Etiology and Clinical Outcomes of Idiopathic Noncirrhotic Portal Hypertension

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Etiology and Clinical Outcomes of Idiopathic Noncirrhotic Portal Hypertension

Etiologie en klinisch beloop van idiopatische niet-cirrotische portale hypertensie

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CHAPTER 1

INTRODUCTION AND AIMS

Idiopathic noncirrhotic portal hypertension (INCPH) is a rare disorder in the Western world (1). Most studies were performed more than 15 years ago enrolling patients for more than a decade earlier (2-4). The last decade only two small cohort studies have been reported in literature (5-6). As a result, many aspects of this disorder remain to be elucidated. This thesis aims to investigate the morphological features, etiology, prevalence and clinical outcome of patients with INCPH.

The international nomenclature about INCPH has been ambiguous. In the Indian subcontinent this condition is known as noncirrhotic portal fibrosis, while in Japan and other Asian countries (7-9), it is referred to as idiopathic portal hypertension. In the Western world this condition has been variably termed hepatoportal sclerosis, idiopathic portal hypertension, incomplete septal cirrhosis and nodular regenerative hyperplasia (4, 10-12). Since all these entities share histopathological characteristics (obliterative vascular lesions) and clinical profile, INCPH can be viewed as a distinct single entity with various pathological aspects, rather than different clinicopathological entities. We therefore suggested a uniform nomenclature **(Chapter 2)**.

Liver biopsy remains essential in the diagnosis of INCPH. First, it is indispensible for the exclusion of liver cirrhosis. Second, morphological signs suggestive for the diagnosis of INCPH have been described in Asian patients. However, the occurrence of these signs in Western patients remains undetermined. We assessed these histopathological features in a large cohort of INCPH patients **(Chapter 3)**. Additionally, morphological signs present in liver specimens of patients with INCPH were compared to specimens of noncirrhotic portal vein thrombosis (PVT), the latter serving as a control group for noncirrhotic portal hypertension. Finally, the pathological features between liver specimens of INCPH with and without HIV were studied.

INCPH has been considered a condition with a benign disease course (5-year survival of nearly 100%). However, long-term follow-up studies and determinants of survival were lacking. We studied the clinical manifestations, outcome and determinants of survival in a large cohort of Western INCPH patients **(Chapter 4)**.

In the Western world, prevalence data are scarce. A histological review of 2500 autopsies demonstrated a prevalence of INCPH histological features of 3%. However, only 5% of these patients had clinical evidence of portal hypertension. We investigated the prevalence of sonographic signs of portal hypertension (cirrhotic and noncirrhotic) in an elderly population-based study. In addition determinants and upper limit of normal spleen size were assessed **(Chapter 5)**.

The occurrence of INCPH has been described increasingly in patients infected with the human immunodeficiency virus (HIV) (13-19). Several aspects regarding this condition remain to be elucidated. We aimed to evaluate the number of patients nationwide diagnosed with this disorder by a systematic survey in all HIV treatment centres in the Netherlands. Furthermore we studied the risk factors, clinical features and outcome of this disorder **(Chapter 6)**.

According to expert opinions, the number of diagnosed HIV-associated INCPH cases is a tip of the iceberg since this disorder can be asymptomatic for a long time. Furthermore, up to now, INCPH has been linked to didanosine treatment. Nevertheless, the role of didanosine in the pathophysiology of INCPH remains controversial as the drug was widely used in HIV treatment in the past and reports on INCPH remain scarce. We assessed the prevalence of HIV-associated INCPH and the association between this disorder and didanosine by systematically screening a large population of HIV-infected patients (with and without exposure to didanosine). Additionally we aimed to identify risk factors for INCPH in didanosine treated HIV patients **(Chapter 7)**.

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CHAPTER 2

IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION: A REVIEW

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ABSTRACT

Idiopathic noncirrhotic portal hypertension (INCPH) is characterized by an increased portal venous pressure gradient in the absence of a known cause of liver disease and portal vein thrombosis. In contrast to the high prevalence of this disorder in India, INCPH is a rare disease in the Western world. The etiology of INCPH can be divided in five categories: chronic infections, exposure to medication or toxins, thrombophilia, immunological disorders and genetic disorders. Multifactorial etiology can also be encountered. Chronic abdominal infection is incriminated as the most important etiological factor in Eastern patients and thrombophilia in the Western patients. The majority of INCPH patients initially present with signs or complications of portal hypertension (mainly variceal bleeding and splenomegaly). These patients usually have preserved liver function. Liver function impairment occurs mainly in the context of intercurrent conditions. INCPH patients are often clinically and radiologically misdiagnosed as liver cirrhosis so that a liver biopsy is indispensable to discriminate cirrhosis from INCPH. The histopathological characteristics of INCPH are heterogeneous, demonstrating overlap between several pathological entities (hepatoportal sclerosis, nodular regenerative hyperplasia and incomplete septal cirrhosis). Even though hemodynamical changes in INCPH patients are not comparable to those in cirrhotics, prophylaxis and treatment of variceal bleeding is recommended to be similar. Anticoagulation therapy must be considered only in patients who develop portal vein thrombosis. INCPH has been considered a disorder with a relatively benign disease course. However, liver failure, hepatic encephalopathy and hepatopulmonary syndrome can occur and are considered indications for liver transplantation.

INTRODUCTION

Portal hypertension is a clinical syndrome defined by a portal-caval venous pressure gradient exceeding 5 mm Hg (1). This increase of portal pressure eventually will lead to the development of collateral circulation and splenomegaly. In the Western world, liver cirrhosis is the most frequent cause of portal hypertension. However, in a variety of disorders portal hypertension develops in the absence of cirrhosis. This condition, referred to as noncirrhotic portal hypertension, is often classified based on the site of obstruction (prehepatic, intrahepatic and suprahepatic portal hypertension) (Table 1). Worldwide, the most common cause of noncirrhotic portal hypertension is schistosomiasis (2). In the Western world, chronic liver diseases such as non-alcoholic steatohepatitis, primary biliary cirrhosis, primary sclerosing cholangitis and congenital hepatic fibrosis together with extrahepatic portal vein thrombosis and Budd-Chiari syndrome are common causes of noncirrhotic portal hypertension (3-5). If all these conditions have been ruled out, the diagnosis of idiopathic noncirrhotic portal hypertension (INCPH) can be made (Table 2) (6).

