 months after the onset of acute infection. An estimated 20%–30% of CHC patients will progress to cirrhosis over a 25–30 year period, with older age at infection (> 40 years) associated with a faster rate of fibrosis progression.²³

The introduction of highly efficacious direct-acting antiviral (DAA) drug regimens has led to a cure for more than 95% of patients. Patients are considered cured if they have sustained virological response (SVR), defined by the absence of detectable HCV RNA in the blood at least 12 weeks after treatment completion.²⁴ SVR is associated with fibrosis improvement and reduced risk of HCC development and all-cause mortality.^{25,26} Patients with established cirrhosis at SVR and patients deemed to be at high-risk require continued surveillance for HCC.²⁷

Chronic Cholestatic Liver Disease

PBC and PSC are the 2 major types of chronic cholestatic liver disease, both leading to bile duct deterioration. PBC is a progressive autoimmune liver disease primarily affecting women, characterized by the presence of antimitochondrial antibodies, and T-lymphocyte-mediated destruction of small intrahepatic bile ducts, and portal inflammation with fibrosis. Histologic fibrosis stage has been shown to predict survival. Without effective therapy, PBC advances by approximately one histological stage every 1.5 years, with a median survival of 9 years.²⁸

PSC is an idiopathic cholestatic hepatobiliary disease characterized by chronic inflammation, progressive fibrosis, and narrowing of bile ducts. PSC is closely associated with inflammatory bowel disease, with ulcerative colitis seen in up to 86% of PSC patients. PSC can be categorized into small-duct and large-duct variants, with large-duct PSC considered to be the more progressive form of the disease and having an increased risk of liver transplant or death. The median time from diagnosis to liver transplant or death ranges from 12 to 21 years.²⁸

Early Detection of Chronic Liver Disease is Needed

Many cases of chronic liver disease are detected only after patients are admitted via the emergency department with end-stage liver disease.²⁹ This highlights the need to identify and manage patients most at risk of developing liver complications at an earlier stage of disease. Studies have shown that patients with chronic liver disease can be identified earlier in primary care using noninvasive tests, including serum-based methods.^{30–33}

ELF

Atellica IM ELF quantifies analytes that are components of the extracellular matrix (ECM), which directly contribute to liver fibrosis. Hyaluronic acid (HA), a glycosaminoglycan produced by hepatic stellate cells, is an essential component within the connective matrix.

Amino-terminal propeptide of type III procollagen (PIIINP), produced by fibroblasts in the liver, is a marker of fibrogenesis and inflammation. Tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) is a circulating inhibitor of matrix metalloproteinase (MMP) enzymes that can inhibit fibrolysis and fibrosis repair. Together, these analytes reflect qualitative and quantitative changes in the ECM associated with progression of liver disease.^{34–37}

The ECM refers to a set of macromolecules that comprises the extracellular scaffolding of the liver. Some ECM markers reflect fibrogenesis and others reflect fibrosis regression, allowing for a dynamic evaluation of ECM activity. The ELF score, derived from an algorithm that combines the individual measurements of HA, PIIINP, and TIMP-1, has been demonstrated to be useful for the assessment of severity of liver fibrosis in patients with chronic liver disease.^{35,37}

ELF for Diagnostic Use (Correlation to Histology)

Histological liver fibrosis stage is known to predict the occurrence of liver-related outcomes in patients with chronic liver disease independent of etiology.^{38–44} Studies have shown that the ELF score correlates with histology in multiple chronic liver disease etiologies, including NAFLD/NASH,^{45–48} ALD,^{33,49} PBC,^{50,51} viral hepatitis,^{52–56} and mixed populations.^{35,57,58} These studies indicate that Atellica IM ELF can be used as an effective surrogate marker for liver fibrosis staging.

ELF for Prognostic Use (Correlation to Disease Progression Events)

Independent of histology, the ELF score has been shown to be useful as a prognostic marker in patients with chronic liver disease to determine the likelihood of progressing to cirrhosis and the occurrence of future liver-related clinical events, including decompensation, HCC, liver transplant, and death. The prognostic ability of the ELF score has been demonstrated in a variety of chronic liver disease etiologies, including NAFLD/NASH,⁵⁹⁻⁶³ ALD,⁶⁴ PBC,^{50,65} PSC,⁶⁶⁻⁶⁹ viral hepatitis,^{56,70-74} and mixed populations.^{57,75-78}

A prognostic risk assessment using Atellica IM ELF, in conjunction with other laboratory findings and clinical assessments, may be useful in determining which patients could benefit from additional examinations, from increased monitoring, and from potential lifestyle changes and treatment interventions.

ELF for Early Identification of Chronic Liver Disease

Additionally, Atellica IM ELF has been used to identify unsuspected advanced fibrosis, including in patients with normal liver function tests.^{30,31} Implementation of a 2-step algorithm consisting of Fibrosis-4 (FIB-4), another marker of liver fibrosis, and Atellica IM ELF led to a substantial improvement in detection of cases of advanced fibrosis and cirrhosis, and a significant reduction of unnecessary referrals from primary care to secondary care.³² These studies emphasize the value of testing with Atellica IM ELF to identify at-risk patients at an early stage of chronic liver disease.

Principles of the Procedure

The reagents that comprise Atellica IM ELF are Atellica IM HA, Atellica IM PIIINP, and Atellica IM TIMP-1. These reagents are based on 2-site sandwich assay methodology and direct chemiluminescent technology, and are run on fully automated Atellica IM Analyzers.

Atellica IM HA reagents use hyaluronic acid binding protein (HABP) for both capture and detection of HA. The Lite Reagent contains HABP conjugated with acridinium ester, and the Ancillary Well Reagent contains HABP conjugated with fluorescein isothiocyanate (FITC). The Solid Phase contains an anti-FITC mouse monoclonal antibody covalently bound to paramagnetic particles.

Atellica IM PIIINP reagents use 2 mouse monoclonal antibodies (MAb). The antibody in the Lite Reagent is an acridinium-ester-labeled anti-PIIINP antibody. The antibody in the Ancillary Well Reagent is a biotin-labeled anti-PIIINP antibody. The Solid Phase contains streptavidin-coated paramagnetic particles.

Atellica IM TIMP-1 reagents use 2 mouse monoclonal antibodies (MAb) that bind to TIMP-1 in the Lite Reagent: an anti-TIMP-1 F(ab)2 antibody labeled with acridinium ester and an FITC-labeled anti-TIMP-1 antibody. The Solid Phase contains an anti-FITC mouse monoclonal antibody covalently bound to paramagnetic particles.

A direct relationship exists between the amount of HA, PIIINP, and TIMP-1 present in the patient sample and the amount of relative light units (RLUs) detected by the system.

For Atellica IM Analyzer systems, the ELF score is calculated using the following equation:

$$\text{ELF score} = 2.278 + 0.851 \ln(C_{\text{HA}}) + 0.751 \ln(C_{\text{PIIINP}}) + 0.394 \ln(C_{\text{TIMP-1}})$$

Concentrations (C) of each of the constituents are in ng/mL.

Reagents

Material Description	Storage	Stability
Atellica IM HA ReadyPack® primary reagent pack^{a, b} Lite Reagent 10.0 mL/reagent pack Hyaluronic acid binding protein (bovine) labeled with acridinium ester (~0.5 µg/mL) in buffer; bovine serum albumin; surfactant; preservatives Solid Phase 10.0 mL/reagent pack Paramagnetic particles coated with mouse monoclonal anti-FITC (~37.8 µg/mL) in buffer; bovine serum albumin; surfactant; sodium azide (< 0.1%) Ancillary Well Reagent 5.0 mL/reagent pack Hyaluronic acid binding protein (bovine) labeled with FITC (~0.55 µg/mL) in buffer; bovine serum albumin; surfactant	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
Atellica IM PIINP ReadyPack® primary reagent pack^{a, b} Lite Reagent 5.0 mL/reagent pack Mouse monoclonal anti-PIINP labeled with acridinium ester (~2.0 µg/mL) in buffer; bovine serum albumin; mouse IgG; surfactant; sodium azide (< 0.1%) Solid Phase 15.0 mL/reagent pack Streptavidin-coated paramagnetic microparticles (~0.225 mg/mL) in buffer; bovine serum albumin; surfactant; sodium azide (< 0.1%) Ancillary Well Reagent 5.0 mL/reagent pack Mouse monoclonal anti-PIINP labeled with biotin (~0.7 µg/mL) in buffer; bovine serum albumin; mouse IgG; surfactant; sodium azide (< 0.1%)	Unopened at 2–8°C Onboard	Until expiration date on product 28 days
Atellica IM TIMP-1 ReadyPack® primary reagent pack^{a, b} Lite Reagent 10.0 mL/reagent pack Mouse monoclonal anti-TIMP-1 labeled with acridinium ester (~0.1 µg/mL) and mouse monoclonal anti-TIMP-1 labeled with FITC (~1.8 µg/mL) in buffer; goat serum; bovine serum albumin; bovine gamma globulin; mouse IgG; surfactant; preservatives Solid Phase 17.5 mL/reagent pack Paramagnetic particles coated with mouse monoclonal anti-FITC (~26.3 µg/mL) in buffer; goat serum; bovine serum albumin; bovine gamma globulin; surfactant; preservatives	Unopened at 2–8°C Onboard	Until expiration date on product 28 days

Material Description	Storage	Stability
Atellica IM ELF CAL^a 2.0 mL/vial After reconstitution, low or high levels of HA, PIINP (bovine), TIMP-1 (human); bovine serum albumin, buffer; sodium azide (< 0.1%); preservative	Lyophilized at 2–8°C	Until the expiration date on the vial label
	Reconstituted at 2–8°C	60 days
	Reconstituted at room temperature	8 hours
Atellica IM Multi-Diluent 10 ReadyPack ancillary reagent pack^{b, c} 5.0 mL/pack Tris buffer; bovine serum albumin; goat serum; mouse serum; sodium azide (< 0.1%); preservatives	Unopened at 2–8°C	Until expiration date on product
	Onboard	28 days
Atellica IM Multi-Diluent 13 ReadyPack ancillary reagent pack^{b, c} 10.0 mL/pack Buffer; surfactant; sodium azide (< 0.1%)	Unopened at 2–8°C	Until expiration date on product
	Onboard	28 days
Atellica IM PW3^{b, d} 50.0 mL/pack Sodium hypochlorite (0.5%), sodium hydroxide (< 0.5%), pH 11.0	Unopened at 2–8°C	Until expiration date on product
	Onboard	100 days

^a Store in an upright position.

^b Prevent exposure to sunlight and heat.

^c Refer to *Optional Materials*.

^d Refer to *Materials Required but Not Provided*.

Warnings and Precautions

For *in vitro* diagnostic use.

For Professional Use.

For Prescription Use Only.

CAUTION

Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

Safety data sheets (SDS) available on [siemens-healthineers.com](https://www.siemens-healthineers.com).



**H312, H412
P280, P273, P312,
P501**

Warning!

Harmful in contact with skin. Harmful to aquatic life with long lasting effects.

Wear protective gloves/protective clothing/eye protection/face protection. Avoid release to the environment. Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Sodium azide (Atellica IM ELF CAL).

**H412
P273, P501**

Harmful to aquatic life with long lasting effects.

Avoid release to the environment. Dispose of contents and container in accordance with all local, regional, and national regulations.

Contains: Sodium hypochlorite (Atellica IM PW3 for Atellica IM PIINP assay).

**Warning! Potential Biohazard**

Contains human source material.

No known test method can ensure that products derived from human source materials will not transmit infection. These materials should be handled using good laboratory practices and universal precautions.⁷⁹⁻⁸¹

CAUTION

This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

Storage and Stability

Store all reagents in an upright position, away from light and heat. Do not use products beyond the expiration date printed on the product labeling.

For information about storage and stability, refer to *Reagents*.

Onboard Stability

Discard products at the end of the onboard stability interval. Do not use products beyond the expiration date printed on the product labeling.

For information about product onboard stability, refer to *Reagents*.

Specimen Collection and Handling

Serum is the recommended specimen type for this assay.

The handling and storage information provided here is based on data or references maintained by the manufacturer. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Collecting the Specimen

- Observe universal precautions when collecting specimens. Handle all specimens as if they are capable of transmitting disease.⁸¹
- Follow recommended procedures for collection of diagnostic blood specimens by venipuncture.⁸²
- Follow the instructions provided with your specimen collection device for use and processing.⁸³
- Allow blood specimens to clot completely before centrifugation.⁸⁴
- Keep tubes capped at all times.⁸⁴

Storing the Specimen

- Specimens may be stored on the clot for up to 48 hours at 2–8°C.
- Samples may be stored at room temperature for up to 24 hours prior to centrifugation.

- Separated samples are stable for 8 hours onboard the system, for 48 hours at room temperature, and for 7 days at 2–8°C.
- Separated samples are stable at ≤ -20°C for up to 12 months. Avoid more than 4 freeze-thaw cycles. Do not store in a frost-free freezer. Thoroughly mix thawed samples and centrifuge them before using.

Transporting the Specimen

Package and label specimens for shipment in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiological agents.

If during shipment, specimens may be subjected to temperatures > 25°C, then ship specimens frozen.

Preparing the Samples

For a single determination, measurement of HA requires 20 µL of sample, measurement of PIIINP requires 20 µL of sample, and measurement of TIMP-1 requires 25 µL of sample. These volumes do not include the unusable volume in the sample container, or the additional volume required when performing duplicates or other tests on the same sample. For a complete list of appropriate sample containers and information about determining the minimum required volume, refer to the system online help.

The sample volume required to perform onboard dilution may differ from the sample volume required to perform a single determination on an undiluted sample. Refer to *Dilutions*.

Do not use specimens with apparent contamination.

Before placing samples on the system, ensure that samples are free of:

- Bubbles or foam.
- Fibrin or other particulate matter.

