Non-alcoholic fatty liver disease

Michael Pavlides
Jeremy Cobbold

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a global health problem, with an estimated prevalence of 30% among adults in industrialized countries, and associated with obesity and type 2 diabetes mellitus. NAFLD is defined histologically and represents a spectrum from simple steatosis to steatohepatitis, fibrosis and cirrhosis. Hepatic triglyceride droplet accumulation is considered the first feature in the natural history; in some patients, this is associated with inflammation and fibrosis, through the interaction of environmental and host factors. Patients with NAFLD are generally asymptomatic and usually present with incidental findings of abnormal liver function tests or an echobright liver on ultrasonography. Non-invasive tests are routinely used to stratify risk of advanced fibrotic disease, and liver biopsy is used in selected cases to assess for evidence of hepatic co-morbidity. Treatment is focused on weight reduction through dietary modifications and exercise. Cardiovascular risk factors should be addressed and the treatment of diabetes mellitus, hypertension and hyperlipidaemia optimized. Liver-specific treatments such as vitamin E and pioglitazone have shown benefit in clinical trials, but have not been widely adopted into clinical practice because of concerns over long-term safety. Several other agents including obeticholic acid are being evaluated in Phase II and III clinical trials.

Keywords Cirrhosis; diabetes mellitus; insulin resistance; metabolic syndrome; MRCP; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; obesity; steatosis

Introduction

Non-alcoholic fatty liver disease (NAFLD) is associated with obesity and the metabolic syndrome. It is the most common cause of liver dysfunction in developed countries, and poses a significant burden on health services.

Michael Pavlides *BSc MB BS MRCP DPhil is Head of Liver Imaging at the Oxford Centre for Clinical Magnetic Resonance Research at the University of Oxford, and an Honorary Consultant Hepatologist at the Oxford University Hospitals NHS Foundation Trust, UK. Competing interests: MP is a shareholder in Perspectum Diagnostics.*

Jeremy Cobbold *MA PhD FRCP is Consultant Hepatologist and Clinical Lead for the Oxford Liver Unit, Oxford University Hospitals NHS Foundation Trust, and Honorary Senior Clinical Lecturer at the University of Oxford, UK. He runs the metabolic hepatology service and has a clinical research interest in NAFLD. Competing interests: JC is on the Advisory Board and consults for NovoNordisk and Intercept has consulted for for Alnylam pharmaceuticals and has received speaker fees for BMS and Intercept Pharmaceuticals.*

Key points

- Routine non-invasive risk stratification involves serum-based tests in the community Fibrosis-4 score, non-alcoholic fatty liver disease (NAFLD) fibrosis score, Enhanced Liver Fibrosis test), and ultrasound elastography in secondary care. MR-based tests (multiparametric MRI, MR elastography) are becoming more common
- Treatments for type 2 diabetes lead to weight loss, which is known to be beneficial for NAFLD
- Several new agents to treat NAFLD are currently in Phase II and III clinical trials
- Guidelines advising on NAFLD have been produced by NICE, the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD)

Definition and epidemiology

NAFLD is defined histologically as the presence of lipid droplets in >5% of hepatocytes in the absence of other causes of fat deposition, such as excessive alcohol consumption, drugs (e.g. methotrexate) and viruses (e.g. hepatitis C). The condition represents a spectrum of pathology ranging from simple steatosis, to steatosis with inflammation and hepatocyte damage (non-alcoholic steatohepatitis (NASH)) with or without fibrosis (Figure 1).

The epidemiology of NAFLD and its different subtypes is difficult to study as NAFLD is classified histologically and liver biopsy is not appropriate for population-level studies. Studies using proton magnetic resonance spectroscopy to quantify intrahepatic triglycerides (triacylglycerols) have estimated a population prevalence of 30%.[1] Using ultrasound and biopsy for disease classification, the prevalence of NAFLD and NASH was estimated at 46% and 12%, respectively (see Further reading).