Table 1 Causes of non-cirrhotic portal hypertension

a) Pre-sinusoidal
Developmental abnormalities
Adult polycystic disease
Hereditary haemorrhagic disease
Arteriovenous fistulas
Congenital hepatic fibrosis
Idiopathic noncirrhotic portal hypertension
Biliary diseases
Primary biliary cirrhosis
Autoimmune cholangiopathy
Primary sclerosing cholangitis
Toxic (vinyl chloride)
Neoplastic occlusion of the portal vein
Lymphoma
Epithelioid haemangioendothelioma
Epithelial malignancies
Chronic lymphocytic leukemia
Granulomatous lesions
Schistosomiasis
Mineral oil granuloma
Sarcoidosis

Table 1 Causes of noncirrhotic portal hypertension *(continued)*

b) Sinusoidal
Fibrosis of space of Disse
Drug induced (methotrexate, amiodarone)
Alcoholic liver damage
Toxic (vinyl chloride, copper)
Metabolic (NASH, Gaucher's disease, Zellweger syndrome)
Inflammatory (viral hepatitis, chronic Q fever, healed CMV)
Amyloid or light chain deposition in Disse' s space
Early non-cirrhotic alcoholic liver disease (defenestration of sinusoidal lining)
Destruction or collaps of sinusoids (acute necroinflammatory diseases)
Infiltrative diseases
Mastocytosis
Agnogenic myeloid metaplasia
Gaucher's disease
Hypertrophy of Kupffer's cells
Parasites (visceral leishmaniasis)
Metabolic (Gaucher's disease)
Compression of sinusoids by markedly hypertrophied hepatocytes
Microvesicular steatosis (alcohol, acute fatty liver of pregnancy)
c) Post-sinusoidal
Veno-occlusive disease
Acute radiation injury
Toxic injury
Drug induced injury
Phlebosclerosis of hepatic veins
Alcoholic liver disease
Chronic radiation injury
Hypervitaminosis A
E-ferol injury
Primary vascular malignancies
Epithelioid haemangioendothelioma
Angiosarcoma
Granulomatous phlebitis
Sarcoidosis
Mycobacterium avium intracellulare
Lipogranulomas
Mineral oil granulomas

Table 2 Diagnostic criteria of idiopathic noncirrhotic portal hypertension*

1) Clinical signs of portal hypertension (any one of the following **)
Splenomegaly/hypersplenism
Oesophageal varices
Ascites (non-malignant)
Increased hepatic venous pressure gradient
Portovenous collaterals
2) Exclusion of cirrhosis on liver biopsy
3) Exclusion of chronic liver disease causing cirrhosis or noncirrhotic portal hypertension †
Chronic viral hepatitis B/C
Non-alcoholic steatohepatitis/alcoholic steatohepatitis
Autoimmune hepatitis
Hereditary hemochromatosis
Wilson's disease
Primary biliary cirrhosis
4) Exclusion of conditions causing noncirrhotic portal hypertension
Congenital liver fibrosis
Sarcoidosis
Schistosomiasis
5) Patent portal and hepatic veins (doppler ultrasound or computed tomography scanning)

* All five criteria must be fulfilled in order to diagnose INCPH.

** Splenomegaly must be accompanied by additional signs of portal hypertension in order to fulfil this criterion.

† Chronic liver disease must be excluded since severe fibrosis might be understaged on liver biopsy

The international nomenclature about this condition is ambiguous. In the Indian subcontinent this condition is known as noncirrhotic portal fibrosis, while in Japan and other Asian countries, it is referred to as idiopathic portal hypertension. In the Western world this condition has been variably termed hepatoportal sclerosis, idiopathic portal hypertension, incomplete septal cirrhosis and nodular regenerative hyperplasia (NRH). Since all these entities share histopathological characteristics (obliterative vascular lesions) and clinical profile, it has been suggested that INCPH can be viewed as a distinct single entity with various pathological aspects, rather than different clinicopathological entities. Agreement on uniform nomenclature is an essential requirement for collaborative studies. We therefore suggest that in future studies, the term INCPH will be used since it covers both the clinical and etiological aspects of the disorder. The purpose of this review is to provide a critical appraisal of the available scientific literature of this disorder in the Western world. Additionally, differences and similarities between Western and Eastern patients will be discussed.

HISTORY AND EPIDEMIOLOGY

At the end of the 19th century, Banti described a syndrome characterized by marked splenomegaly and anemia in the absence of hematological disease (7). In retrospect, it becomes clear that the patient cohort studied by Banti comprised patients with cirrhosis, INCPH and tropical splenomegaly syndrome due to chronic malaria. Subsequently, a panel of Indian experts denominated splenomegaly in patients without liver pathology or chronic malaria as noncirrhotic portal fibrosis (8, 9). In India, INCPH incidence estimates as high as 23% have been reported (10, 11). In the Western world, INCPH might be responsible for 3-5% of cases of portal hypertension (12). A histological review of 2500 autopsies demonstrated a prevalence of INCPH histological features of 3%. However, only 5% of these had evidence of portal hypertension (13). Concerning INCPH in the Western world, most studies were performed more than 15 years ago enrolling patients for more than a decade earlier (14-16). The last decade only two small cohort studies have been reported in literature (6, 17). Based on these limited data, a male predominance with a median age of 40 years has been described. Exceptionally, INCPH has been reported in children (18).