Remove particulates by centrifugation according to CLSI guidance and the collection device manufacturer's recommendations.⁸⁴

Procedures

Materials Provided

The following materials are provided:

REF	Contents	Number of Tests
11208118	1 ReadyPack primary reagent pack containing Atellica IM HA Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack primary reagent pack containing Atellica IM PIIINP Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack primary reagent pack containing Atellica IM TIMP-1 Lite Reagent and Solid Phase Atellica IM HA master curve card Atellica IM PIIINP master curve card Atellica IM TIMP-1 master curve card 1 vial of Atellica IM ELF CAL low calibrator CAL L 1 vial of Atellica IM ELF CAL high calibrator CAL H Atellica IM ELF CAL calibrator assigned value cards CAL LOT VAL	50

Materials Required but Not Provided

The following materials are required to perform Atellica IM ELF, but are not provided:

REF	Description
	Atellica IM Analyzer ^a
10995566	Atellica IM ELF™ QC (quality control material)
10995666	Atellica IM PW3 (probe wash) PW 3 (For Atellica IM PIIINP)

^a Additional system fluids are required to operate the system: Atellica IM Wash, Atellica IM Acid, Atellica IM Base, and Atellica IM Cleaner. For system fluid instructions for use, refer to the Document Library.

Optional Materials

The following materials may be used to perform Atellica IM ELF, but are not provided:

REF	Description
11208708	Atellica IM ELF™ MCM (master curve material)
10995640	Atellica IM Multi-Diluent 10 (diluent) DIL (For Atellica IM TIMP-1)
10995643	Atellica IM Multi-Diluent 13 (diluent) DIL (For Atellica IM HA and Atellica IM PIIINP)

Assay Procedure

Measurements for HA, PIIINP, and TIMP-1 must be obtained within 8 hours of one another for the ELF score to be valid.

The Atellica IM ELF score is automatically calculated by the Atellica IM Analyzer and is reported along with results for HA, PIIINP, and TIMP-1.

Refer to *Calculation of Results* for more information.

Measurement of HA

The system automatically performs the following steps:

1. Dispenses 20 µL of sample into a cuvette.
2. Dispenses 100 µL of Ancillary Well Reagent and 200 µL of Solid Phase, then incubates for 18 minutes at 37°C.
3. Separates, aspirates, then washes the cuvette with Atellica IM Wash 1.
4. Resuspends the particles in 115 µL of Atellica IM Wash 1 and incubates for approximately 7 minutes.
5. Dispenses 200 µL of Lite Reagent, then incubates for 18 minutes at 37°C.
6. Separates, aspirates, then washes the cuvette with Atellica IM Wash 1.
7. Dispenses 300 µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescent reaction.
8. Reports results.

Measurement of PIIINP

The system automatically performs the following steps:

1. Dispenses 20 µL of sample into a cuvette.
2. Dispenses 300 µL of Solid Phase and 100 µL of Ancillary Well Reagent, then incubates for 3 minutes at 37°C.
3. Dispenses 100 µL of Lite Reagent, then incubates for 6 minutes at 37°C.
4. Separates, aspirates, then washes the cuvette with Atellica IM Wash 1.
5. Dispenses 300 µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescent reaction.
6. Reports results.

Measurement of TIMP-1

The system automatically performs the following steps:

1. Dispenses 25 µL of sample into a cuvette.
2. Dispenses 350 µL of Solid Phase and 200 µL of Lite Reagent, then incubates for 3 minutes at 37°C.
3. Separates, aspirates, then washes the cuvette with Atellica IM Wash 1.
4. Dispenses 300 µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescent reaction.
5. Reports results.

Preparing the Reagents

All reagents are liquid and ready to use. Before loading the packs onto the system, reagents require mixing. For information about mixing the reagents, refer to the system online help.

Preparing the System

Ensure that sufficient reagent packs are loaded onto the system. Refer to *Materials Provided* and *Materials Required but Not Provided* for guidance about required reagents.

For information about loading products, refer to the system online help.

Master Curve Definition

Before initiating calibration on each new lot of reagent, enter the master curve values by scanning the master curve cards. For information about defining the master curve, refer to the system online help.

Performing Calibration

For calibration of Atellica IM ELF, use the calibrators provided with each kit to calibrate measurements of HA, PIIINP and TIMP-1.

Note Calibrators provided in an assay kit must only be used with the reagent lot provided in the same kit.

Calibration Frequency

Perform a calibration if one or more of the following conditions exist:

- When changing lot numbers of primary reagent packs.
- At the end of the lot calibration interval, for a specified lot of calibrated reagent on the system.

- At the end of the pack calibration interval, for calibrated reagent packs on the system.
- When indicated by quality control results.
- After major maintenance or service, if indicated by quality control results.

At the end of the onboard stability interval, replace the reagent pack on the system with a new reagent pack. Recalibration is not required, unless the lot calibration interval is exceeded.

Reagent	Stability Interval	Days
HA	Onboard Stability	28
	Pack Calibration Interval	14
	Lot Calibration Interval	24
PIINP	Onboard Stability	28
	Pack Calibration Interval	14
	Lot Calibration Interval	24
TIMP-1	Onboard Stability	28
	Pack Calibration Interval	14
	Lot Calibration Interval	24

Follow government regulations or accreditation requirements for calibration frequency. Individual laboratory quality control programs and procedures may require more frequent calibration.

Preparing the Calibrators

Prepare calibrators using the following steps:

1. Add 2.0 mL of special reagent water into each vial. Replace cap.

Note For information about reagent water requirements, refer to the system online help.

2. Let the vials stand for 15–20 minutes at room temperature to allow the lyophilized material to dissolve.
3. Gently mix and invert the vials to ensure homogeneity of the material.

Note Use calibrators within the stability limits specified in *Reagents* and discard any remaining material.

Calibration Procedure

Note Dispose of any calibrator that remains in the sample container after 8 hours. Do not refill or reuse sample containers. Do not return any calibrator material back into the original container.

The required sample volume for testing depends on several factors. For information about sample volume requirements, refer to the system online help.

Use the following lot-specific materials to perform calibration:

- For the master curve and assay test definitions, refer to the lot-specific master curve and test definition sheet **MC TDEF** provided with the assay reagents.
- Calibrators provided in an assay kit must only be used with reagents from that assay kit lot. Do not use calibrators from one assay kit lot with reagents from a different assay kit lot.

- For the calibrator definitions, refer to the calibrator assigned value sheet

CAL	LOT	VAL
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 provided with the calibrator materials
- Generate lot-specific barcode labels to use with the calibrator samples.

For instructions about how to perform the calibration procedure, refer to the system online help.

Performing Quality Control

For quality control of Atellica IM ELF, use the Atellica IM ELF QC at least once during each day that samples are analyzed. For the assigned values, refer to the quality control assigned value sheet provided.

Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

In addition, perform quality control:

- Following a valid calibration
- With use of a new lot of reagent
- When troubleshooting test results that do not match clinical conditions or symptoms

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system online help.

Taking Corrective Action

If the quality control results do not fall within the expected control interval, do not report results. Perform corrective actions in accordance with established laboratory protocol. For suggested protocol, refer to the system online help.

Results

Calculation of Results

The ELF score is a unitless numerical value, derived from an algorithm that combines the individual quantitative measurements of HA, PIIINP, and TIMP-1.

The ELF score is automatically calculated by the Atellica IM Analyzer and is reported along with results for HA, PIIINP, and TIMP-1.

Dilutions

HA

Serum HA measurements performed using Atellica IM ELF are validated from 3.00–1000.00 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with HA levels > 1000.00 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

- Load Atellica IM Multi-Diluent 13.
- Ensure that sufficient sample volume is available. Refer to the table below.
- Select the appropriate dilution factor.

For automatic dilutions enter a Dilution Point ≤ 1000 ng/mL.

Sample	Dilution	Sample Volume (μ L)
Serum	1:5	40

PIIINP

Serum PIIINP measurements performed using Atellica IM ELF are validated from 1.00–150.00 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with PIIINP levels > 150.00 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

- Load Atellica IM Multi-Diluent 13.
- Ensure that sufficient sample volume is available. Refer to the table below.
- Select the appropriate dilution factor.

For automatic dilutions enter a Dilution Point ≤ 150 ng/mL.

Sample	Dilution	Sample Volume (μ L)
Serum	1:5	30

TIMP-1

Serum TIMP-1 measurements performed using Atellica IM ELF are validated from 5.0–1300.0 ng/mL. For information about dilution options, refer to the system online help.

Dilute and retest serum samples with TIMP-1 levels > 1300.0 ng/mL to obtain accurate results.

For automated dilutions, perform the following activities:

- Load Atellica IM Multi-Diluent 10.
- Ensure that sufficient sample volume is available. Refer to the table below.
- Select the appropriate dilution factor.

For automatic dilutions enter a Dilution Point ≤ 1300 ng/mL.

Sample	Dilution	Sample Volume (μ L)
Serum	1:5	25

Interpretation of Results

Interpret the ELF score using the following guidelines:

ELF Score	Risk of Disease Progression (Development of Cirrhosis or Liver-Related Events)
< 9.80	Lower
≥ 9.80 – < 11.30	Mid ^a
≥ 11.30	Higher

^a In the Mid group, the risk of disease progression is similar to the pre-test risk. Pre-test risk refers to the likelihood of disease progression in the overall intended use population without considering the ELF score.

Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

The following information pertains to limitations of the assay:

- Measurements of HA, PIIINP, and TIMP-1 must be obtained within 8 hours of one another for the ELF score to be valid.
- Atellica IM ELF is limited to the detection of HA, PIIINP, and TIMP-1 in human serum.
- Only use results obtained on the Atellica IM Analyzer to calculate ELF scores.
- Do not use hemolyzed samples.
- Do not use samples that contain fluorescein. Samples with fluorescein may cause falsely depressed results. Evidence suggests that patients undergoing retinal fluorescein angiography can retain amounts of fluorescein in the body for up to 72 hours post-treatment. In cases of patients with renal insufficiency, including many diabetics, retention could be longer.⁸⁵
- Patient samples may contain heterophilic antibodies that could react in immunoassays and cause falsely elevated or depressed results. This assay is designed to minimize interference from heterophilic antibodies.^{86,87}
- The reagents for HA, PIIINP, and TIMP-1 have only been clinically validated in terms of their combined contribution to the ELF score. Clinical decisions should not be based on individual measurements of HA, PIIINP, or TIMP-1.
- Always interpret results in conjunction with the patient's medical history, clinical presentation, and other findings.

Expected Values

The reagent formulations used on the Atellica IM Analyzer are the same as those used on the ADVIA Centaur systems. Expected values were established using the ADVIA Centaur XP system and confirmed by assay comparison. Refer to *Assay Comparison*.

Expected values of ADVIA Centaur ELF were established on the ADVIA Centaur XP system with 594 samples from U.S. blood donors of known gender, ethnicity, and age. Study subjects were selected to be representative of the U.S. population. Although subjects were apparently healthy and presumably free of viral hepatitis, the absence of liver disease (including cryptic fibrosis) and other conditions associated with liver fibrosis (including metabolic syndrome) were not confirmed.

Reference intervals were determined by calculating the 5th and 95th percentiles of the distribution of values in accordance with the CLSI guideline EP28-A3c.⁸⁸ In populations with $n < 120$, a robust method was used to determine reference intervals.

Gender	Ethnicity	N	Age Range	Mean	Median	5 th Percentile	95 th Percentile
Female	African American	68	27–62	7.62	7.66	6.38	8.92
Female	Caucasian	86	18–64	7.74	7.77	6.63	8.81
Female	All	154	18–64	7.69	7.75	6.53	8.69
Male	African American	236	18–65	7.64	7.66	6.19	8.92
Male	Caucasian	198	19–66	7.85	7.81	6.87	9.01
Male	All ^a	440	24–53	7.74	7.74	6.46	8.94
Both	African American	304	18–65	7.64	7.68	6.21	8.83
Both	Caucasian	284	18–66	7.82	7.79	6.83	8.97
Both	All ^a	594	18–66	7.72	7.74	6.51	8.90

^a Includes an additional 6 Hispanic male subjects.

A published study conducted using 183 apparently healthy East Asian female and male subjects demonstrated the following ELF scores: mean of 7.75, median of 7.82, 5th percentile of 5.95, and 95th percentile of 8.73.⁸⁹

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference interval for the diagnostic evaluation of patient results.⁸⁸ Consider these values as guidance only.

Performance Characteristics

The reagent formulations used on the Atellica IM Analyzer are the same as those used on the ADVIA Centaur system. Some performance characteristics for the Atellica IM assay were established using the ADVIA Centaur XP system.

Measuring Interval

HA

3.00–1000.00 ng/mL

The lower limit of the measuring interval is defined by the limit of quantitation (LoQ). Report results below the measuring interval as < 3.00 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

PIIINP

1.00–150.00 ng/mL

The lower limit of the measuring interval is defined by the LoQ. Report results below the measuring interval as < 1.00 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

TIMP-1

5.0–1300.0 ng/mL

The lower limit of the measuring interval is defined by the LoQ. Report results below the measuring interval as < 5.0 ng/mL. When sample results exceed the measuring interval, refer to *Dilutions*.

Detection Capability

Detection capability was determined in accordance with CLSI Document EP17-A2 using the Atellica IM Analyzer.⁹⁰

Method	HA (ng/mL)	PIIINP (ng/mL)	TIMP-1 (ng/mL)	ELF Score ^a
Limit of Blank (LoB)	1.00	0.50	3.0	2.19
Limit of Detection (LoD)	2.00	0.75	4.0	3.20
Limit of Quantitation (LoQ)	3.00	1.00	5.0	3.85

^a The detection capability limits for the ELF score are based on a theoretical calculation incorporating the established detection capability limits derived individually for HA, PIIINP and TIMP-1.

The LoB corresponds to the highest measurement that is likely to be observed for a blank sample with a probability of 95%.

The LoD corresponds to the lowest concentration of HA, PIIINP, or TIMP-1 that can be detected with a probability of 95%.

The LoQ corresponds to the lowest amount of HA, PIIINP, or TIMP-1 in a sample at which the total CV is $\leq 20\%$.

Clinical Performance

The performance of Atellica IM ELF to assess disease severity was evaluated in studies of subjects with NASH, ALD, PSC, PBC, chronic HBV infection (CHB), chronic HCV infection (CHC), and in a study of mixed chronic liver disease etiologies.

The prognostic performance of Atellica IM ELF was determined by evaluating the likelihood of disease progression according to baseline ELF score. Disease progression endpoints, specific to the particular study, include development of histologically staged cirrhosis, hepatic decompensation, liver transplantation, hepatocellular carcinoma, and death.