Pathogenesis

NAFLD is characterized by two pathological features:

1. Liver fat accumulation
2. Development of inflammation and fibrosis.

Liver fat accumulation

Obesity and peripheral insulin resistance are key risk factors for NAFLD as they lead to changes in hepatic metabolic pathways (e.g. increased *de novo* lipogenesis and decreased β-oxidation), resulting in intrahepatic triglyceride accumulation. Despite intrahepatic lipid accumulation, most patients do not develop inflammation and fibrosis.

Development of NASH and fibrosis

It is not entirely clear why some patients develop inflammation (NASH) and fibrosis. This progression results from the interactions of multiple pathogenic environmental and host mechanisms or 'multiple parallel hits' (see Further reading). An increasing sedentary lifestyle, positive calorie balance and refined

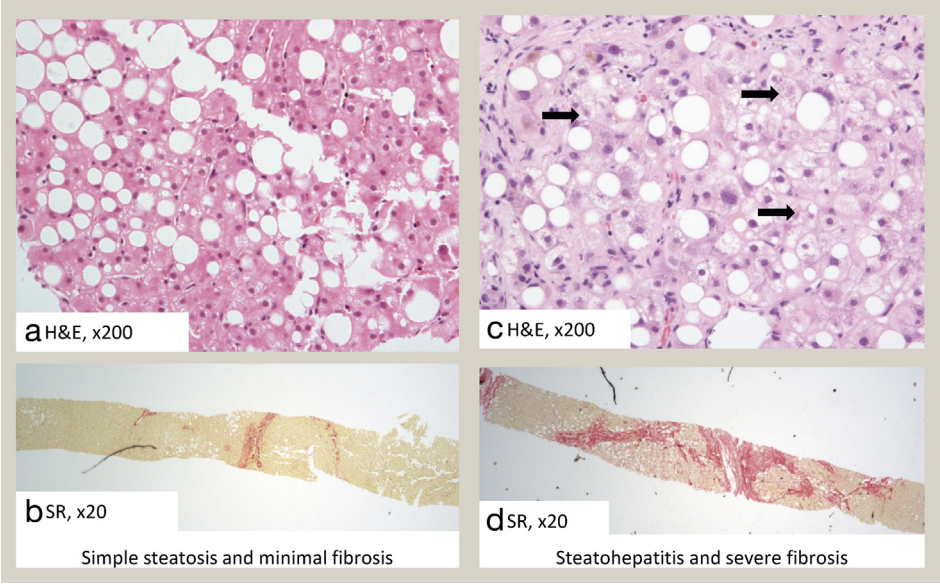

Figure 1 The histological spectrum of non-alcoholic fatty liver disease. The figure shows high magnification (×200) histological sections stained with haematoxylin and eosin (H&E) from patients with (**a**) simple steatosis and (**c**) steatohepatitis, with prominent ballooning of hepatocytes (black arrows). The bottom row shows low magnification (×20) histological sections stained with Sirius red (SR) fibrosis stain from the same patients. The patient with simple steatosis has mild fibrosis (**b**), while the patient with steatohepatitis has severe fibrosis (**d**).

sugars have been implicated. Genetic factors such as polymorphisms in the patatin-like phospholipase 3 (*PNPLA3*) gene are also associated with disease occurrence and progression.[2]

Gut microbiota produce short-chain fatty acids that can be absorbed and provide excess energy. At the same time they can produce toxic substances and excess endogenous alcohol that are delivered to the liver through the portal circulation, where they can induce inflammation and fibrosis. The disease phenotype can be transmitted with transfer of gut microbiota from diseased animals to healthy controls. Other pathogenic mechanisms implicated in the pathogenesis of NASH include endocrine factors from adipose tissue (adiponectin, leptin), cytokines (interleukin-6, tumour necrosis factor-α), bile acid metabolism and endoplasmic reticulum stress.