ETIOLOGY

Many theories on the development of INCPH have been proposed, signifying limited understanding of the disease process. Theoretically, the etiology of INCPH can be divided in five categories: chronic infections, exposure to medication or toxins, genetic disorders, thrombophilia and immunological disorders. Multifactorial etiology can also be encountered. One could speculate that the difference in worldwide prevalence of INCPH can be explained by a difference in genetic predisposition and area-specific diseases. In Western INCPH patients, a combination of disorders is often present.

Immunological disorders

INCPH has frequently been reported in association with immunological disorders (17, 19, 20). Various theories have been given to explain these associations. In patients with systemic sclerosis, a fibrogenetic process has been suggested as etiological factor in the development of INCPH (21). Alternatively, in systemic lupus erythematosus patients, immunoglobulin interference with prostacyclin formation has been designated to increase microthrombosis vulnerability (22). IgA anticardiolipin antibodies elevation, hypothetically leading to obliteration of small vessels, has been demonstrated in the majority of celiac disease related INCPH (19). Another immunological disorder with a high prevalence of INCPH is primary hypogammaglobulinemia. Malamut et al. demonstrated histological features of INCPH in 70% of these patients (23).

Infections

Bacterial infection of the gut with repeated septic embolization and subsequent obstruction of small portal veins may be involved in the etiology of INCPH. This theory is supported by the high prevalence of INCPH in low socioeconomic areas with a high abdominal infection rate at birth and in early childhood (24). In addition, animal studies demonstrated that injection of E. coli into the portal vein results in the development clinical and histological characteristics of INCPH (25).

INCPH has been reported increasingly in patients with human immunodeficiency virus (HIV) infection (26-33). It remains a matter of debate whether a component of highly active anti-retroviral therapy (HAART) or the presence of hypercoagulability is playing a role in the development of HIV related INCPH. Regarding the etiological role of HAART, prolonged exposure to didanosine has been assigned a potential role in its development. In a small cohort of HIV patients with cryptogenic liver disease, long term didanosine treatment was observed in the majority of INCPH patients (27). Additionally, 2 recent case series reported long term exposure of didanosine in 7/8 and 12/12 HIV patients with INCPH (32, 33). Its exposure has been shown to be associated with increased cardiovascular risk in HIV-infected patients, probably due to a pro-thrombotic state secondary to enhancement of pro-inflammatory mediators (34). Additionally, endothelial and mitochondrial damage of the portal system due to didanosine have been postulated in the pathophysiology of INCPH. Despite these hypotheses, it is difficult to conclude on the etiological role of didanosine, as the drug was widely used in the treatment of HIV in the past. Alternatively, a high prevalence of pre-existing hypercoagulability (mainly protein S deficiency), possibly leading to vascular obstruction, has been reported in patients with HIV-related INCPH (26, 28, 29). This association remains controversial since it has not been demonstrated consistently (32, 33).

Medication and Toxins

Several medications and chemicals have been alleged to cause INCPH. Among those, azathioprine, 6- thioguanine and arsenic as Fowler`s solution are the most frequently reported drugs associated with this disorder (35-37). Key et al. described the development portal hypertension in five chronic myeloid leukemia patients treated with busulphan and 6-thioguanine (38). However, since INCPH has also been associated with hematological diseases outside the setting of cytotoxic treatment, the association between this treatment and INCPH is not completely established (39). Currently, the most commonly used immunosuppressive drugs associated with the development of histological and clinical signs of INCPH are thiopurines (azathioprine and 6-mercaptopurine) (40, 41). Although it is tempting to incriminate drug intake and chemical exposure as primary etiological factors, only a small minority of the patients treated with the above mentioned drugs or exposed to these chemicals develop clinical or histological signs of

INCPH. It appears that an underlying susceptibility is needed in order to develop this disorder when exposed to the above described agents.

Genetic disorders

Reports on familial aggregation of INCPH and occurrence of its histological features in several congenital disorders (Adams-Oliver syndrome and Turner`s disease) suggest a genetic background for this disorder (18, 42-45). The high prevalence of HLA-DR3 positivity in these families supports an immunogenetic basis of this disorder (43).

Thrombophilia

Hillaire et al. identified a 54% prevalence of prothrombotic disorders in a small patient cohort (6). An additional argument supporting the thrombophilia theory is the high prevalence and incidence of portal vein thrombosis in Western patients with INCPH. On the basis of clinical and histological data from INCPH patients, thrombophilia might be indicated the underlying vulnerability necessary for the development of this disorder (46-49).

PATHOPHYSIOLOGY

Portal hemodynamics have been described to be different between INCPH and cirrhosis. A dual theory implicating both increased splenic blood flow and intrahepatic obstruction has been hypothesized regarding the development of INCPH (Figure 1). Based on earlier studies, it has been speculated that the primary cause of INCPH is not related to hepatic abnormalities but rather to an increased portal venous flow secondary to splenomegaly (50, 51). An overproduction of NO, liberated in the sinus lining spleen cells, has been designated to be responsible for the dilatation of splenic sinuses and subsequently massive splenomegaly in INCPH patients (52). In these patients, liver specimens demonstrate normal histopathology. Observed disease remission after splenectomy supports the pathogenetic significance of splenomegaly in INCPH (53-55). In patients with more advanced disease, increased intrahepatic resistance due to obliteration of the portal venous microcirculation presumably will lead to a further elevation of portal hypertension. Thrombophilia, immunological disorders and infections have been indicated as potential initiating lesions for the portal venous obliteration (6, 49, 56, 57). However, since no supportive data are available, this theory remains an area of conjecture. An additional role has been attributed to endothelin-1 in the pathophysiology of INCPH. It has been speculated that an increased production of the latter increases vascular resistance and stimulates periportal collagen production (58).