For studies with biopsied subjects, the diagnostic performance of Atellica IM ELF was determined by evaluating the correlation of ELF measurements to histology staging.

The information provided should not be used to infer risk in patient populations other than those described.

NAFLD (NASH) F3 - Bridging Fibrosis: Progression to Cirrhosis

A prospective multicenter international clinical trial was performed in NASH subjects with biopsy-confirmed bridging fibrosis (F3; Ishak 3-4) to evaluate the safety and efficacy of an investigational drug (NCT01672866).^{59,62,91}

The study included subjects with chronic liver disease due to NASH defined as macrovesicular steatosis involving $> 5\%$ of hepatocytes on a liver biopsy with associated lobular inflammation and Stage 3-4 fibrosis by Ishak score. The intended follow-up period of this trial was 240 weeks, but it was discontinued after the Week 96 interim analysis. For the subjects included in the analysis, the median follow-up time was 27.8 months (Interquartile range (IQR): 22.1-34.7 months). Due to the lack of drug effect observed in this study, subjects from all treatment arms were pooled for the analysis.

There were a total of 196 subjects in this study with biopsies performed at one or both of the Week 48 and Week 96 visits in addition to a baseline biopsy. ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 196) are described in the following table.

Table 1. NASH F3: Demographics

Age in years (median [IQR]) ^a	55 (48–59) ^b
Sex (N [%]) ^c	
Female:	113 (62%)
Male:	83 (38%)
Diabetes mellitus (N [%])	126 (64%)
BMI in kg/m ² (median [IQR])	33.7 (30.3–38.4) ^b
Ishak score (N [%])	
Ishak 3:	113 (58%)
Ishak 4:	83 (42%)
ELF score, median (median [IQR])	9.7 (9.1–10.4)

^a Interquartile range.

^b Calculations were performed using the enrolled study population (N = 217) which includes subjects without ELF scores and subjects without follow-up biopsy.

^c Number of subjects.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of progression to cirrhosis (defined histologically as Ishak 5 or 6) up to 3.9 years following enrollment.

The risk of progression to cirrhosis was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

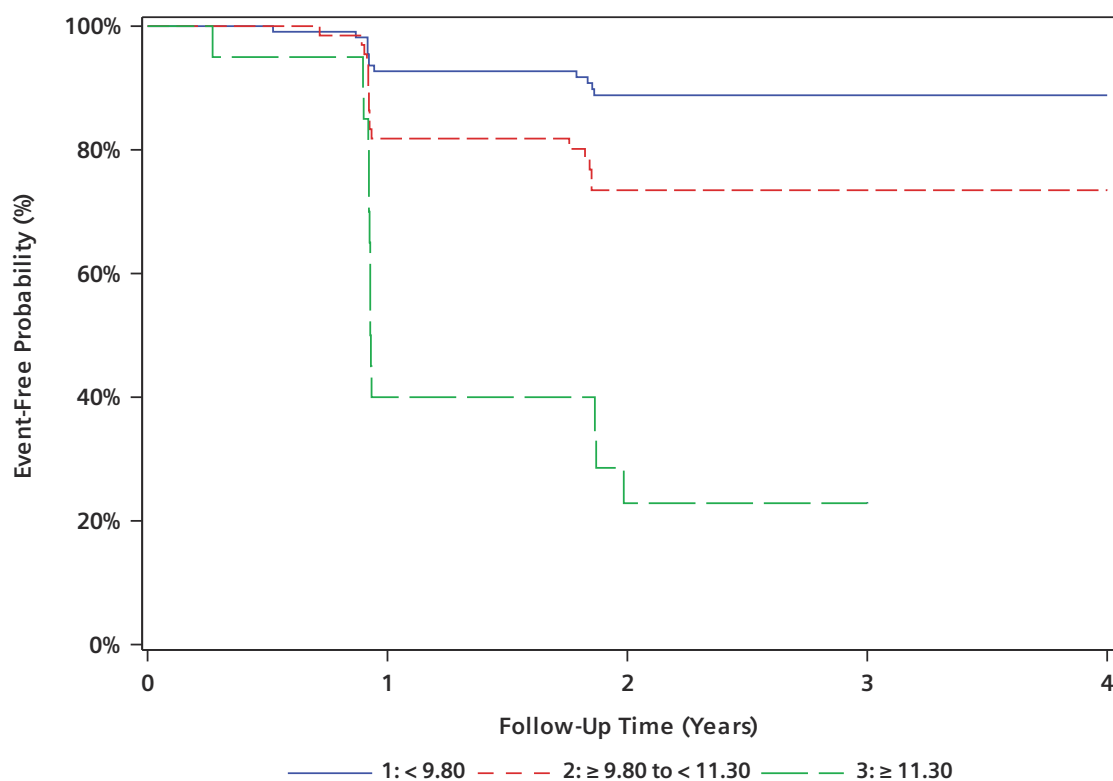
Table 2. NASH F3: Prognostic performance

Risk Group (ELF Score)	N ^a	Progression to Cirrhosis		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	110	12	98	10.9% (5.8%, 18.3%)	0.42 (0.26, 0.70)	1.00
Mid (≥ 9.80 to < 11.30)	66	17	49	25.8% (15.9%, 38.0%)	1.20 (0.82, 1.89)	2.61 (1.24, 5.46)
Higher (≥ 11.30)	20	15	5	75.0% (50.9%, 91.3%)	10.36 (4.30, 28.83)	10.31 (4.79, 22.17)
All	196	44	152	22.4% (16.8%, 28.9%)	—	—

^a Number of subjects.

^b Confidence interval.

Kaplan-Meier curves up to 4 years were plotted for each of the risk groups to display the proportion of subjects without a liver-related event over time.



ELF Score	Number at Risk (Years)				
	0	1	2	3	4
1: < 9.80	110	100	86	33	0
2: ≥ 9.80 to < 11.30	66	54	42	8	0
3: ≥ 11.30	20	8	4	0	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict progression to cirrhosis.

Table 3. NASH F3: Clinical performance for progression to cirrhosis

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	72.7% (32/44) (57.2%, 85.0%)	64.5% (98/152) (56.3%, 72.1%)	37.2% (32/86) (27.0%, 48.3%)	89.1% (98/110) (81.7%, 94.2%)
11.30	34.1% (15/44) (20.5%, 49.9%)	96.7% (147/152) (92.5%, 98.9%)	75.0% (15/20) (50.9%, 91.3%)	83.5% (147/176) (77.2%, 88.7%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

NAFLD (NASH) F4 - Compensated Cirrhosis: Progression to Liver-Related Events

A prospective multicenter international clinical trial was performed in NASH subjects with biopsy-confirmed compensated cirrhosis (F4; Ishak 5-6) to evaluate the safety and efficacy of an investigational drug (NCT01672879).^{59,62,91}

The study included subjects with Stage 5-6 cirrhosis by Ishak score and liver biopsy consistent with NASH or cryptogenic cirrhosis. The intended follow-up period of this trial was 244 weeks, but it was discontinued after the week 96 interim analysis. For the subjects included in the analysis, the median follow-up time was 29.0 months (Interquartile range (IQR): 21.7–33.7 months).

Due to the lack of drug effect observed in this study, subjects from all treatment arms were pooled for the analysis. ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 250) are described in the following table.

Table 4. NASH F4: Demographics

Age in years (median [IQR]) ^a	55 (48–59) ^b
Sex (N [%]) ^c	
Female:	159 (64%)
Male:	91 (36%)
Cirrhosis subpopulation (N [%])	
NASH cirrhosis:	151 (60%)
Cryptogenic cirrhosis: ^d	99 (40%)
Diabetes Mellitus (N [%])	170 (68%)
BMI in kg/m ² (median [IQR])	33.6 (29.7–38.2) ^b
HVPG in mm Hg (median [IQR])	12 (9–16) ^b
HVPG ≥ 10 mm Hg (N [%])	175 (68%) ^b
Ishak score (N [%])	
Ishak 5:	86 (34%)
Ishak 6:	164 (66%)
ELF score, median (median [IQR])	10.7 (9.9–10.7)

^a Interquartile range.

^b Calculations were performed using the enrolled study population (N = 258) which includes subjects without ELF scores.

^c Number of subjects.

^d The study excluded other causes of liver disease including viral hepatitis and alcoholic liver disease.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events up to 3.8 years following enrollment. The clinical endpoints were:

- Death
- Liver transplantation
- Qualification for liver transplantation (Model for End-Stage Liver Disease (MELD) ≥ 15)
- Esophageal variceal bleeding requiring hospitalization
- Clinically apparent ascites requiring treatment
- Hepatic encephalopathy of at least Grade 2 (West Haven criteria) requiring treatment
- Newly diagnosed varices in a subject without prior varices

The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

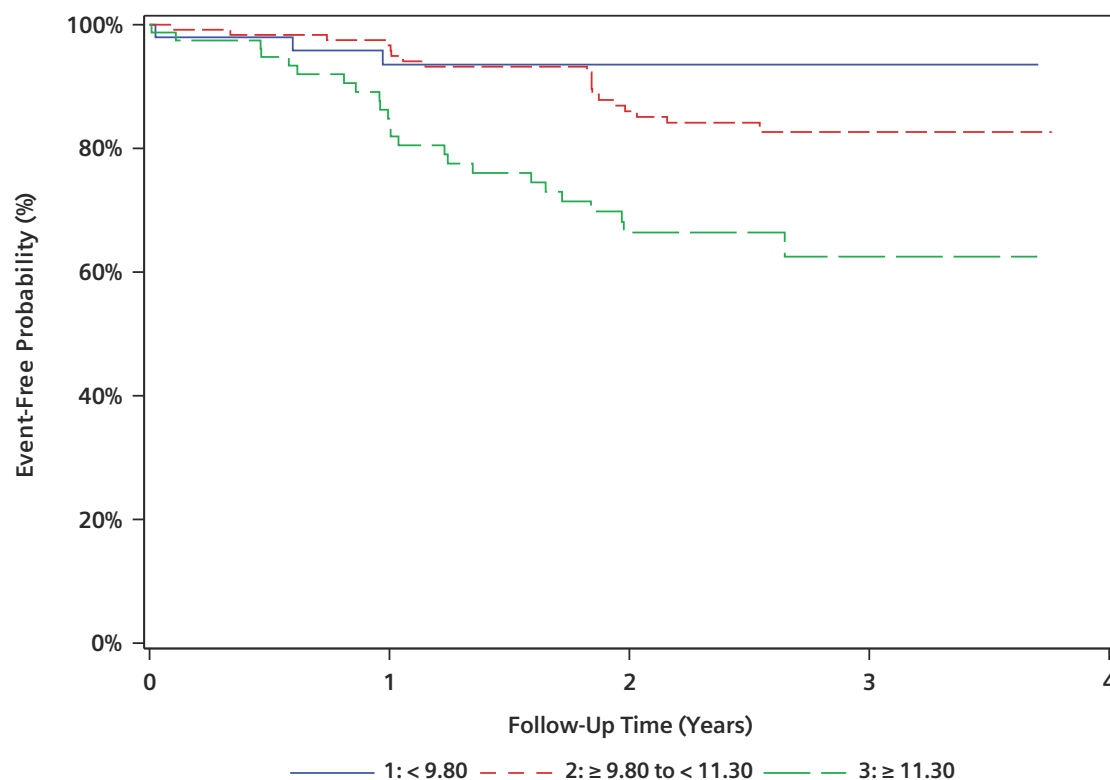
Table 5. NASH F4: Prognostic performance

Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	49	3	46	6.1% (1.3%, 16.9%)	0.29 (0.09, 0.89)	1.00
Mid (≥ 9.80 to < 11.30)	122	19	103	15.6% (9.6%, 23.2%)	0.82 (0.56, 1.18)	2.42 (0.72, 8.18)
Higher (≥ 11.30)	79	24	55	30.4% (20.5%, 41.8%)	1.94 (1.35, 2.77)	6.13 (1.84, 20.38)
All	250	46	204	18.4% (13.8%, 23.8%)	—	—

^a Number of subjects.

^b Confidence interval.

Kaplan-Meier curves up to 4 years were plotted for each of the risk groups to display the proportion of subjects without a liver-related event over time.



ELF Score	Number at Risk (Years)				
	0	1	2	3	4
1: < 9.80	49	41	36	6	0
2: ≥ 9.80 to < 11.30	122	113	94	22	0
3: ≥ 11.30	79	58	39	9	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events.

Table 6. NASH F4: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV (95% CI) ^b	NPV (95% CI) ^c
9.80	93.5% (43/46) (82.1%, 98.6%)	22.5% (46/204) (17.0%, 28.9%)	21.4% (43/201) (15.9%, 27.7%)	93.9% (46/49) (83.1%, 98.7%)
11.30	52.2% (24/46) (36.9%, 67.1%)	73.0% (149/204) (66.4%, 79.0%)	30.4% (24/79) (20.5%, 41.8%)	87.1% (149/171) (81.2%, 91.8%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

NAFLD: Correlation to Histology

A prospective clinical trial was performed in Japanese NAFLD patients who underwent biopsy for histological staging. ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 410) are described in the following table.

Table 7. NAFLD: Demographics

Age in years (median [IQR]) ^a		61 (50–68)
Sex (N [%]) ^b	Female:	235 (57%)
	Male:	175 (43%)
BMI in kg/m ² (median [IQR])		27.9 (25.4–31.4)
HbA1c % (median [IQR])		6.2% (5.7–6.9)
Liver fibrosis stage (N [%])	Brunt F0:	41 (10%)
	Brunt F1:	115 (28%)
	Brunt F2:	112 (27%)
	Brunt F3:	112 (27%)
	Brunt F4:	30 (7%)
ELF score, median (median [IQR])		10.3 (9.4–11.0)

^a Interquartile range.

^b Number of subjects.

An analysis was performed to evaluate the correlation of ELF scores to Brunt staging at baseline. Sensitivity and specificity were calculated for various ELF cutoffs in relation to the presence of significant fibrosis ($\geq$ F2), advanced fibrosis ($\geq$ F3) and cirrhosis ($\geq$ F4).