Natural history

Liver disease

Although NASH is often considered a hallmark of more progressive disease, the presence of advanced fibrosis is the strongest predictor of clinical outcomes, and patients without NASH can also progress to more advanced fibrosis. A prospective cohort study with a mean follow-up of 26.4 years indicated that only patients with advanced stages of fibrosis (bridging) were at increased risk of all-cause mortality (hazard ratio (HR) 3.28, $p < 0.0001$) compared with the reference population,[3] whereas patients with NASH and milder forms of fibrosis (F0-2) did not have an increased mortality risk (HR 1.41, $p = 0.072$). A separate study found that fibrosis predicted mortality and liver transplantation, and advanced fibrosis predicted liver-related events (see Further reading).

Paired biopsy studies suggest that NASH can be dynamic, with both progression and regression of disease without intervention. Patients with non-alcoholic fatty liver (simple steatosis) and NASH can progress over time. The development of diabetes mellitus is a risk factor for progressive fibrosis.

Patients with cirrhosis resulting from NAFLD are at increased risk of developing hepatocellular carcinoma (HCC). It is estimated that 1–22% of all HCC arises in patients with NAFLD. In NAFLD, HCC can sometimes develop in patients without cirrhosis. Even though patients with NAFLD can have a lower risk than patients with other liver diseases (e.g. hepatitis C), the impact from HCC arising in patients with NAFLD is anticipated to be much greater because of the high prevalence of NAFLD.

Cardiovascular disease and all-cause mortality

Cardiovascular disease and malignancies are the two leading causes of death in patients with NAFLD, with liver disease only in third place. This is a consistent finding in studies looking at the long-term prognosis of patients with NAFLD (see Further reading).

Clinical features

NAFLD is usually asymptomatic before the development of cirrhosis and complications (ascites, variceal bleeding, hepatic encephalopathy). Patients can present with non-specific symptoms such as right upper quadrant pain or lethargy. Most patients are identified incidentally, through abnormal liver function tests (e.g. mild elevation of aminotransferase values) or abnormal radiology tests (e.g. bright echotexture on ultrasound suggesting fatty infiltration).

Management

Patients with NAFLD/NASH are at increased risk of both liver-related and cardiovascular morbidity and mortality. The effective management of NAFLD should therefore address cardiometabolic risk factors with a structured approach, ideally involving a multidisciplinary team.[4] Management can be based

in primary and secondary/tertiary care, with non-invasive risk stratification tools available to aid appropriate onward referral.

Advice on the management of NAFLD is available from national bodies (e.g. National Institute for Health and Care Excellence; www.nice.org.uk/guidance/ng49) and international liver disease academic organizations (European Association for the Study of the Liver (EASL; The management of non-alcoholic liver disease), American Association for the Study of Liver Disease (AASLD; Non-alcoholic fatty liver disease, diagnosis and management)). Preventive strategies require a socioeconomic approach to health promotion and combating the obesogenic environment from an early age.

Diagnosis and staging of liver disease

A firm diagnosis of NAFLD is required to inform further management. In practice, after exclusion of other liver aetiologies such as autoimmune or chronic viral hepatitis, a diagnosis of NAFLD is made in patients with:

- elevated liver aminotransferase values
- and/or features of fatty liver on ultrasonography in the absence of any other causes of chronic liver disease
- in the context of at least one feature of the metabolic syndrome (obesity, hypertension, dyslipidaemia, type 2 diabetes mellitus (T2DM)/impaired fasting glycaemia).

Liver biopsy can be required to make a diagnosis in atypical presentations and, more commonly, make the diagnosis in the investigation of patients with persistent mild elevation of aminotransferase values of unknown cause. Table 1 summarizes what tests should be performed in patients with suspected NAFLD.

NAFLD and coexistent liver disease: NAFLD is now so prevalent that the presence of other causes of chronic liver disease does not exclude a fatty liver component. In cases where NAFLD is suspected to coexist with other conditions, liver biopsy can be required for further assessment, as steatosis can be an additional driver of disease progression (e.g. haemochromatosis, hepatitis B virus).

Risk stratification: once the diagnosis of NAFLD has been established, it is important to stratify risk. More advanced liver disease confers higher morbidity and mortality. The presence of fibrosis in particular has prognostic implications.[3] Of note, serum aminotransferase concentration does not correlate with disease severity, and cirrhosis can be present with normal liver function tests.