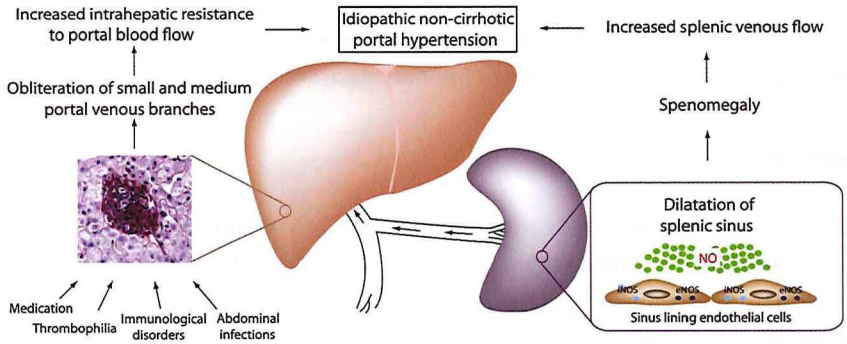

Figure 1 Hypothesized pathophysiology of idiopathic noncirrhotic portal hypertension. Presumably, two mechanisms are involved in the development of INCPH. First, diffuse and strong expression of inducible nitric oxide synthetase (iNOS) and endothelial nitric oxide synthetase (eNOS) in the sinus lining cells of the spleen have been demonstrated. Subsequently, liberation of NO will lead to dilatation of splenic sinuses and splenomegaly. The increased splenic venous flow secondary to splenomegaly will contribute to an increase in portal hypertension. Second, obliterative portal venopathy is a characteristic morphological feature in INCPH. This obliteration of the portal venous microcirculation presumably leads to an increase intrahepatic resistance. There is no consensus regarding the specific mechanisms of this injury. Potential initiating lesions of obliterative portal venopathy are thrombophilia, immunological disorders, infections and medication. Due to lack of supportive data, this theory remains speculative.

CLINICAL PRESENTATION

The majority of INCPH patients initially presents with signs or complications of portal hypertension. In a large Indian study, 72% of patients with INCPH presented with gastrointestinal hemorrhage, only a minority (14%) presented with splenomegaly (11, 59). In contrast, a low prevalence of upper gastrointestinal bleeding as initial manifestation has been reported in Japanese and Western patients of which the majority presented with splenomegaly or liver test disturbances (6, 60). Compared to spleen enlargement in other causes of portal hypertension (liver cirrhosis and portal vein thrombosis), a massive and disproportional large spleen is observed in patients with INCPH. In a large review on Indian INCPH patients, clinical splenomegaly was the most common initial symptom at the time of diagnosis (68.9%) (10). In addition, 5.3% of these patients reported dragging pain caused by a huge mass. A minority of the INCPH patients (30%) demonstrated impaired liver function at initial presentation in the context of gastrointestinal bleeding or in association with severe concurrent diseases. In general, liver function improved after controlling these associated conditions (6). Hepatic encephalopathy has rarely been reported in INCPH (61-63). Ascites has been described in 50% of INCPH patients (6). Comparable to liver failure, transient ascites occurs mainly in the presence of intercurrent conditions and mostly resolves after controlling the triggering events. Chronic ascites is described in association with renal failure and insulin dependent diabetes mellitus in a

minority of the patients. Until recently, the hepatopulmonary syndrome was considered to be a rare complication in INCPH patients (64). However, two recent prospective studies reported a 10% prevalence of this pulmonary disorder in these patients (65, 66).

DIAGNOSIS

Since there is no single test that can be regarded the gold standard to diagnose INCPH, its diagnosis remains a challenge. Even in renowned hepatology centers, INCPH patients are frequently misdiagnosed as having liver cirrhosis. Krasinskas et al. demonstrated that the majority of INCPH patients undergoing liver transplantation carried a pretransplantation diagnosis of cirrhosis (63).

The initial assessment in patients with liver test disturbances or detected esophageal varices is typically performed with abdominal ultrasonography. Nodularity of the liver surface and thickening of the portal vein walls are sonographic features of INCPH (Figure 2) (10, 13, 46-48). However, these manifestations are not specific for INCPH and can also be observed in patients with liver cirrhosis. Recently, promising data have been published regarding discrimination between liver cirrhosis and INCPH with transient elastography (67). The mean liver stiffness in a large cohort of INCPH patients was 9.2 kPa, being significantly lower compared to the observed values in patients with liver cirrhosis (>14 kPa) (68). As a result, the finding of liver stiffness values <14 kPa in the presence of clear signs of portal hypertension should raise the suspicion of INCPH.

Currently, liver biopsy remains essential in the diagnosis of INCPH. It is indispensible for the exclusion of liver cirrhosis since based on radiological examinations INCPH patients

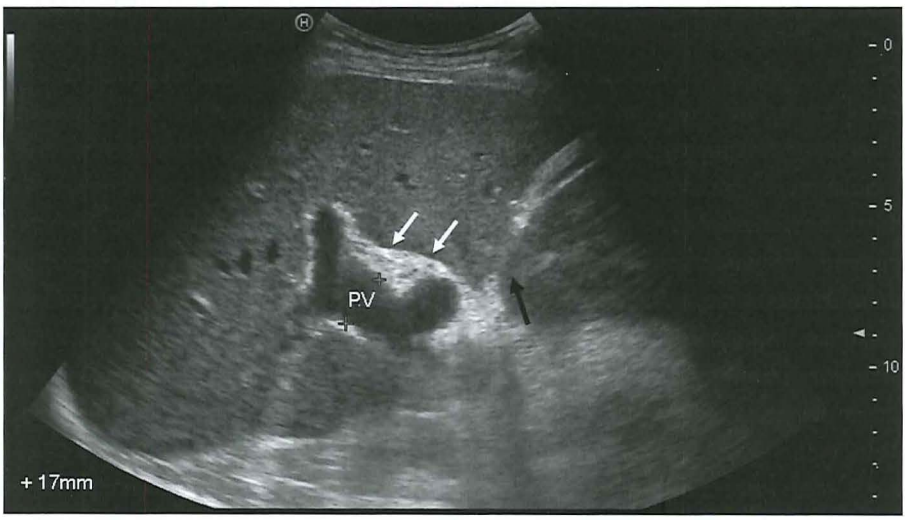

Figure 2 Abdominal ultrasound in a patient with INCPH demonstrating liver border nodularity (black arrow), dilatation of portal vein (PV) and thickened portal vein wall (white arrow).

are indistinguishable of cirrhotics. If liver cirrhosis and additional liver diseases known to cause portal hypertension histologically have been excluded, the pathologist has to look carefully for the discrete pathological characteristics of INCPH.