Table 8. NAFLD: Diagnostic performance

Fibrosis Severity	Estimate	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Significant Fibrosis (Brunt F0–F1 versus F2–F4)	Sensitivity	98.8% (251/254)	95.7% (243/254)	81.9% (208/254)	59.4% (151/254)	27.9% (71/254)
	95% CI ^a	(96.6%, 99.8%)	(92.4%, 97.8%)	(76.6%, 86.4%)	(53.1%, 65.5%)	(22.5%, 33.9%)
	Specificity	3.8% (6/156)	34.0% (53/156)	66.7% (104/156)	84.6% (132/156)	97.4% (152/156)
	95% CI	(1.4%, 8.2%)	(26.6%, 42.0%)	(58.7%, 74.0%)	(78.0%, 89.9%)	(93.6%, 99.3%)
Advanced Fibrosis (Brunt F0–F2 versus F3–F4)	Sensitivity	99.3% (141/142)	99.3% (141/142)	90.8% (129/142)	71.1% (101/142)	40.1% (57/142)
	95% CI	(96.1%, 99.9%)	(96.1%, 99.9%)	(84.8%, 95.0%)	(62.9%, 78.4%)	(32.0%, 48.7%)
	Specificity	3.0% (8/268)	23.5% (63/268)	51.1% (137/268)	72.4% (194/268)	93.3% (250/268)
	95% CI	(1.3%, 5.8%)	(18.6%, 29.0%)	(45.0%, 57.2%)	(66.6%, 77.6%)	(89.6%, 96.0%)
Cirrhosis (Brunt F0–F3 versus F4)	Sensitivity	100% (30/30)	100% (30/30)	96.7% (29/30)	83.3% (25/30)	56.7% (17/30)
	95% CI	(88.4%, 100%)	(88.4%, 100%)	(82.8%, 99.9%)	(65.3%, 94.4%)	(37.4%, 74.5%)
	Specificity	2.4% (9/380)	16.8% (64/380)	39.2% (149/380)	60.5% (230/380)	84.7% (322/380)
	95% CI	(1.1%, 4.4%)	(13.2%, 21.0%)	(34.3%, 44.3%)	(55.4%, 65.5%)	(80.7%, 88.2%)

^a Confidence interval.

ALD: Progression to Liver-Related Events and Correlation to Histology

A prospective clinical trial was performed in Danish primary care and secondary care patients at risk of alcoholic liver disease.^{33,64} Two analyses were performed for the subjects included in this study.

A prognostic analysis was performed for a cohort of subjects followed-up for clinical outcomes. For this analysis, the median follow-up time was 3.0 years (Interquartile range (IQR): 1.5–4.8 years).

A separate diagnostic analysis was performed for a cohort of subjects who underwent biopsy for histological staging. ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the prognostic analysis (N = 418) and diagnostic analysis (N = 350) are described in the following table.

Table 9. ALD: Demographics

	Prognostic cohort (N = 418)	Diagnostic cohort (N = 350) ^a
Age in years (median [IQR]) ^b	57 (50–64)	— ^a
Sex (N [%]) ^c		
Female:	108 (26%)	— ^a
Male:	310 (74%)	— ^a
Patient population (N [%])		
Primary Care:	201 (48%)	— ^a
Secondary Care:	217 (52%)	— ^a
Abstaining drinking at enrollment (N [%])	183 (44%)	— ^a
BMI in kg/m ² (median [IQR]) ^d	27.2 (24.0–30.8)	— ^a
Liver fibrosis stage (N [%])		
Kleiner F0:	35 (10%)	35 (10%)
Kleiner F1:	125 (35%)	120 (34%)
Kleiner F2:	106 (30%)	103 (29%)
Kleiner F3:	27 (8%)	26 (7%)
Kleiner F4:	66 (18%)	66 (19%)
Not Available:	59 (14%)	0 (0%)
ELF score, median (median [IQR])	9.3 (8.6–10.4)	—

^a Calculations were performed using the entire enrolled study population (N = 418)

^b Interquartile range.

^c Number of subjects.

^d BMI information is not available for 2 subjects.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events following enrollment. The clinical endpoints were events indicative of hepatic decompensation.

The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 10. ALD: Prognostic performance

Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI) ^c
		Yes	No			
Lower (< 9.80)	159	4	155	2.5 (0.7, 6.3)	0.12 (0.05, 0.30)	1.00
Mid (≥ 9.80 to < 11.30)	44	12	32	27.3 (15.0, 42.8)	1.73 (0.97, 3.08)	7.48 (3.05, 18.35)

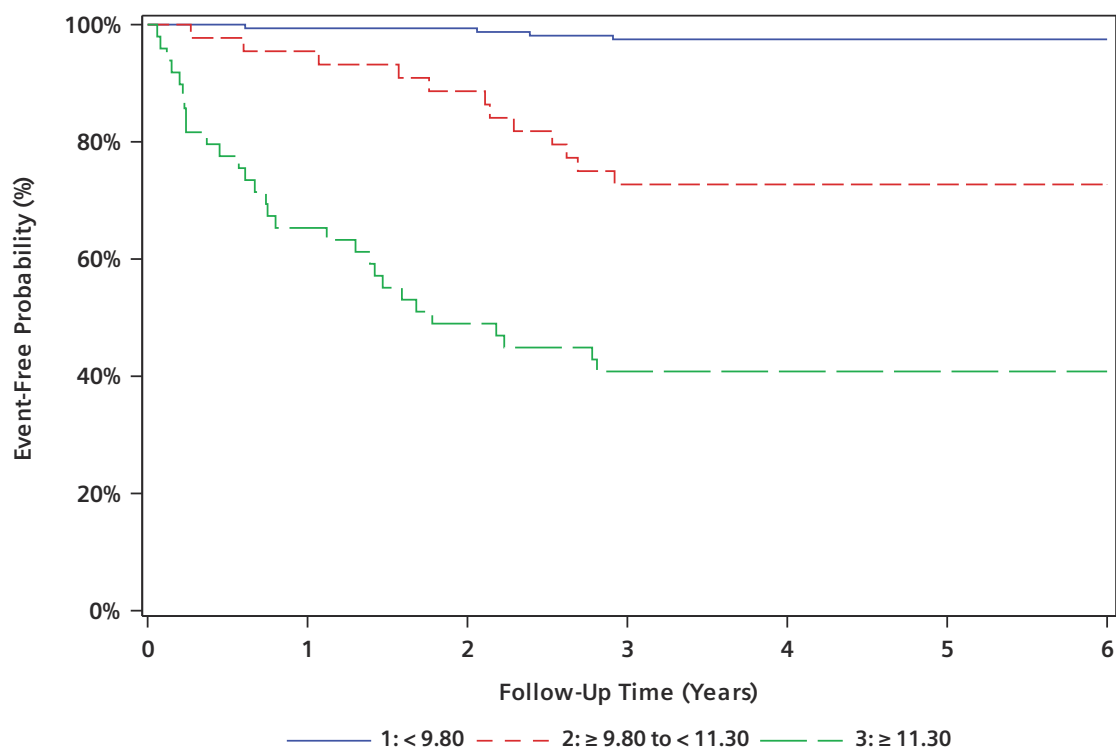
Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI) ^c
		Yes	No			
Higher (≥ 11.30)	49	29	20	59.2 (44.2, 73.0)	6.67 (4.17, 10.67)	24.51 (10.74, 55.95)
All	252	45	207	17.9 (13.3, 23.2)	—	—

^a Number of subjects with at least 3 years of follow-up or with an event occurring earlier than 3 years.

^b Confidence interval.

^c Based on the overall study population of 418 subjects.

Kaplan-Meier curves up to 6.5 years were plotted for each of the risk groups to display the proportion of subjects without hepatic decompensation over time.



ELF Score	Number at Risk (Years)						
	0	1	2	3	4	5	6
1: < 9.80	159	158	158	155	110	65	1
2: ≥ 9.80 to < 11.30	44	42	39	32	23	13	0
3: ≥ 11.30	49	32	24	20	17	13	1

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events in subjects at 3 years following enrollment (N = 252).

Table 11. ALD: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	91.1% (41/45) (78.8%, 97.5%)	74.9% (155/207) (68.4%, 80.6%)	44.1% (41/93) (33.8%, 54.8%)	97.5% (155/159) (93.7%, 99.3%)
11.30	64.4% (29/45) (48.8%, 78.1%)	90.3% (187/207) (85.5%, 94.0%)	59.2% (29/49) (44.2%, 73.0%)	92.1% (187/203) (87.5%, 95.4%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

An analysis was performed to evaluate the correlation of ELF scores to Kleiner staging at baseline. Sensitivity and specificity were calculated for various ELF cutoffs in relation to the presence of significant fibrosis ($\geq$ F2), advanced fibrosis ($\geq$ F3) and cirrhosis ($\geq$ F4).

Table 12. ALD: Diagnostic performance

Fibrosis Severity	Estimate (95% CI) ^a	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Significant Fibrosis (Kleiner F0–F1 versus F2–F4)	Sensitivity	96.9% (189/195)	87.2% (170/195)	63.6% (124/195)	49.2% (96/195)	32.3% (63/195)
	(95% CI)	(93.4%, 98.9%)	(81.7%, 91.5%)	(56.4%, 70.4%)	(42.0%, 56.5%)	(25.8%, 39.4%)
	Specificity	23.2% (36/155)	61.9% (96/155)	88.4% (137/155)	96.8% (150/155)	98.1% (152/155)
	(95% CI)	(16.8%, 30.7%)	(53.8%, 69.6%)	(82.3%, 93.0%)	(92.6%, 98.9%)	(94.5%, 99.6%)
Advanced Fibrosis (Kleiner F0–F2 versus F3–F4)	Sensitivity	100.0% (92/92)	97.8% (90/92)	88.0% (81/92)	76.1% (70/92)	59.8% (55/92)
	95% CI	(96.1%, 100.0%)	(92.4%, 99.7%)	(79.6%, 93.9%)	(66.1%, 84.4%)	(49.0%, 69.9%)
	Specificity	16.3% (42/258)	46.1% (119/258)	76.4% (197/258)	88.0% (227/258)	95.7% (247/258)
	95% CI	(12.0%, 21.4%)	(39.9%, 52.4%)	(70.7%, 81.4%)	(83.4%, 91.7%)	(92.5%, 97.9%)

Fibrosis Severity	Estimate (95% CI) ^a	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Cirrhosis (Kleiner F0-3 versus F4)	Sensitivity	100.0% (66/66)	100.0% (66/66)	93.9% (62/66)	83.3% (55/66)	69.7% (46/66)
	95% CI	(94.6%, 100.0%)	(94.6%, 100.0%)	(85.2%, 98.3%)	(72.1%, 91.4%)	(57.2%, 80.4%)
	Specificity	14.8% (42/284)	42.6% (121/284)	71.8% (204/284)	83.8% (238/284)	93.0% (264/284)
	95% CI	(10.9%, 19.5%)	(36.8%, 48.6%)	(66.2%, 77.0%)	(79.0%, 87.9%)	(89.3%, 95.7%)

^a Confidence interval.

PSC: Progression to Cirrhosis and Correlation to Histology

A prospective multicenter international clinical trial was performed in large-duct PSC subjects to evaluate the safety and efficacy of an investigational drug (NCT01672853).^{68,69} The study included subjects with chronic cholestatic liver disease for 6 months or more. ELF measurements were performed on an ADVIA Centaur XP system. Two analyses were performed for the subjects included in this study.

A prognostic analysis was performed for the cohort of subjects (N = 191) followed for the progression to histologically staged cirrhosis. For this analysis, the median follow-up time was 23.0 months (Interquartile range (IQR): 22.7–23.1 months). A diagnostic analysis was performed for the overall cohort of subjects (N = 234) to evaluate the correlation of ELF to histology. Due to the lack of drug effect observed in this study, subjects from all treatment arms were pooled for the analysis.

Baseline clinical characteristics for the clinical trial subjects included in the prognostic analysis and diagnostic analysis are described in the following table.

Table 13. PSC: Demographics

	Prognostic cohort (N = 191)	Diagnostic cohort (N = 234)
Age in years (median [IQR]) ^a	— ^b	45 (37–52)
Sex (N [%]) ^c		
Female:	69 (36%)	85 (36%)
Male:	122 (64%)	149 (64%)
Ulcerative colitis (N [%])	— ^b	112 (48%)
BMI in kg/m ² (median [IQR])	— ^b	25.2 (23–29)

	Prognostic cohort (N = 191)	Diagnostic cohort (N = 234)
Ishak score (N [%])		
Ishak 0:	19 (10%)	20 (9%)
Ishak 1:	38 (20%)	40 (17%)
Ishak 2:	51 (27%)	55 (24%)
Ishak 3:	51 (27%)	57 (24%)
Ishak 4:	32 (17%)	37 (16%)
Ishak 5:	N/A ^d	10 (4%)
Ishak 6:	N/A	15 (6%)
ELF score, median (median [IQR])	9.2 (8.5–10.2)	9.5 (8.6–10.4)

^a Interquartile range.

^b Calculations were performed using the entire enrolled study population (N = 234).

^c Number of subjects.

^d N/A = not applicable.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of progression to cirrhosis (defined histologically as Ishak 5 or 6) up to 2.3 years following enrollment.

The risk of progression to cirrhosis was assessed according to absolute risk, likelihood ratio, and hazard probability of progression after 48 and 96 weeks.

Table 14. PSC: Prognostic performance

Risk Group (ELF Score)	N ^a	Progression to Cirrhosis		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Progression after 48 weeks (95% CI)	Progression after 96 weeks (95% CI)
		Yes	No				
Lower (< 9.80)	124	10	114	8.1% (3.9%, 14.3%)	0.38 (0.22, 0.64)	3.2% (1.3%, 7.9%)	8.6% (5.3%, 13.8%)
Mid (≥ 9.80 to < 11.30)	55	18	37	32.7% (20.7%, 46.7%)	2.09 (1.36, 3.22)	20.0% (10.2%, 39.3%)	38.9% (28.0%, 54.1%)
Higher (≥ 11.30)	12	8	4	66.7% (34.9%, 90.0%)	8.61 (2.74, 27.04)	58.3% (26.7%, 99.9%)	83.3% (46.3%, 99.9%)
All	191	36	155	18.8% (13.6%, 25.1%)	—	—	—

^a Number of subjects.