Liver biopsy for assessment of NAFLD: liver biopsy is the reference standard for NAFLD evaluation. It allows the differentiation of simple steatosis from steatohepatitis, and staging of fibrosis, particularly in the setting of clinical trials. Furthermore, it can exclude coexistent liver disease, especially when there is diagnostic uncertainty (e.g. the presence of liver autoantibodies).

However, liver biopsy is limited by costs and risks of complications. It is not appropriate for most patients with NAFLD who are expected to have simple steatosis. As a result, non-invasive tests are increasingly being developed and used for risk stratification, to identify those at low risk of significant or advanced fibrosis and to reduce the reliance on liver biopsy for routine diagnosis and staging.

Clinical, biochemical and radiological assessments for patients with suspected NAFLD

Exclusion of other liver pathology	**Rationale**
Alcohol and medication history	To exclude alcoholic fatty liver disease and drug causes of steatosis
Serology for chronic viral hepatitis (B,C)	Viral hepatitis can cause secondary fatty liver or can coexist with NAFLD
Serum ferritin and transferrin saturations	Iron indices can be elevated in NAFLD and can be associated with more aggressive disease
α-Antitrypsin concentration	To exclude α_1-antitrypsin deficiency
Ceruloplasmin	In patients <45 years old, to exclude Wilson's disease
Autoimmune screen (ANA, AMA, SMA, LKMA, immunoglobulins)	To exclude autoimmune liver disease. Note that low titres ($\leq$1:160) of ANA can be seen in patients with NAFLD
Liver ultrasound	To look for macroscopic evidence of fatty liver (bright liver echotexture) and complications (e.g. HCC, features of portal hypertension such as splenomegaly)
Other tests to consider (MRCP, CT, OGD, coeliac serology)	MRCP should be considered in patients with features of cholestatic disease. If cirrhosis is suspected, OGD for screening of oesophageal varices in indicated. CT can be needed to assess for HCC or vascular abnormalities. Coeliac disease is associated with mildly elevated aminotransferase concentrations
Tests to assess metabolic syndrome	
Weight, height, waist $\pm$ hip circumference	To identify obesity
Blood pressure	To identify and quantify essential hypertension
HbA_{1c}, fasting plasma glucose, urinalysis	To assess for impaired fasting glycaemia, diabetes mellitus and nephropathy
Plasma lipid profile (fasting)	To identify dyslipidaemia
Risk stratification	
Simple serum-based tests	e.g. FIB-4, NAFLD fibrosis score; used to rule out individuals with high risk for advanced disease
Direct fibrosis test	e.g. ELF test; used in primary or secondary care as a screening test for advanced fibrosis
Imaging/elastography	e.g. Transient elastography; used to identify patients at high risk of advanced fibrosis who would benefit from liver biopsy

ANA, antinuclear antibody; AMA, antimitochondrial antibody; CT, computed tomography; LKMA, liver–kidney microsomal antibody; MRCP, magnetic resonance cholangiopancreatography; OGD, oesophago-gastro-duodenoscopy; SMA, smooth muscle antibody.

Table 1

Medications with potential secondary beneficial effects on the liver

Agent	Usual indication	Potential secondary beneficial effect
Metformin	T2DM	Weight loss, decreased insulin resistance, decreased liver triglyceride content. Evidence of reduced HCC/malignancy risk
GLP-1 analogues (e.g. liraglutide)	T2DM	Weight loss, decreased appetite, decreased insulin resistance and potential improvement of liver disease severity
ARB (e.g. losartan)	Hypertension	Hepatic antifibrotic effects in experimental models
SGLT2 inhibitors (e.g. empagliflozin)	T2DM	Weight loss, reduced cardiovascular mortality, decreased liver fat and liver enzymes in experimental models

ARB, angiotensin receptor blocker.