PATHOLOGY

Gross pathology

Macroscopic features in INCPH are mainly based on examination of resection specimens from liver transplantation (46, 48, 49, 63, 69). The majority of these liver explants demonstrate organizing old thrombi (occluding or mural) in the large portal vein branches, nodular appearance, atrophy and dysmorphy. In contrast, recent thrombi are rarely seen (70). Contrasting, in some patients gross appearance is normal.

Histopathology

Historically, INCPH has been classified in 4 different histological categories namely: idiopathic portal hypertension, NRH, partial nodular transformation (PNT) and incomplete septal cirrhosis (13, 24, 47, 48, 71-74). The presence of fibrotic portal tracts and thin fibrous septa in the absence of cirrhosis are pathological criteria for idiopathic portal hypertension (47, 73). In NRH, the parenchyma shows micronodular transformation, with central hyperplasia and an atrophic rim in the absence of fibrosis (Figure 3) (71). PNT is characterized by the presence of noncirrhotic, grossly visible parenchymal nodules located in the perihilar region of the liver around the large portal tracts (74). By definition, these nodules are larger than those in NRH and diagnosis is only possible on resection specimens (75). Nodules macroscopically exhibiting PNT may show histological features similar to those in NRH (i.e. small hyperplastic nodules). Finally, incomplete septal cirrhosis is characterized by slender "incomplete" septal fibrosis that demarcates the parenchyma into conspicuous nodules with small hypoplastic portal tracts and hyperplastic hepatocytes (76, 77). Recently, this classification in different categories has been questioned (46). First, histopathological examination of whole livers from Western patients with INCPH demonstrated the concomitant presence of the different features in one specimen. Furthermore, pathological examination of livers resected at transplantation or autopsy failed to categorize the specimens according to the proposed classification due to the heterogeneity of the lesions (46, 48, 63, 78). As a result, in the Western world, INCPH is viewed as a single clinical entity with various pathological features rather than separate clinicopathological entities. Although no pathognomonic histological findings exist in INCPH, frequently observed morphological features include the following: obliterative portal venopathy (luminal narrowing or obliteration of small portal venous branches accompanied by dense deposits of elastic fibers) (Figure 4B), increased number of portal vascular channels, dilated portal veins

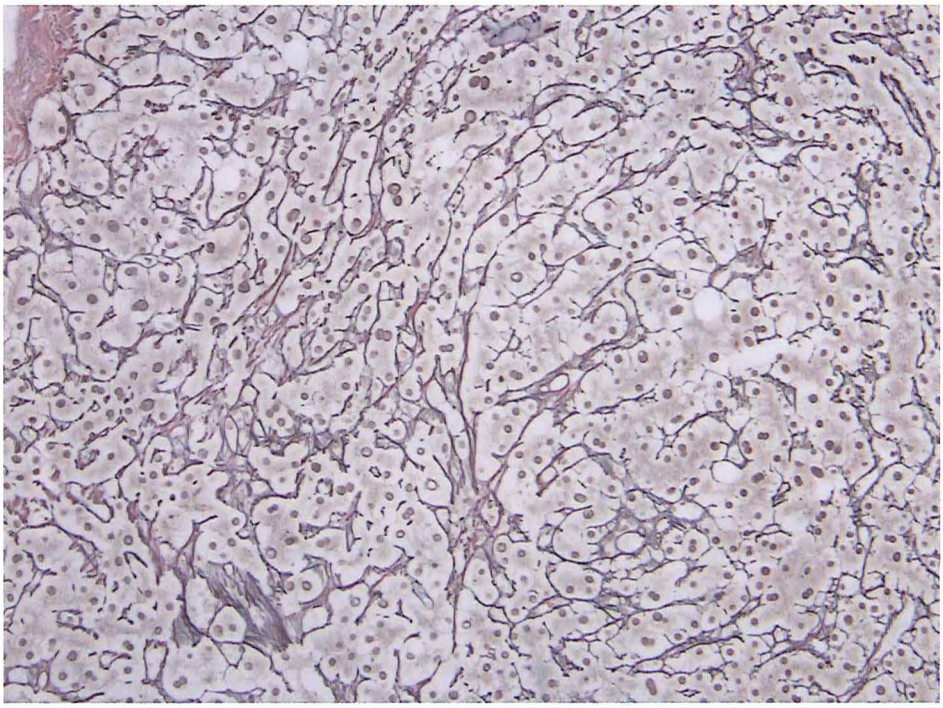

Figure 3 Liver biopsy demonstrating nodular regenerative hyperplasia. The liver parenchyma shows micronodular transformation and an atrophic rim without fibrosis. (Gomori stain. x400.)

herniating into the surrounding parenchyma (paraportal shunt vessels) (Figure 4C), sinusoidal dilatation (megasinusoids) and periportal/perisinusoidal fibrosis (6, 13, 46, 47, 63, 76, 79, 80). Considering its high prevalence in INCPH liver specimens, obliterative portal venopathy is generally regarded the primary lesion in the development of the intrahepatic hemodynamical changes (6, 24, 81). According to Wanless, this obliteration of portal venules results in disturbed intrahepatic circulation and subsequently parenchymal remodeling as observed in NRH and PNT (development of hepatocytic atrophy in the areas with reduced portal venous blood supply and compensatory hyperplasia in the best perfused areas) (13). The additional morphological features of INCPH can be regarded as intrahepatic microcirculatory disturbances. For instance, the increased number of portal vascular channels and the paraportal shunt vessels (regarded the histological equivalent of the portal vein cavernoma) are believed to shunt blood from the obliterated portal segments towards unaffected tracts. Other morphological findings however are at variance with Wanless's obstructive portal vasculopathy theory. In the largest retrospective study on Western INCPH patients up to now, abnormal portal vessels were found in less than half of the cases. Furthermore, periportal and perisinusoidal fibrosis were more frequently observed in the absence than in the presence of portal vessel alterations. Therefore, Hillaire et al. suggested an alternative hypothesis in