^b Confidence Interval.

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict progression to cirrhosis.

Table 15. PSC: Clinical performance for progression to cirrhosis

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	72.2% (26/36) (54.8%, 85.8%)	73.5% (114/155) (65.9%, 80.3%)	38.8% (26/67) (27.1%, 51.5%)	91.9% (114/124) (85.7%, 96.1%)
11.30	22.2% (8/36) (10.1%, 39.2%)	97.4% (151/155) (93.5%, 99.3%)	66.7% (8/12) (34.9%, 90.1%)	84.4% (151/179) (78.2%, 89.3%)

^a Confidence Interval.

^b Positive predictive value.

^c Negative predictive value.

An analysis was performed to evaluate the correlation of ELF scores to Ishak staging at baseline. Sensitivity and specificity were calculated for various ELF cutoffs in relation to the presence of significant fibrosis ($\geq$ Ishak 3), advanced fibrosis ($\geq$ Ishak 4) and cirrhosis ($\geq$ Ishak 5).

Table 16. PSC: Diagnostic performance

Fibrosis Severity	Estimate	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Significant Fibrosis (Ishak 0–2 versus 3–6)	Sensitivity	95.8% (114/119)	82.4% (98/119)	58.8% (70/119)	37.8% (45/119)	19.3% (23/119)
	95% CI ^a	(90.5%, 98.6%)	(74.3%, 88.7%)	(49.4%, 67.8%)	(29.1%, 47.2%)	(12.7%, 27.6%)
	Specificity	29.6% (34/115)	57.4% (66/115)	80.0% (92/115)	91.3% (105/115)	97.4% (112/115)
	95% CI	(21.4%, 38.8%)	(47.8%, 66.6%)	(71.5%, 86.9%)	(84.6%, 95.7%)	(92.6%, 99.5%)
Advanced Fibrosis (Ishak 0–3 versus 4–6)	Sensitivity	95.2% (59/62)	90.3% (56/62)	69.4% (43/62)	54.8% (34/62)	29.0% (18/62)
	95% CI	(86.5%, 99.0%)	(80.1%, 96.4%)	(56.3%, 80.4%)	(41.7%, 67.5%)	(18.2%, 41.9%)
	Specificity	20.9% (36/172)	47.1% (81/172)	70.9% (122/172)	87.8% (151/172)	95.3% (164/172)
	95% CI	(15.1%, 27.8%)	(39.4%, 54.8%)	(63.5%, 77.6%)	(81.9%, 92.3%)	(91.0%, 98.0%)

Fibrosis Severity	Estimate	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Cirrhosis (Ishak 0–4 versus 5–6)	Sensitivity	100% (25/25)	96.0% (24/25)	76.0% (19/25)	68.0% (17/25)	40.0% (10/25)
	95% CI	(86.3%, 100%)	(79.6%, 99.9%)	(54.9%, 90.6%)	(46.5%, 85.0%)	(21.1%, 61.3%)
	Specificity	18.7% (39/209)	41.1% (86/209)	64.6% (135/209)	81.8% (171/209)	92.3% (193/209)
	95% CI	(13.6%, 24.6%)	(34.4%, 48.1%)	(57.7%, 71.1%)	(75.9%, 86.8%)	(87.9%, 95.6%)

^a Confidence interval.

PSC: Progression to Liver Transplant or Death

A retrospective multicenter trial was performed with specimens collected from Canadian and European large-duct PSC patients with follow-up information recorded in their medical charts.⁶⁷ ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 498) are described in the following table.

Table 17. PSC: Demographics

Age in years (median [IQR]) ^a	33 (25–44)
Sex (N [%]) ^b	
Female:	195 (39%)
Male:	303 (61%)
Autoimmune hepatitis (N [%])	44 (9%)
Inflammatory bowel disease (N [%])	
All (including unspecified IBD):	355 (71%)
Ulcerative colitis:	270 (54%)
Crohn's disease:	59 (12%)
ELF score, median (median [IQR])	9.1 (8.2–10.5)

^a Interquartile range.

^b Number of subjects.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver transplantation or all-cause mortality up to 10 years following enrollment.

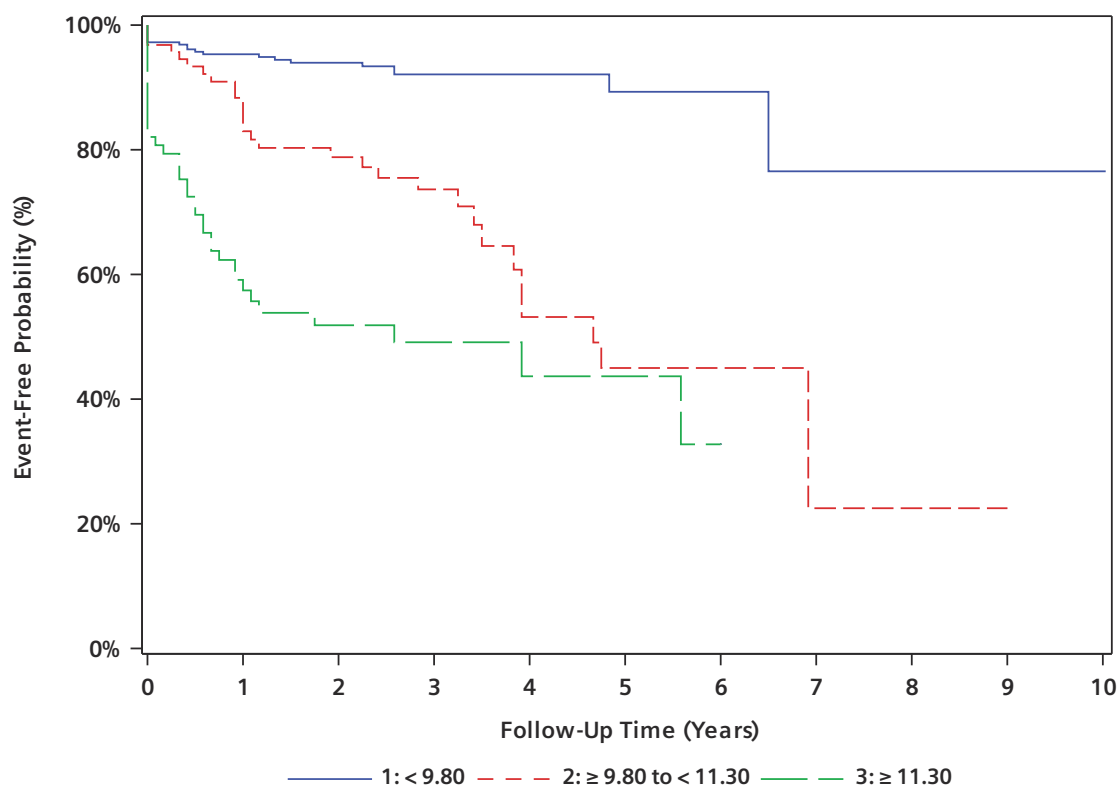
The risk of liver transplant or death was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 18. PSC: Prognostic performance

Risk Group (ELF Score)	N ^a	Liver Transplant or Death		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	326	22	304	6.7% (4.3%, 10.0%)	0.34 (0.23, 0.49)	1.00
Mid (≥ 9.80 to < 11.30)	94	29	65	30.9% (21.7%, 41.2%)	2.08 (1.43, 3.02)	4.34 (2.50, 7.56)
Higher (≥ 11.30)	78	37	41	47.4% (36.0%, 59.1%)	4.20 (2.87, 6.15)	9.12 (5.35, 15.52)
All	498	88	410	17.7% (14.4%, 21.3%)	—	—

^a Number of subjects.^b Confidence interval.

Kaplan-Meier curves up to 10 years were plotted for each of the risk groups to display the proportion of subjects without a liver-related event over time.



ELF Score	Number at Risk (Years)										
	0	1	2	3	4	5	6	7	8	9	10
1: < 9.80	326	221	179	115	52	28	8	5	4	3	2
2: ≥ 9.80 to < 11.30	94	66	52	36	14	9	2	1	1	1	0
3: ≥ 11.30	78	35	24	15	8	4	1	0	0	0	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events.

Table 19. PSC: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	75.0% (66/88) (64.6%, 83.6%)	74.1% (304/410) (69.6%, 78.3%)	38.4% (66/172) (31.1%, 46.1%)	93.3% (304/326) (90.0%, 95.7%)
11.30	42.0% (37/88) (31.6%, 53.0%)	90.0% (369/410) (86.7%, 92.7%)	47.4% (37/78) (36.0%, 59.1%)	87.9% (369/420) (84.3%, 90.8%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

PBC: Progression to Liver-Related Events and Correlation to Histology

A prospective multicenter trial in the United States was performed to evaluate the efficacy of low-dose methotrexate in combination with ursodiol in subjects with established but not decompensated PBC (NCT00006168).⁹²

All subjects were positive for anti-microbial antibody, had serum alkaline phosphatase levels at least 1.5 times the upper limit of normal, and liver biopsy with histological findings compatible with PBC diagnosis. Due to the lack of effect of methotrexate observed in this study, subjects from all treatment arms were pooled for the analysis.

Two analyses were performed for the subjects included in this study. A prognostic analysis was performed at 5 years for the cohort of subjects followed-up for clinical outcomes with survival curves generated for all patients with follow-up up to 10 years. The median follow-up time for the prognostic population was 6.0 years (Interquartile range (IQR): 4.2–7.5 years). A separate diagnostic analysis was performed for the cohort of 152 subjects who underwent biopsy for histological staging.

There were a total of 161 subjects in this study with banked samples available for ELF testing. ELF measurements were performed on an Immuno 1 analyzer.

Baseline clinical characteristics for the clinical trial subjects included in the prognostic analysis (N = 161) and diagnostic analysis (N = 152) are described in the following table.

Table 20. PBC: Demographics

	Prognostic cohort (N = 161) ^a	Diagnostic cohort (N = 152)
Age in years (median [IQR]) ^b	52 (46–58)	52 (46–58)
Sex (N [%])		
Female:	150 (93%)	—
Male:	11 (7%)	—

		Prognostic cohort (N = 161) ^a	Diagnostic cohort (N = 152)
Ishak score (N [%])	Ishak 0:	8 (5%)	8 (5%)
	Ishak 1:	25 (16%)	25 (16%)
	Ishak 2:	55 (34%)	55 (36%)
	Ishak 3:	35 (22%)	35 (23%)
	Ishak 4:	18 (11%)	18 (12%)
	Ishak 5:	7 (4%)	7 (5%)
	Ishak 6:	4 (3%)	4 (3%)
	Unavailable:	9 (6%)	—
ELF score, median (median [IQR])		9.2 (8.4–10.1)	9.1 (8.4–10.0)

^a Number of subjects

^b Interquartile range.

The following analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events up to 5 years following enrollment. The clinical endpoints were death, liver transplantation and events indicative of hepatic decompensation.

The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 21. PBC: Prognostic performance

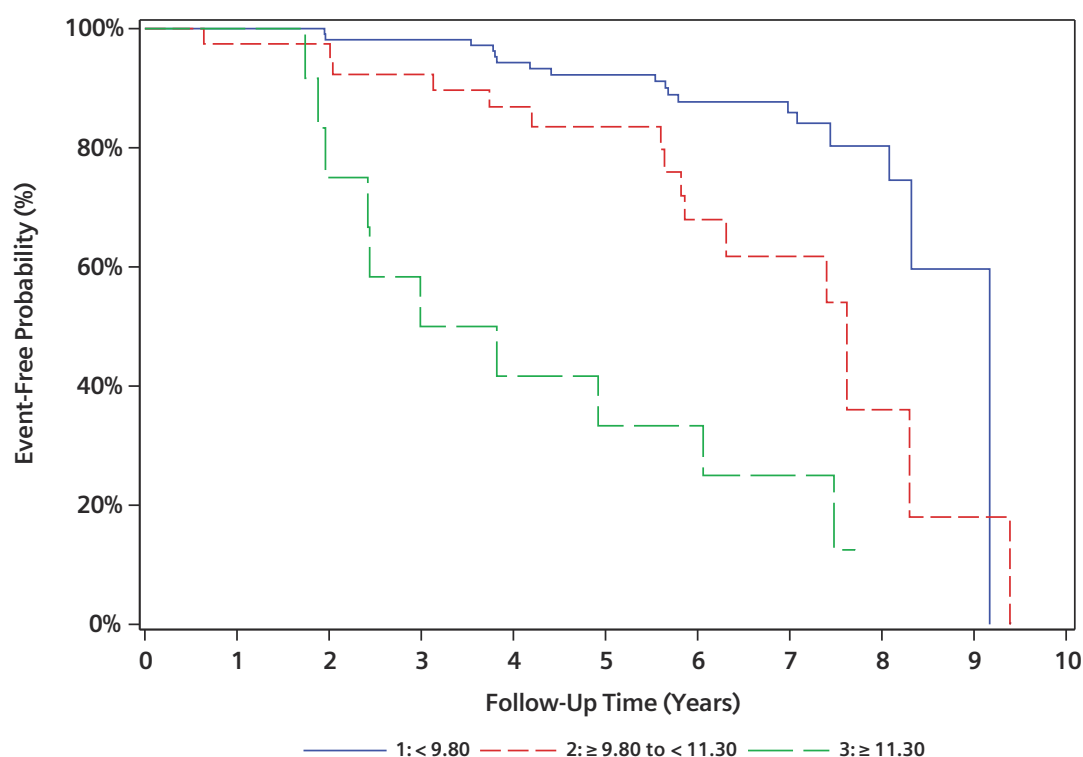
Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI) ^c
		Yes	No			
Lower (< 9.80)	94	8	86	8.5% (3.7%, 16.1%)	0.48 (0.27, 0.85)	1.00
Mid (≥ 9.80 to < 11.30)	30	6	24	20.0% (7.7%, 38.6%)	1.30 (0.60, 2.80)	2.31 (0.89, 7.39)
Higher (≥ 11.30)	12	8	4	66.7% (34.9%, 90.1%)	10.36 (3.42, 31.45)	12.31 (4.59, 33.03)
All	136	22	114	16.2% (10.4%, 23.5%)	—	—

^a Number of subjects with at least 5 years of follow-up or with an event occurring earlier than 5 years.