Table 2

Non-invasive assessments of NAFLD: non-invasive assessment is now part of routine care. Clinical and biochemical factors associated with progressive disease have been identified (age >45 years, body mass index (BMI) >30 kg/m^2, T2DM or impaired fasting glycaemia, aspartate aminotransferase to alanine aminotransferase ratio >1). In primary care, serum-based tests are used to rule out individuals at risk of having advanced fibrosis. Examples include the NAFLD fibrosis score and the Fibrosis-4 (FIB-4) score, based on blood markers and clinical features, which are useful to exclude advanced liver fibrosis and have high negative predictive values. The Enhanced Liver Fibrosis (ELF) test incorporates metabolites of fibrogenesis in a proprietary algorithm and is recommended as a community screening test for advanced fibrosis in the UK (www.nice.org.uk/guidance/ng49).

In secondary care, non-invasive tests can be used for further risk stratification, for selecting patients for liver biopsy and for monitoring disease progression. Transient elastography as a measure of liver stiffness, which is associated with liver fibrosis, is used to stratify disease severity in NAFLD. Magnetic resonance elastography, mainly available in the USA, can also assess liver stiffness, whereas multiparametric magnetic resonance imaging[5] appears promising for NAFLD evaluation as it can concurrently assess three variables (inflammation/fibrosis, steatosis, iron) associated with more aggressive disease.

Lifestyle assessment

Lifestyle factors including diet and physical exercise should be assessed in detail. A 7-day food diary helps to identify dietary habits that can be improved. Direct questioning about consumption of soft drinks, fruit juice and snacks is often required as they are frequently overlooked when recalling food intake.

Therapeutic interventions: lifestyle modification remains the mainstay of treatment. Programmes including diet and exercise have improved hepatic steatosis and insulin resistance in research studies and specialized multidisciplinary clinics. Weight loss improves histological features of NAFLD severity (NASH, fibrosis), and any treatments that can lead to weight loss are considered beneficial (see Further reading). Calorie-counting, such as is advocated by commercial weight loss programmes, and newer smartphone applications focus attention on intake. Physical activity and energy expenditure can be monitored using accelerometers or smartphone applications.

Metabolic syndrome

Assessment: the components of the metabolic syndrome (T2DM or impaired fasting glycaemia, hypertension, obesity, dyslipidaemia) should be assessed in detail, as liver disease severity is associated with the number of the metabolic syndrome components present.

Therapeutic interventions: the management of metabolic syndrome components should be optimized (e.g. weight loss, treatment of hypertension, dose adjustments of lipid-lowering medication, glycaemic control in T2DM). Diabetes therapies associated with weight loss in the context of improved glycaemic control, such as glucagon-like peptide 1 (GLP-1) analogues (e.g. liraglutide or exenatide) and sodium glucose co-transporter 2 (SGLT-2) inhibitors, are associated with improved cardiovascular mortality; these are favoured where possible over agents that are associated with weight gain, such as insulin and sulfonylureas. Where possible, agents with actual or potential secondarily beneficial effects on the liver should be chosen (Table 2). Use of statins is encouraged in the context of NAFLD to reduce cardiovascular risk, and mildly deranged liver biochemistry is not a contraindication to use.

Liver-specific drug treatments

Despite numerous candidate drugs and some positive results from clinical trials, this remains an area of unmet need. Vitamin E and pioglitazone have shown some promise but concerns relating to the long-term safety of these medications means that they have not been widely adopted into clinical practice. An increased all-cause mortality has been reported with vitamin E, while pioglitazone is associated with weight gain.

Obeticholic acid improved histological indices of steatohepatitis and fibrosis in a recent study (see Further reading). However, patients on obeticholic acid developed a worse plasma lipid profile and had higher insulin resistance, raising questions about long-term cardiovascular safety. A large Phase III study is underway and should provide further data in this field.

Surgical strategies

Bariatric surgery: such surgery leads to dramatic and sustained weight loss, improvement or resolution of T2DM and improvement of histological indices including fibrosis.

Liver transplantation: this can be indicated in patients who develop cirrhosis and complications. NAFLD accounts for an

increasing proportion of patients referred for transplantation. As cardiovascular disease often coexists with end-stage liver disease in individuals with NAFLD, particular care should be taken in assessment and optimization before transplantation. Weight management may also be needed, as a BMI >40 kg/m^2 is a relative contraindication for transplantation. ◆

KEY REFERENCES

1 Wong VW, Chu WC, Wong GL, et al. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut* 2012; **61:** 409–15.

2 Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40:** 1461–5.