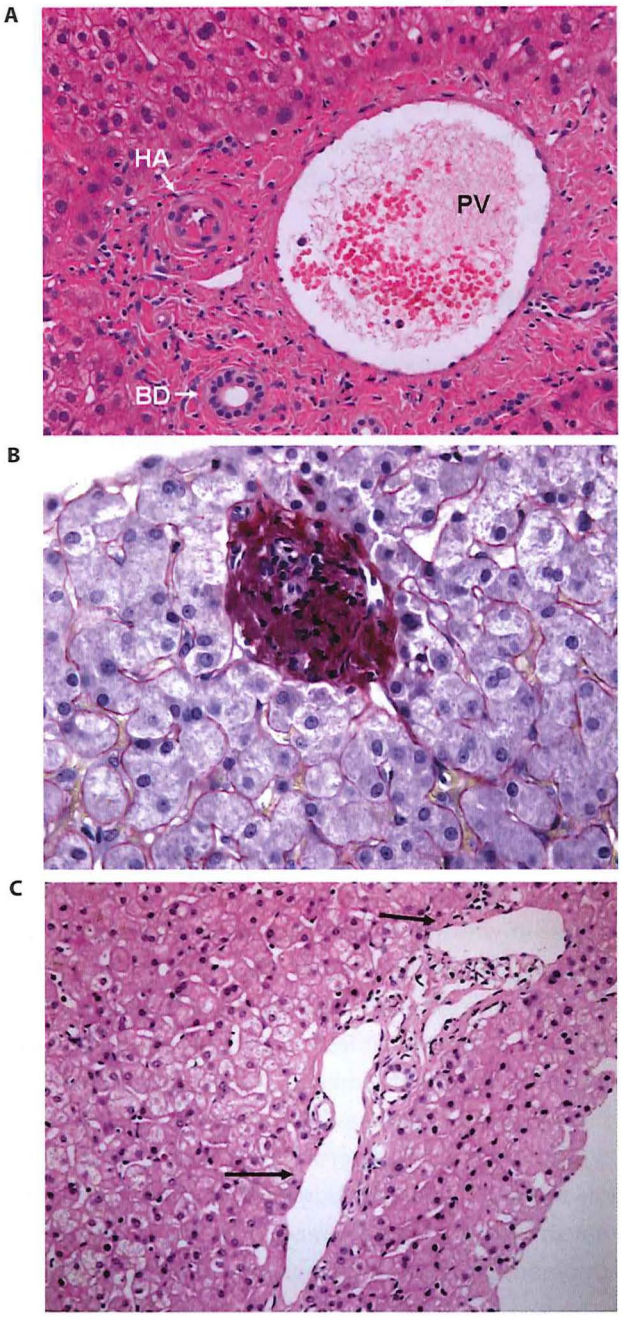

Figure 4 (A) Normal portal tract showing a branch of the hepatic artery (HA) and the interlobular bile duct (BD) of approximately the same size. The branch of the portal vein (PV) is more than three times the diameter of the artery (haematoxylin and eosin stain. x400.) (B) Fibrotic portal tract with obliterated small portal vein (Sirius Red stain. x400.) (C) Portal tract demonstrating paraportal shunt vessels (black arrow). (haematoxylin and eosin stain. x160.)

which the causative process of the disease (a prothrombotic disorder) acts directly on the sinusoidal and portal vein wall inducing fibrosis, obstruction and secondary alterations in the architecture (6). Notwithstanding the occurrence or development of INCPH in patients with these histological features, a significant amount of patients with the described histological characteristics are observed in patients without clinical signs of portal hypertension (40, 82).

NATURAL COURSE AND PROGNOSIS

Current data suggest that, despite liver function impairment occurring in the context of esophageal hemorrhage or infection, the mortality of variceal hemorrhage in INCPH is significantly lower than that observed in cirrhotic patients (6, 16, 60, 76). None of the patients described by Hillaire et al. died due to esophageal bleeding. As a result, isolated INCPH is regarded a relative benign disorder (5-year survival of nearly 100%) (24). Contrasting with this view, progression to liver failure (occurring late in disease course) requiring liver transplantation has been reported increasingly (17, 49, 63, 78). Cazals-hatem et al. reported the development of severe liver failure in 7/59 patients with obliterative portal venopathy during a median follow-up of 8.6 years (49). Liver function impairment and ascites in these patients can possibly be explained by a reduction in portal flow and subsequently atrophy of the peripheral hepatic parenchyma. In addition, the lack of compensatory arterial changes worsens ischemia and contributes to liver failure (83). The demonstration of obliterated large portal veins in explanted livers from INCPH patients transplanted because of liver failure supports this hypothesis (49). However, since no clear data are available, this hypothesis is speculative.
In comparison to patients with liver cirrhosis, a high incidence of portal vein thrombosis has been reported in INCPH patients (6, 32, 84, 85). In patients with HIV related INCPH, a substantially higher incidence of portal vein thrombosis (75%) has been documented (32, 85, 86) raising the possibility that HIV infection or its treatment may play a separate role in the development of portal vein thrombosis. A trend towards portal vein thrombosis being associated with poor prognosis has been reported (6). As a result, we believe that early diagnosis by regular screening of portal vein patency and subsequently institution of anticoagulation therapy is strongly suggested. Considering the high incidence of portal vein thrombosis in INCPH, the occurrence of its histological features of in patients with portal vein thrombosis and the high prevalence of prothrombotic disorders in both conditions it can be hypothesized that these 2 entities are different presentations of single disorder.