^b Confidence interval.

^c Based on the overall study population of 161 subjects.

Kaplan-Meier curves up to 10 years were plotted for each of the risk groups to display the proportion of subjects without hepatic decompensation over time.



ELF Score	Number at Risk (Years)										
	0	1	2	3	4	5	6	7	8	9	10
1: < 9.80	110	107	105	105	95	86	67	48	17	1	0
2: ≥ 9.80 to < 11.30	39	38	38	35	31	24	14	10	2	1	0
3: ≥ 11.30	12	12	9	6	5	4	4	2	0	0	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events in subjects at 5 years following enrollment (N = 136).

Table 22. PBC: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV (95% CI) ^b	NPV (95% CI) ^c
9.80	63.6% (14/22) (43.2%, 81.8%)	75.4% (86/114) (67.2%, 82.9%)	33.3% (14/42) (20.1%, 48.1%)	91.5% (86/94) (84.9%, 96.3%)
11.30	36.4% (8/22) (18.2%, 56.8%)	96.5% (110/114) (92.2%, 99.2%)	66.7% (8/12) (39.4%, 89.3%)	88.7% (110/124) (82.5%, 93.7%)

^a Confidence interval.

^b Positive predictive value

^c Negative predictive value

An analysis was performed to evaluate the correlation of ELF scores to Ishak staging at baseline. Sensitivity and specificity were calculated for various ELF cutoffs in relation to the presence of significant fibrosis ($\geq$ Ishak 3), advanced fibrosis ($\geq$ Ishak 4) and cirrhosis ($\geq$ Ishak 5).

Table 23. PBC: Diagnostic performance

Fibrosis Severity	Estimate (95% CI) ^a	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Significant Fibrosis (Ishak 0–2 versus 3–6)	Sensitivity	87.5% (56/64)	71.9% (46/64)	53.1% (34/64)	28.1% (18/64)	15.6% (10/64)
		(76.8%, 94.4%)	(59.2%, 82.4%)	(40.2%, 65.7%)	(17.6%, 40.8%)	(7.8%, 26.9%)
	Specificity	30.7% (27/88)	56.8% (50/88)	86.4% (76/88)	96.6% (85/88)	100.0% (88/88)
		(21.3%, 41.4%)	(45.8%, 67.3%)	(77.4%, 92.7%)	(90.4%, 99.3%)	(95.9%, 100%)
Advanced Fibrosis (Ishak 0–3 versus 4–6)	Sensitivity	93.1% (27/29)	82.8% (24/29)	65.6% (19/29)	34.5% (10/29)	24.1% (7/29)
		(77.2%, 99.1%)	(64.2%, 94.1%)	(45.7%, 82.1%)	(17.9%, 54.3%)	(10.3%, 43.5%)
	Specificity	26.8% (33/123)	51.2% (63/123)	78.0% (96/123)	91.1% (112/123)	97.6% (120/123)
		(19.2%, 35.6%)	(42.0%, 60.3%)	(69.7%, 85.0%)	(84.6%, 95.4%)	(93.0%, 99.5%)
Cirrhosis (Ishak 0–4 versus 5–6)	Sensitivity	81.8% (9/11)	81.8% (9/11)	72.7% (8/11)	36.4% (4/11)	18.2% (2/11)
		(48.2%, 97.7%)	(48.2%, 97.7%)	(39.0%, 94.0%)	(10.9%, 69.2%)	(2.3%, 51.8%)
	Specificity	23.4% (33/141)	46.8% (66/141)	73.0% (103/141)	87.9% (124/141)	94.3% (133/141)
		(16.7%, 31.3%)	(38.4%, 55.4%)	(64.9%, 80.2%)	(81.4%, 92.8%)	(89.1%, 97.5%)

^a Confidence interval.

CHB: Progression to Liver-Related Events

A prospective clinical trial was performed in Korean subjects with chronic HBV infection.⁷⁰ For the subjects included in the analysis, the median follow-up time was 2.9 years (Interquartile range (IQR): 0.5–5.0 years). ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 170) are described in the following table.

Table 24. CHB: Demographics

Age in years (SD)		45 (15)
Sex (N [%])		
	Female:	67 (39%)
	Male:	103 (61%)
HbeAg positive (N [%])		45 (26%)
HBV DNA >10 ⁵ copies/mL (N [%])		33 (19%)
Antiviral therapy (N [%])		
	Any:	82 (48%)
	Entecavir:	41 (24%)
	Lamivudine:	31 (18%)
	Telbivudine:	5 (3%)
	Clevudine:	3 (2%)
	Adefovir:	2 (1%)
Batts and Ludwig's fibrosis stage (N [%])		
	F0-1:	49 (29%)
	F2:	36 (21%)
	F3:	38 (22%)
	F4:	47 (28%)
ELF score (median [SD])		9.6 (1.7)

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events following enrollment. The clinical endpoints were death, hepatocellular carcinoma, and events indicative of hepatic decompensation.

The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 25. CHB: Prognostic performance

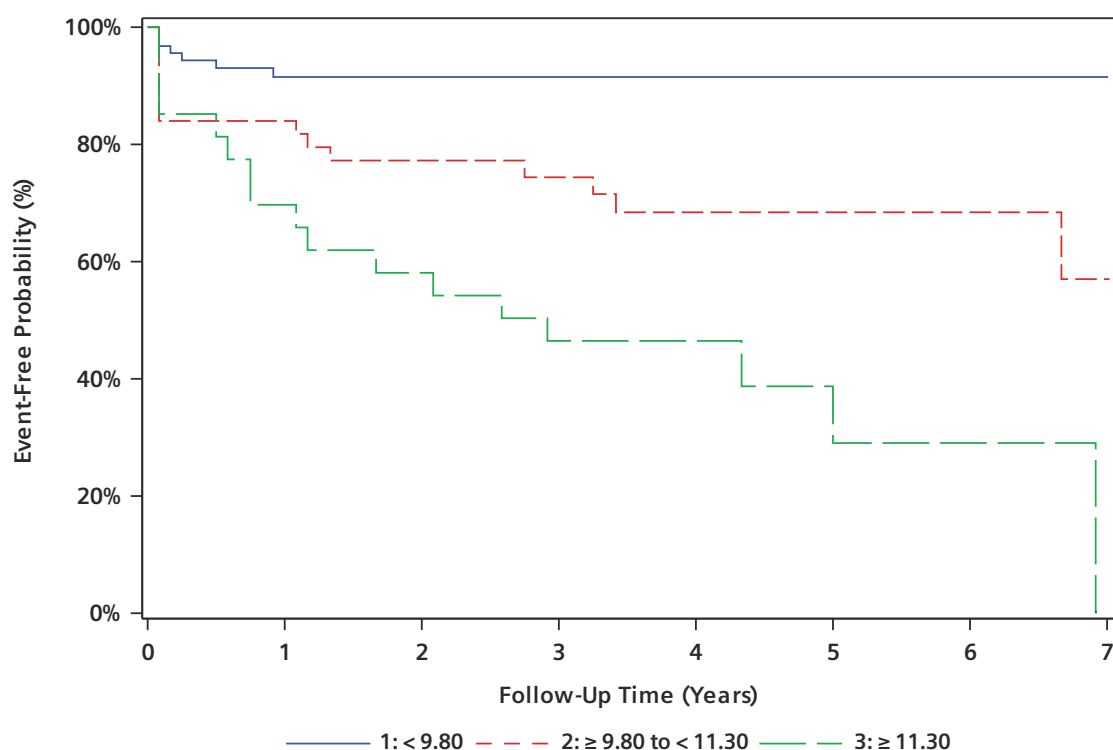
Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	93	7	86	7.5% (3.1%, 14.9%)	0.27 (0.14, 0.54)	1.00
Mid (≥ 9.80 to < 11.30)	50	15	35	30.0% (17.9%, 44.6%)	1.44 (0.88, 2.34)	3.88 (1.58, 9.54)

Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Higher (≥ 11.30)	27	17	10	63.0% (42.4%, 80.6%)	5.71 (2.85, 11.44)	9.01 (3.72, 21.82)
All	170	39	131	22.9% (16.9%, 30.0%)	—	—

^a Number of subjects.

^b Confidence interval.

Kaplan-Meier curves up to 7 years were plotted for each of the risk groups to display the proportion of subjects without hepatic decompensation event over time.



ELF Score	Number at Risk (Years)							
	0	1	2	3	4	5	6	7
1: < 9.80	93	57	51	46	36	29	17	4
2: ≥ 9.80 to < 11.30	50	38	32	26	12	10	8	3
3: ≥ 11.30	27	18	15	12	8	4	1	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events in subjects at 7 years following enrollment (N = 398).

Table 26. CHB: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	82.1% (32/39) (68.6%, 92.5%)	65.6% (86/131) (57.3%, 41.6%)	41.6% (32/77) (30.9%, 52.6%)	92.5% (86/93) (86.2%, 97.0%)
11.30	43.6% (17/39) (28.7%, 59.1%)	92.4% (121/131) (87.1%, 96.3%)	63.0% (17/27) (44.5%, 79.7%)	84.6% (121/143) (78.2%, 90.1%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

CHB: Progression to Hepatocellular Carcinoma

A prospective clinical trial was performed in subjects from Hong Kong with chronic HBV infection.⁷² The study included subjects on antiviral medication scored as intermediate risk (11–19) or high risk (≥ 20) using liver stiffness measurement hepatocellular carcinoma (LSM-HCC), a scoring system composed of age, serum albumin, HBV DNA levels, and LSM. For the subjects included in the analysis, the median follow-up time was 10.7 years (Interquartile range (IQR): 9.4–11.2 years). ELF measurements were performed on an ADVIA Centaur XP system.

Baseline clinical characteristics for the clinical trial subjects included in the analysis (N = 453) are described in the following table.

Table 27. CHB: Demographics

Age in years (median [IQR]) ^a	53 (42–64)
Sex (N [%]) ^b	
Female:	116 (26%)
Male:	337 (74%)
HbeAg positive (N [%])	155 (34%)
HBV DNA, 10 IU/mL (mean [SD])	5.6 (1.6)
Maintained virologic response (N [%])	388 (86%)
Antiviral therapy (N [%])	
Entecavir:	414 (91%)
Tenofovir disoproxil fumarate:	39 (9%)
Anti-viral treatment duration, months (mean [SD])	47.2 (13.3)
Transient elastography (LSM), kPa (median [range])	14.0 (9.0–16.1)
ELF score, median (median [IQR])	10.1 (8.6–11.6)

^a Interquartile range

^b Number of subjects.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events following enrollment. The clinical endpoint was hepatocellular carcinoma (HCC).

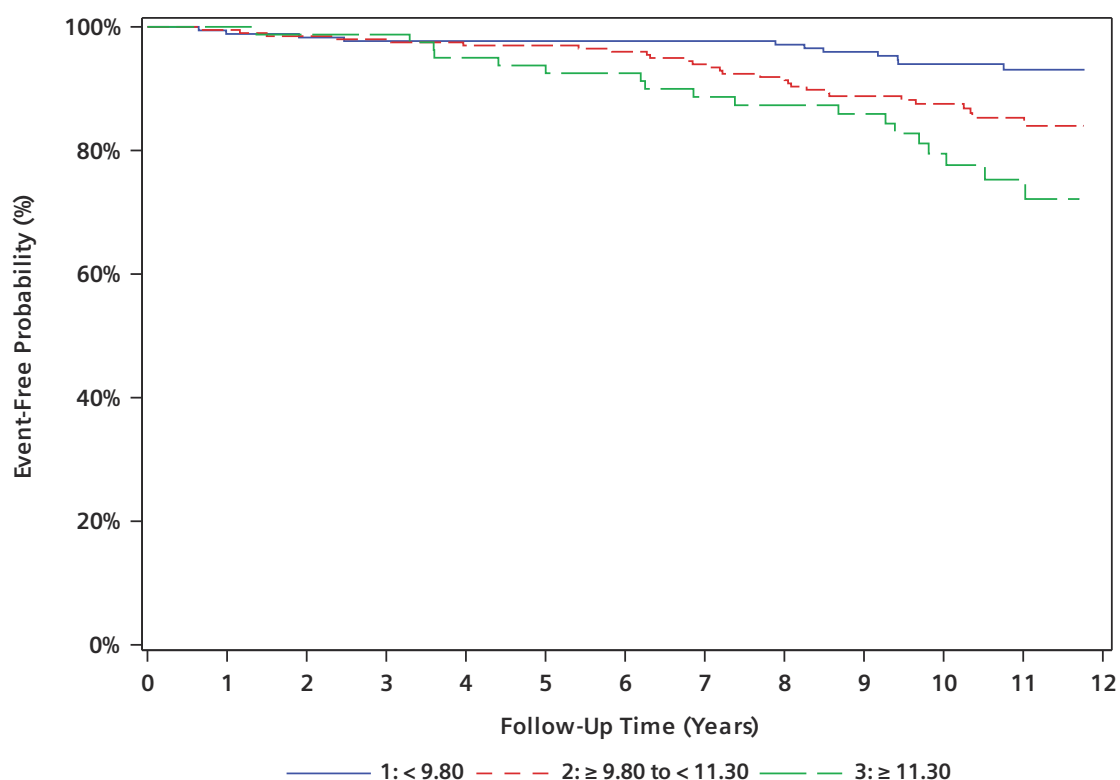
The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 28. CHB: Prognostic performance

Risk Group (ELF Score)	N ^a	HCC		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI)
		Yes	No			
Lower (< 9.80)	174	11	163	6.3% (3.2%, 11.0%)	0.61 (0.36, 1.04)	1.00
Mid (≥ 9.80 to < 11.30)	199	20	179	10.1% (6.2%, 15.1%)	1.01 (0.72, 1.43)	1.70 (0.81, 3.54)
Higher (≥ 11.30)	80	14	66	17.5% (9.9%, 27.6%)	1.92 (1.18, 3.13)	3.21 (1.46, 7.06)
All	453	45	408	9.9% (7.3%, 13.1%)	—	—

^a Number of subjects.^b Confidence interval.

Kaplan-Meier curves up to 12 years were plotted for each of the risk groups to display the proportion of subjects without hepatic decompensation over time.