3 Ekstedt M, Hagstrom H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatol* 2015; **61:** 1547–54.

4 Moolla A, Motohashi K, Marjot T, et al. A multidisciplinary approach to the management of NAFLD is associated with improvement in markers of liver and cardio-metabolic health. *Frontline Gastroenterol* 2019; **0:** 1–10. https://doi.org/10.1136/flgastro-2018-101155.

5 Pavlides M, Banerjee R, Tunnicliffe EM, et al. Multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease severity. *Liver Int* 2017; **37:** 1065–73.

FURTHER READING

Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterol* 2015; **149**. 389–397.e10.

Hyysalo J, Mannisto VT, Zhou Y, et al. A population-based study on the prevalence of NASH using scores validated against liver histology. *J Hepatol* 2014; **60:** 839–46.

Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385:** 956–65.

Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatol* 2010; **52:** 1836–46.

Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterol* 2015; **149**. 367–78.e5.

TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 56-year-old man presented for review because of an alanine aminotransferase result of 56 U/litre (5–35). He felt well but said that his GP had been monitoring his liver blood tests for some years. He had no history of significant alcohol intake. He was taking amlodipine for hypertension.

On clinical examination, the body mass index was 32 kg/m^2.

Investigation

- Ultrasonography of the liver showed an echogenic appearance

What are the most appropriate investigations to establish the most likely diagnosis (select one)?

A. Percutaneous liver biopsy
B. Magnetic resonance imaging of the liver
C. Transient elastography (FibroScan®) with measurement of the controlled attenuation parameter
D. Blood pressure measurement, waist circumference, fasting plasma lipids and HbA_{1c}
E. A chronic liver disease screen including hepatitis B and C serology, liver autoantibodies, serum ferritin concentration, plasma lipid profile and glycated haemoglobin (HbA_{1c})

Question 2

A 48-year-old British-Bangladeshi man presented for review 6 weeks after the finding of an alanine aminotransferase (ALT) of 48 U/litre (5–35) on annual blood tests for monitoring of diabetes mellitus. A screen of blood tests had been negative for hepatic co-morbidity.

Investigations

- Ultrasonography of the liver showed a bright liver echotexture
- Alanine aminotransferase 30 U/litre (5–35)

What is the most appropriate approach to risk stratification for the likely diagnosis?

A. Reassure and recheck liver function tests in 1 year
B. Risk stratification is not required because the alanine aminotransferase has returned to normal
C. Arrange magnetic resonance elastography
D. Refer for liver biopsy
E. FIB-4 algorithm ± Enhanced Liver Fibrosis test or FibroScan®

Question 3

A 62-year-old woman presented for review. She had been found to have non-alcoholic steatohepatitis (NASH) with advanced fibrosis but not cirrhosis on liver biopsy. Four years previously, she had been found to have type 2 diabetes mellitus. She was taking metformin 1 g 12-hourly, amlodipine 5 mg daily and simvastatin 20 mg daily.

On clinical examination, the body mass index was 33 kg/m^2. Blood pressure measured in primary care was 145/88 mmHg and 143/92 mmHg.

Investigation

- HbA_{1c} 63 mmol/mol (20–42)

What is the most appropriate change to her management?

A. Initiation of a sulfonylurea such as gliclazide and twice-daily blood sugar monitoring
B. Initiate a sulfonylurea such as gliclazide, increase dose of amlodipine to 10 mg daily
C. Initiate a GLP-1 analogue such as liraglutide, increase dose of amlodipine to 10 mg daily
D. Initiate a glucagon-like peptide-1 (GLP-1) analogue such as liraglutide, change amlodipine to ramipril
E. Initiation of a basal bolus regimen of insulin with close monitoring (four times daily) of blood sugar measurement and addition of a β-adrenoceptor blocker medication such as atenolol