The development of hepatocellular carcinoma in INCPH patients remains a matter of debate. Notwithstanding, the reporting of liver cell atypia and pleomorphism in nodular regenerative hyperplasia liver specimens, a causal relationship between he-

patocellular carcinoma and INCPH has not been proven (39, 87). Nzeako et al. studied the association between NRH and hepatocellular carcinoma in 342 non-cirrhotic patients (88). In the majority of the patients, the concurrence of NRH and hepatocellular carcinoma could be attributed to additional factors known to be associated with the development of NRH (portal vein thrombosis, chemotherapy, radiotherapy) and the study failed to indicate this disorder as the underlying condition of hepatocellular carcinoma. As a result, hepatocellular carcinoma surveillance in patients with INCPH is not recommended.

TREATMENT

Treatment and prophylaxis of variceal gastrointestinal bleeding

Hemodynamics and consequently the management and prophylaxis of variceal bleeding in patients with INCPH are not entirely comparable to these in cirrhotic patients. Currently, scientific data on management and prophylaxis (primary and secondary) of variceal bleeding in INCPH patients are scarce. Nevertheless, we recommend to follow the guidelines of prophylaxis and management of cirrhotic variceal bleeding in patients with INCPH (89). Endoscopic sclerotherapy has been proven to be effective in controlling acute variceal bleeding of esophageal hemorrhage in 95% of INCPH patients (90). No scientific data have been published regarding endoscopic band ligation in these patients. However, considering the proven inferiority of sclerotherapy compared to endoscopic variceal ligation in cirrhotic patients, the latter treatment is currently also regarded the most appropriate endoscopic treatment in INCPH patients. Despite the fact that data regarding combination of endoscopic treatment with vasoactive drugs and antibiotic prophylaxis are lacking, we recommend applying these treatments in INCPH patients considering the effectivity in cirrhotics. Based on Indian studies, emergency shunt surgery because of unmanageable bleeding was only required in 5% of cases with acute variceal bleeding (91). Despite the alleged safety and efficacy of shunt surgery, INCPH patients with uncontrollable hemorrhage are currently preferentially treated with transjugular intrahepatic portosystemic shunt (TIPS) because of its lower invasiveness (24). Taking into account the preserved liver function in these patients, the complications of this procedure observed in patients with cirrhosis (such as hepatic encephalopathy) are expected to be rare. However, no data are available. Concerning secondary prophylaxis of variceal bleeding in INCPH patients, smaller studies have demonstrated a reduction of bleeding rate by endoscopic therapy (90, 92).

Gastric varices are seen in nearly 25% of Indian INCPH patients (93). Portal hypertensive gastropathy is uncommon at initial presentation and is a rare cause of upper gastrointestinal bleeding (94). In patients with liver cirrhosis nonselective β-blockers have shown to reduce gastric mucosal blood flow and to decrease of recurrent bleeding in a

randomized controlled trial (95). In keeping with this, comparable treatment is applied in INCPH induced portal hypertensive gastropathy.

Treatment of splenomegaly and hypersplenism

Patients with INCPH have a massive splenomegaly leading to increased portal venous flow and subsequently to portal hypertension. As a result, splenectomy and partial splenic embolization have demonstrated to decrease portal hypertension in these patients (96, 97). In selected INCPH patients (abdominal discomfort, hypersplenism, patients) these interventions can be regarded effective therapeutic modalities.

Oral anticoagulation

Based on the high prevalence of thrombophilia and incidence of portal vein thrombosis in INCPH patients, several authors incriminated thrombosis of small intrahepatic portal veins as an important etiological factor in the development of this disorder (6, 30). Additionally, a trend towards poor prognosis has been reported in INCPH patients developing portal vein thrombosis (6). As a result, anticoagulation therapy has been proposed by several authors in order to prevent disease progression and to maintain portal vein patency (6, 32, 98). However, considering the fact that gastrointestinal bleeding is the main complication of INCPH and the uncertain role of thrombophilia in the pathogenesis, this treatment is still a matter of debate and cannot be generally implicated until more solid data are present. Nonetheless, we believe that anticoagulation therapy must be considered in patients with underlying prothrombotic conditions and in patients who develop portal vein thrombosis.

Liver transplantation

Generally, patients with isolated INCPH have a normal liver function and the complications of portal hypertension can be managed successfully with endoscopic therapy and shunting. However, several reports describing liver transplantation in patients with INCPH have been published. The reported indications requiring liver transplantation in these patients were medical unmanageable portal hypertension, hepatopulmonary syndrome, hepatic encephalopathy and progressive hepatic failure (49, 63, 78). Recently, Karsinskas et al. described a small cohort of INCPH patients treated with liver transplantation (63). The main indication for liver transplantation was medically unmanageable severe portal hypertension; a minority was listed because of hepatic encephalopathy. Notwithstanding the fact that resistant bleeding in INCPH patients, should be treated with portosystemic shunting before considering the option of liver transplantation, only 2 patients underwent pretransplantation portosystemic shunting procedures (TIPS and mesocaval shunt). Presumably, the high frequency of cirrhosis misdiagnoses in these patients led to early referral for liver transplantation. In order to prevent unnecessary

liver transplantation in these patients, early discrimination between cirrhosis and INCPH is extremely important. Based on small sized cohorts (with limited follow-up), the post-transplantation outcome in these patients is good and INCPH tends not to recur (63, 99, 100).