ELF Score	Number at Risk (Years)												
	0	1	2	3	4	5	6	7	8	9	10	11	12
1: < 9.80	174	172	171	170	170	170	169	168	167	154	134	81	0
2: ≥ 9.80 to < 11.30	199	198	196	195	193	192	190	184	178	161	128	67	0
3: ≥ 11.30	80	80	79	79	76	75	73	67	65	56	43	24	0

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict HCC in subjects at 12 years following enrollment (N = 398).

Table 29. CHB: Clinical performance to predict HCC

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	75.6% (34/45) (60.5%, 87.1%)	40.0% (163/408) (35.2%, 44.9%)	12.2% (34/279) (8.6%, 16.6%)	93.7% (163/174) (89.0%, 96.8%)
11.30	31.1% (14/45) (18.2%, 46.6%)	83.8% (342/408) (79.9%, 87.3%)	17.5% (14/80) (9.9%, 27.6%)	91.7% (342/373) (88.4%, 94.3%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

Mixed Etiologies: Progression to Liver-Related Events and Correlation to Histology

A prospective multicenter trial in Europe as performed in subjects with chronic liver disease of varying etiologies.^{35,57,75} Two analyses were performed for the subjects included in this study. A prognostic analysis was performed for a cohort of subjects followed-up for clinical outcomes. For this analysis, the median follow-up time was 6.9 years (Interquartile range (IQR): 5.4–7.7 years). A separate diagnostic analysis was performed for a cohort of subjects who underwent biopsy for histological staging. ELF measurements were performed on an Immuno 1 analyzer.

Baseline clinical characteristics for the clinical trial subjects included in the prognostic analysis (N = 457) and diagnostic analysis (N = 921) are described in the following table.

Table 30. Mixed Etiologies: Demographics

	Prognostic cohort (N = 457)	Diagnostic cohort (N = 921)
Age in years (median [IQR]) ^a	42 (34–51)	43 (35–54)
Sex (N [%]) ^b		
Female:	152 (33%)	340 (37%)
Male:	305 (67%)	581 (63%)
Disease etiology (N [%])		
Viral hepatitis (HBV):	25 (5%)	48 (5%)
Viral hepatitis (HCV):	198 (43%)	431 (47%)
ALD:	86 (19%)	55 (6%)
NAFLD:	44 (10%)	93 (10%)
Autoimmune liver disease (AIH):	26 (6%)	36 (4%)
Autoimmune liver disease (PBC):	18 (4%)	32 (3%)
Autoimmune liver disease (PSC):	8 (2%)	16 (2%)
Other:	52 (11%)	210 (23%)

	Prognostic cohort (N = 457)	Diagnostic cohort (N = 921)
Alcohol consumption (N [%])		
Abstinent:	152 (33%)	—
Males: < 3 units/day; Females: < 2 units/day	201 (44%)	—
Males: 3 to < 6 units/day; Females: 2 to < 4 units/day	58 (13%)	—
Males: ≥ 6 units/day; Females: ≥ 4 units/day	46 (10%)	—
Ishak score (N [%])		
Ishak 0:	107 (23%)	227 (25%)
Ishak 1:	99 (22%)	201 (22%)
Ishak 2:	55 (12%)	133 (14%)
Ishak 3:	45 (10%)	112 (12%)
Ishak 4:	45 (10%)	86 (9%)
Ishak 5:	18 (4%)	55 (6%)
Ishak 6:	51 (11%)	107 (12%)
Unavailable:	37 (8%)	—
ELF score, median (median [IQR])	8.6 (7.8–9.6)	8.5 (7.7–9.6)

^a Interquartile range.

^b Number of subjects.

An analysis was performed to evaluate the prognostic ability of ELF scores at baseline to predict the risk of liver-related events following enrollment. The clinical endpoints were death, liver transplantation, hepatocellular carcinoma, and events indicative of hepatic decompensation.

The risk of liver-related events was assessed according to absolute risk, likelihood ratio, and Cox proportional hazard ratio.

Table 31. Mixed Etiologies: Prognostic performance

Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI) ^c
		Yes	No			
Lower (< 9.80)	306	10	296	3.3% (1.8%, 5.9%)	0.27 (0.16, 0.47)	1.00
Mid (≥ 9.80 to < 11.30)	55	13	42	23.6% (14.4%, 36.4%)	2.49 (1.45, 4.26)	8.01 (3.51, 18.28)

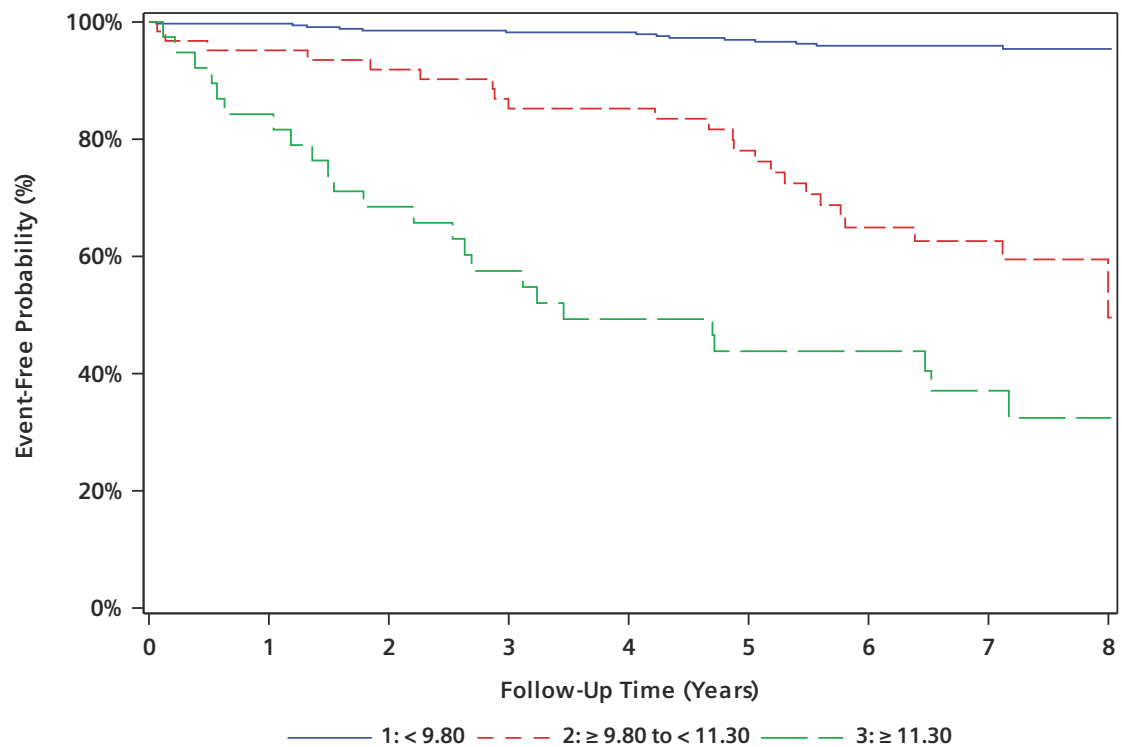
Risk Group (ELF Score)	N ^a	Liver-Related Event		Absolute Risk (95% CI) ^b	Likelihood Ratio (95% CI)	Cox Proportional Hazard Ratio (95% CI) ^c
		Yes	No			
Higher (≥ 11.30)	37	21	16	56.8% (40.9%, 71.3%)	10.56 (5.97, 18.67)	25.81 (12.11, 55.00)
All	398	44	354	11.1% (8.3%, 14.5%)	—	—

^a Number of subjects with at least 5 years of follow-up or with an event occurring earlier than 5 years.

^b Confidence interval.

^c Based on the overall study population of 457 subjects.

Kaplan-Meier curves up to 8 years were plotted for each of the risk groups to display the proportion of subjects without hepatic decompensation over time.



ELF Score	Number at Risk (Years)								
	0	1	2	3	4	5	6	7	8
1: < 9.80	356	340	330	321	312	296	266	183	69
2: ≥ 9.80 to < 11.30	62	59	56	51	50	42	33	21	5
3: ≥ 11.30	39	32	25	21	18	16	16	8	4

Sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the ability of ELF scores to predict liver-related events in subjects at 5 years following enrollment (N = 398).

Table 32. Mixed Etiologies: Clinical performance to predict liver-related events

Cutoff	Sensitivity (95% CI) ^a	Specificity (95% CI)	PPV ^b (95% CI)	NPV ^c (95% CI)
9.80	77.3% (34/44) (62.2%, 88.5%)	83.6% (296/354) (79.3%, 87.3%)	37.0% (34/92) (27.1%, 47.7%)	96.7% (296/306) (94.1%, 98.4%)
11.30	47.7% (21/44) (32.5%, 63.3%)	95.5% (338/354) (92.8%, 97.4%)	56.8% (21/37) (39.5%, 72.9%)	93.6% (338/361) (90.9%, 95.9%)

^a Confidence interval.

^b Positive predictive value.

^c Negative predictive value.

An analysis was performed to evaluate the correlation of ELF scores to Ishak staging at baseline. Sensitivity and specificity were calculated for various ELF cutoffs in relation to the presence of significant fibrosis ($\geq$ Ishak 3), advanced fibrosis ($\geq$ Ishak 4) and cirrhosis ($\geq$ Ishak 5).

Table 33. Mixed Etiologies: Diagnostic performance

Fibrosis Severity	Estimate (95% CI) ^a	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Significant Fibrosis (Ishak 0–2 versus 3–6)	Sensitivity	80.3% (289/360)	61.7% (222/360)	41.9% (151/360)	27.5% (99/360)	16.9% (60/360)
		(75.8%, 84.3%)	(56.4%, 66.7%)	(36.8%, 47.2%)	(22.9%, 32.4%)	(13.0%, 20.9%)
	Specificity	59.5% (334/561)	83.8% (470/561)	94.1% (528/561)	97.9% (549/561)	98.9% (555/561)
		(55.3%, 63.6%)	(80.5%, 86.7%)	(91.8%, 95.9%)	(96.3%, 98.9%)	(97.9%, 99.6%)
Advanced Fibrosis (Ishak 0–3 versus 4–6)	Sensitivity	87.9% (218/248)	73.0% (181/248)	53.6% (133/248)	36.3% (90/248)	23.0% (57/248)
		(83.2%, 91.7%)	(67.0%, 78.4%)	(47.2%, 60.0%)	(30.3%, 42.6%)	(17.9%, 28.7%)
	Specificity	55.7% (375/673)	80.4% (541/673)	92.4% (622/673)	96.9% (652/673)	98.7% (664/673)
		(51.9%, 59.5%)	(77.2%, 83.3%)	(90.2%, 94.3%)	(95.3%, 98.1%)	(97.5%, 99.4%)

Fibrosis Severity	Estimate (95% CI) ^a	ELF Score				
		8.30	9.00	9.80	10.50	11.30
Cirrhosis (Ishak 0–4 versus 5–6)	Sensitivity	92.6% (150/162)	82.1% (133/162)	65.4% (106/162)	46.3% (75/162)	30.9% (50/162)
		(87.4%, 96.1%)	(75.3%, 87.7%)	(57.6%, 72.7%)	(38.4%, 54.3%)	(23.8%, 38.6%)
	Specificity	51.8% (393/759)	76.3% (579/759)	89.7% (681/759)	95.3% (723/759)	97.9% (743/759)
		(48.2%, 55.4%)	(73.1%, 79.3%)	(87.3%, 91.8%)	(93.5%, 96.7%)	(96.9%, 98.8%)

^a Confidence interval.

Precision

Precision was determined in accordance with CLSI Document EP05 A3 using the Atellica IM Analyzer.⁹³ The Atellica IM ELF assay and each of the component assays were calibrated according to their individual calibration intervals. For each assay, samples were assayed in 2 replicates, 2 times a day, for 20 days (n = 80).

The following results are representative of the performance of the assay:

Atellica IM ELF

Sample	Mean ELF Score	Repeatability		Within-Laboratory Precision	
		SD ^a (ELF Score)	CV ^b (%)	SD (ELF Score)	CV (%)
1	9.54	0.02	0.2	0.04	0.4
2	7.67	0.04	0.5	0.08	1.0
3	7.40	0.04	0.6	0.10	1.3
4	11.59	0.04	0.4	0.05	0.4
5	12.53	0.05	0.4	0.06	0.5
6	13.68	0.03	0.2	0.05	0.4
7	7.91	0.02	0.3	0.04	0.5

^a Standard deviation.

^b Coefficient of variation.

Atellica IM ELF was designed to have the following precision:

ELF Score	Precision	
	Repeatability (Within-Run)	Within-Laboratory (Total Precision)
≥ 5.00	≤ 0.15 SD ^a	≤ 0.20 SD

^a Standard deviation.

Atellica IM HA

Sample	Mean (ng/mL)	Repeatability		Within-Laboratory Precision	
		SD ^a (ng/mL)	CV ^b (%)	SD (ng/mL)	CV (%)
1	38.46	0.760	2.0	1.573	4.1
2	45.21	1.092	2.4	2.658	5.9
3	9.07	0.400	4.4	0.747	8.2
4	172.27	7.971	4.6	9.342	5.4
5	825.71	44.687	5.4	52.452	6.4
6	481.97	17.057	3.5	24.983	5.2
7	19.03	0.468	2.5	0.807	4.2

^a Standard deviation.

^b Coefficient of variation.

Atellica IM HA was designed to have the following precision.

Concentration Interval	Precision	
	Repeatability (Within-Run)	Within-Laboratory (Total Precision)
Atellica IM HA ≥ 15.00 ng/mL	≤ 1.20 SD ≤ 8.0% CV	≤ 1.50 SD ≤ 10.0% CV

Atellica IM PIINP

Sample	Mean (ng/mL)	Repeatability		Within-Laboratory Precision	
		SD ^a (ng/mL)	CV ^b (%)	SD (ng/mL)	CV (%)
1	59.76	1.148	1.9	1.649	2.8
2	1.74	0.071	4.1	0.089	5.1
3	7.24	0.122	1.7	0.618	8.5
4	24.22	0.324	1.3	0.693	2.9
5	20.39	0.338	1.7	0.559	2.7
6	104.81	1.998	1.9	3.580	3.4
7	6.58	0.107	1.6	0.168	2.5

^a Standard deviation.

^b Coefficient of variation.

Atellica IM PIINP was designed to have the following precision.

Concentration Interval	Precision	
	Repeatability (Within-Run)	Within-Laboratory (Total Precision)
Atellica IM PIINP ≥ 2.00 ng/mL	≤ 0.16 SD ≤ 8.0% CV	≤ 0.20 SD ≤ 10.0% CV

Atellica IM TIMP-1

Sample	Mean (ng/mL)	Repeatability		Within-Laboratory Precision	
		SD ^a (ng/mL)	CV ^b (%)	SD (ng/mL)	CV (%)
1	15.6	0.23	1.5	0.42	2.7
2	81.1	1.26	1.6	2.38	2.9
3	89.1	1.92	2.2	2.60	2.9
4	626.5	11.41	1.8	22.86	3.6
5	325.2	7.19	2.2	13.91	4.3
6	849.0	20.20	2.4	28.64	3.4
7	76.1	1.23	1.6	1.97	2.6

^a Standard deviation.^b Coefficient of variation.

Atellica IM TIMP-1 was designed to have the following precision.

Concentration Interval	Precision	
	Repeatability (Within-Run)	Within-Laboratory (Total Precision)
Atellica IM TIMP-1 ≥ 25.0 ng/mL	≤ 2.50 SD ≤ 10.0% CV	≤ 3.00 SD ≤ 12.0% CV

Reproducibility

Reproducibility was evaluated according to CLSI document EP05-A3⁹³ using the Atellica IM Analyzer. A reproducibility study was conducted at 3 sites using 1 reagent lot. The study was run over 5 days, 2 runs per day, and 3 replicates per run for serum pools. Reproducibility data was pooled across 3 sites (N = 90).

Sample (N = 90)	Mean ELF Score	Repeatability		Between-Run		Between-Day		Between-Site		Reproducibility	
		SD ^a (ELF Score)	CV ^b (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)	SD (ELF Score)	CV (%)
Serum 1	7.43	0.05	0.6	0.05	0.7	0.00	0.0	0.03	0.4	0.07	1.0
Serum 2	11.54	0.04	0.4	0.06	0.5	0.00	0.0	0.09	0.8	0.12	1.0
Serum 3	12.62	0.05	0.4	0.04	0.3	0.00	0.0	0.10	0.8	0.12	1.0
Serum 4	9.87	0.05	0.5	0.02	0.2	0.02	0.2	0.06	0.6	0.08	0.8
Serum 5	12.46	0.05	0.4	0.07	0.5	0.00	0.0	0.09	0.8	0.13	1.0
Serum 6	13.63	0.04	0.3	0.06	0.4	0.00	0.0	0.07	0.5	0.10	0.7
Serum 7	9.34	0.05	0.6	0.04	0.5	0.00	0.0	0.04	0.4	0.08	0.8
Serum 8	7.91	0.06	0.7	0.01	0.2	0.02	0.2	0.06	0.8	0.08	1.1

^a Standard deviation.^b Coefficient of variation.

Precision data presented above are representative of results obtained by testing of multiple reagent lots.

Assay Comparison

Assay comparison testing was determined using the Passing-Bablok regression model in accordance with CLSI Document EP09-A3.⁹⁴

The relationship between ADVIA Centaur ELF performed on the ADVIA Centaur XP system (y), and the ELF Test performed on the Immuno 1 system (x) is described by the following equation:

Specimen Type	Comparative Assay (x)	Regression Equation	Sample Interval	N ^a	r ^b
Serum	Immuno 1	$y = 1.00x - 0.01$	6.49–13.25	163	0.977

^a Number of samples tested.

^b Correlation coefficient.

In an additional assay comparison study the relationship between Atellica IM ELF performed on the Atellica IM Analyzer (y), and the ADVIA Centaur ELF performed on the ADVIA Centaur XP system (x) is described by the following equation:

Specimen Type	Comparative Assay (x)	Regression Equation	Sample Interval	N ^a	r ^b
Serum	ADVIA Centaur XP system	$y = 1.01x - 0.22$	6.73–13.65	409	0.997

^a Number of samples tested.

^b Correlation coefficient.

The assay is designed to have a correlation coefficient of ≥ 0.95 and a slope of 1.00 ± 0.10 .

Interferences

Interference testing was performed in accordance with CLSI Documents EP07-ed3⁹⁵ and EP37-ed1⁹⁶ using the ADVIA Centaur XP system. Testing demonstrated $\leq 10.0\%$ change in results in the measurements of HA, PIIINP, and TIMP-1 and ≤ 0.30 change in ELF score for each of the following substances.

Therapeutic Substance	Substance Test Concentration
Acetaminophen	200 µg/mL (1.32 mmol/L)
Acetylsalicylic acid	652 µg/mL (3.62 mmol/L)
Azathioprine	2.58 µg/mL (9.31 µmol/L)
Biotin ^a	150 ng/mL (0.61 µmol/L)
Cetirizine	4.35 µg/mL (11.18 µmol/L)
Diphenhydramine	774 ng/mL (3.03 µmol/L)
Disulfiram	1.14 µg/mL (3.84 µmol/L)
Fluorescein ^b	850 ng/mL (2.56 µmol/L)
Glyburide	720 ng/mL (1.45 µmol/L)
Hydroxyzine	267 ng/mL (0.71 µmol/L)
Ibuprofen	219 µg/mL (1.06 mmol/L)
Interferon α2a	10 ng/mL
Interferon α2b	10 ng/mL
Ledipasvir	969 ng/mL (1.09 µmol/L)

Therapeutic Substance	Substance Test Concentration
Liraglutide	168 ng/mL (44.86 nmol/L)
Loratadine	87 ng/mL (0.27 µmol/L)
Mesalamine	20.4 µg/mL (133.42 µmol/L)
Metformin	12 µg/mL (92.88 µmol/L)
Methotrexate	1.36 mg/mL (2.99 mmol/L)
Obeticholic Acid	540 ng/mL (1.28 µmol/L)
Pioglitazone	4.76 µg/mL (13.38 µmol/L)
Ribavirin	25 µg/mL (102.37 µmol/L)
Rifampicin	48 µg/mL (58.56 µmol/L)
Sofosbuvir	1.85 µg/mL (3.50 µmol/L)
Tenofovir	978 ng/mL (3.40 µmol/L)
Tolazamide	45 µg/mL (144.45 µmol/L)
Ursodiol (UDCA)	169 µg/mL (430.51 µmol/L)

- ^a The concentration listed reflects the lowest concentration of the drug that caused a ≤ 0.30 change in the ELF score. For biotin, there was $\leq 10.0\%$ change in results in the measurements of HA, PIIINP, and TIMP-1 at 3500 ng/mL, 65 ng/mL, and 3500 ng/mL, respectively.
- ^b For fluorescein, there was $\leq 10.0\%$ change in the measurements of HA, PIIINP, and TIMP-1 at 500 ng/mL, 300,000 ng/mL, and 250 ng/mL, respectively.

Hemolysis, Icterus, Lipemia (HIL)

Substance	Substance Test Concentration
Hemoglobin	1000 mg/dL (10 g/L)
Bilirubin, conjugated	60 mg/dL (712 µmol/L)
Bilirubin, unconjugated	60 mg/dL (1026 µmol/L)
Lipemia (Intralipid)	3500 mg/dL (35 g/L)

Endogenous Substances

Substance	Substance Test Concentration
Cholesterol	400 mg/dL (10 mmol/L)
Fructose	18 mg/dL (999 µmol/L)
Glucose	1000 mg/dL (56 mmol/L)
Protein (Albumin)	6 g/dL (60 g/L)
Protein (Total)	15 g/dL (150 g/L)

Biotin Interference

The following information summarizes the effect of biotin interference on the ELF score.

	Biotin Test Level (ng/mL)					
	50	65	75	150	300	1200
ELF Score	Bias (change in ELF score)					
7.44	-0.08	-0.10	-0.12	-0.28	-0.53	-1.95
9.90	-0.05	-0.10	-0.13	-0.28	-0.42	-1.59
12.51	-0.07	-0.05	-0.08	-0.20	-0.38	-1.28
13.80	-0.13	-0.10	-0.11	-0.28	-0.60	-1.71

Biotin interference was observed for the measurement of PIIINP, but not for HA or TIMP-1. The following information summarizes biotin interference for measurement of PIIINP:

	Biotin Test Level (ng/mL)					
	50	65	75	150	300	1200
PIIINP (ng/mL)	% Bias					
6.97	-8.0	-6.9	-10.9	-26.0	-49.6	-92.2
10.20	-4.5	-6.5	-9.2	-22.0	-43.7	-87.9
18.64	-5.7	-5.7	-9.7	-21.1	-37.6	-81.4
105.23	-9.3	-8.0	-9.2	-23.6	-53.0	-88.5

Specimens that contain biotin at a concentration of 150 ng/mL demonstrate a less than or equal to 0.30 change in results for ADVIA Centaur ELF. Biotin concentrations greater than this may lead to falsely depressed results for patient samples.

The recommended adult daily dietary intake for biotin is 30 µg/day. Over-the-counter dietary supplements promoted for use in hair, skin, and nail health may contain 5–100 mg of biotin, with recommendations to take multiple pills per day. Pharmacokinetic studies in healthy adults have shown that, in subjects ingesting 5 mg, 10 mg, and 20 mg of biotin, serum concentrations of biotin can reach up to 73 ng/mL, 141 ng/mL, and 355 ng/mL, respectively.⁹⁷ Subjects who take up to 300 mg of biotin per day may have plasma biotin levels as high as 1160 ng/mL.⁹⁸ These studies were performed in a small number of apparently healthy subjects. Clearance of biotin could be different in other patient populations, such as in patients with impaired renal function, which could lead to higher concentrations of biotin in serum or plasma.

Cross-Reactivity

Cross-reactivity was determined in accordance with CLSI Document EP07-ed3.⁹⁵ The potential cross reactant was added to a human serum-based sample at the concentrations indicated and were evaluated for potential interference. Cross-reactivity was calculated as follows:

$$\% \text{ cross reactivity} = \frac{(\text{concentration of spiked sample} - \text{concentration of unspiked sample})}{\text{concentration of compound}} \times 100$$

No significant cross reactivity was observed in the presence of the following substances:

Test Protein	ADVIA Centaur Reagent	Test Concentration (µg/mL)	Cross-Reactivity (%)
Chondroitin Sulfate A	HA	1000	≤ 0.07%
Chondroitin Sulfate B	HA	1000	≤ 0.01%
Chondroitin Sulfate C	HA	1000	≤ 0.01%
Fibronectin	HA	500	≤ 0.01%
Heparin Sulfate	HA	200	≤ 0.01%
Laminin	PIIINP	500	≤ 0.15%
Type I Collagen	PIIINP	2000	≤ 0.07%
Type IV Collagen	PIIINP	200	≤ 0.23%
Type VI Collagen	PIIINP	50	≤ 0.31%
Matrix metalloproteinase-1 (MMP-1) (activated)	TIMP-1	2.5	≤ 0.79%
MMP-2 (activated) ^a	TIMP-1	5	≤ 1.55%
MMP-2 (latent)	TIMP-1	5	≤ 0.19%
MMP-3 (latent)	TIMP-1	5	≤ 0.13%
MMP-9 (activated)	TIMP-1	5	≤ 0.45%
MMP-9 (latent)	TIMP-1	3.5	≤ 0.97%
TIMP-2	TIMP-1	4	≤ 0.08%

^a Activated MMP-2 does not have significant interference with the ELF score. In the presence of 5 µg/mL activated MMP-2 change in ELF score was ≤ 0.30.

Linearity

Linearity testing was performed in accordance with CLSI Document EP06-A⁹⁹ using the Atellica IM Analyzer. For individual measurements of HA, PIIINP, and TIMP-1, a sample containing high levels of analyte was mixed in various proportions with a sample containing low levels of analyte. The resulting mixtures were tested. Atellica IM ELF components of HA, PIIINP, and TIMP-1 were all found to be linear within their respective measuring intervals.

ADVIA Centaur Reagent	Measuring Interval
HA	3.00–1000.00 ng/mL
PIIINP	1.00–150.00 ng/mL
TIMP-1	5.0–1300.0 ng/mL

High-Dose Hook Effect

High HA concentrations can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of HA, patient samples with HA concentrations above the measuring interval and as high as 25,000 ng/mL will report > 1000.00 ng/mL.

High PIIINP concentrations can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of PIIINP, patient samples with PIIINP concentrations above the measuring interval and as high as 2500 ng/mL will report > 150.00 ng/mL.

High TIMP-1 concentrations can cause a paradoxical decrease in the RLUs (high-dose hook effect). For measurements of TIMP-1, patient samples with TIMP-1 concentrations above the measuring interval and as high as 30,000 ng/mL will report > 1300.0 ng/mL.

Results were established using the ADVIA Centaur XP system.

Standardization

HA, PIIINP, and TIMP-1 reagents that comprise Atellica IM ELF are traceable to internal standards. Currently no reference standards are available for these reagents. Assigned values of calibrators and controls are traceable to these internal standards.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

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Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Symbol Title	Symbol	Symbol Title
	Manufacturer		Authorized representative in the European Community
	Use-by date		Authorized representative in Switzerland
	Catalog number		Batch code

Symbol	Symbol Title	Symbol	Symbol Title
	Consult Instructions for Use		Contains sufficient for <n> tests
<.../eifu>	Internet URL address to access the electronic instructions for use	Rev. XX	Version of Instructions for Use
	In vitro diagnostic medical device	Rev.	Revision
RxOnly	Prescription device (US only)		
	CE Marking with Notified Body		Unique Device Identifier
			CE Marking
	Temperature limit		Keep away from sunlight
	Upper limit of temperature		Lower limit of temperature
	Do not re-use		Do not freeze
	Recycle		This way up
	Biological risks		Caution
	Common Units		International System of Units
YYYY-MM-DD	Date format (year-month-day)	YYYY-MM	Date format (year-month)
	Document face up ^a		Handheld barcode scanner
	Target		Mixing of substances
	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.		Interval
	Unique material identification number		Material
	Type of control		Name of control
	Quality control lot value		Calibrator lot value

^a Indicates Assay-eNote

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