CONCLUSIONS

Data on the etiology and management of INCPH are scarce and currently applied diagnostic and therapeutic algorithms are based on personal experience or data from limited number of patients. The nomenclature concerning this clinical disorder has been ambiguent and mainly depended on the present histological features. In order to facilitate future studies and subsequently enhance our understanding of the disease, we propose INCPH as a uniform nomenclature for this disorder independent of the observed histopathological features. In Eastern patients abdominal infectious disease has been incriminated an important role in the development of INCPH, however in Western patients such a risk factor is lacking. Hypercoagulability may play an important role in INCPH. Despite the fact that data regarding treatment of variceal bleeding in INCPH patients are lacking, we recommend to follow the guidelines regarding cirrhotic variceal bleeding in these patients. In general, prognosis and survival of INCPH patients is good. However, liver failure might occur. Prospective multicentre cohort studies are needed to acquire reliable data regarding the treatment and clinical outcome of this challenging disorder.

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CHAPTER 3

HISTOLOGICAL FEATURES IN WESTERN PATIENTS WITH IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION

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ABSTRACT

Aims

In the Western world, idiopathic noncirrhotic portal hypertension (INCPH) is a rare disease. This study aimed to investigate the histopathological features in Western INCPH patients and to assess pathological differences between liver specimens of INCPH with or without HIV.

Methods and results

Biopsies of 70 INCPH patients (of which 15 HIV-infected) were compared to 23 patients with noncirrhotic portal vein thrombosis, (PVT) which served as a control group for noncirrhotic portal hypertension. Phlebosclerosis, nodular regeneration (NR), sinusoidal dilatation, paraportal shunting vessels, perisinusoidal fibrosis and portal tracts remnants were the most prevalent morphological features of INCPH. There were significant ($p<0.01$) morphological differences between INCPH and PVT liver specimens with regard to portal tracts remnants (46% versus 0%), phlebosclerosis, (95% versus 65%), portal vein dilatation (34% versus 78%) and nodular regeneration (56% versus 22%). The degree of NR correlated with the severity of phlebosclerosis ($p<0.01$). NR was seen more frequently in the HIV-INCPH group, compared to the non-HIV-infected patients ($p<0.001$).

Conclusion

Portal tracts remnants, phlebosclerosis and nodular regeneration are typical features of INCPH. Sinusoidal dilatation, paraportal shunting vessels and increased portal and parenchymal vessels might represent pressure-related morphological signs of portal hypertension. Finally, more nodular regeneration was observed in HIV-associated INCPH.

INTRODUCTION

Idiopathic noncirrhotic portal hypertension (INCPH) is a clinical entity consisting of intrahepatic portal hypertension in the absence of cirrhosis and other causes of liver disease (1). In contrast to the high prevalence of this disorder in India and Japan, INCPH is a rare disease in the Western world. As a result, currently available clinical and morphological data are mainly derived from Asian cohorts (2-3). Extending these study results to Western INCPH patients would be incorrect, since different pathophysiological mechanisms have been postulated. In the latter, thrombophilia leading to thrombosis of the small portal vein branches and certain drugs have been proposed as possible pathogenic mechanisms, whereas in Asian patients, vascular damage due to enteric and congenital infections is considered to be the main etiology (4). Furthermore, recent data have shown that the long-term prognosis in Western INCPH patients is poor in comparison with a 5-year survival of 100% in Asian patients (5).

Historically, INCPH has been classified in 4 different histological categories, namely: idiopathic portal hypertension, nodular regenerative hyperplasia (NRH), partial nodular transformation and incomplete septal cirrhosis (2, 4, 6-11). Other names used in the context of INCPH include obliterative portal venopathy and hepatoportal sclerosis (8, 12) . The applicability of the different histological categories is a matter of controversy and histological overlap between the different subgroups has been reported frequently (2, 13-14).

Liver biopsy remains essential in the diagnosis of INCPH. It is indispensable for the exclusion of liver cirrhosis, since radiological examinations do not reliably differentiate INCPH patients from cirrhotic patients (5, 15).

The diagnostic accuracy of the histopathological features reported in Asian patients has not been studied sufficiently in Western INCPH patients. Furthermore, despite the fact that uniform definitions are of major importance, there is no agreement on which morphological features are distinctive for INCPH.

Therefore, the aim of our study was to investigate the histopathological features reported in Asian INCPH patients in a large cohort of Western INCPH. Furthermore, we compared the morphological signs in liver specimens of patients with INCPH to specimens of noncirrhotic portal vein thrombosis (PVT), the latter serving as a control group for noncirrhotic portal hypertension. Finally, we compared the pathological features of liver specimens of INCPH patients with HIV to those without HIV.

MATERIALS AND METHODS

Patient selection

All patients diagnosed with INCPH in 2 European tertiary referral centres (Erasmus MC University Hospital Rotterdam and University Hospital Leuven) between 1992 and 2010 were included in the study. Patient selection was done from a clinical and a liver pathology database. The diagnosis of INCPH was considered when all of the following criteria were fulfilled: (1) clinical evidence of portal hypertension (any of the following features: esophageal varices, hypersplenism and ascites); (2) radiological examination (abdominal CT or Doppler ultrasound) demonstrating patent portal and hepatic veins at initial diagnosis; (3) liver biopsy showing no cirrhosis; (4) exclusion of liver disease known to cause portal hypertension in the absence of cirrhosis (chronic viral hepatitis, alcoholic liver disease, non-alcoholic steatohepatitis (NASH), hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis and Wilson`s disease); (5) exclusion of exposure to copper sulphate, vinyl chloride monomer, past angiography with thorium sulphate, exposure to Spanish toxic oil (1). Patients with the presence of steatosis, ballooning degeneration of hepatocytes and lobular inflammatory infiltrates were considered as potential (N)ASH patients and therefore also excluded. HIV-associated INCPH was diagnosed when all these criteria were met in an HIV-infected person.

The biopsies of 23 patients known to have recent (< 1 year) noncirrhotic PVT were selected from a clinical database and from a liver pathology database. Relevant clinical data were recorded from patient files. The study protocol was approved by the local medical ethical committees.

Histological evaluation

Systematic liver biopsy evaluation was performed by two experienced liver pathologists (JV and MK). Archived hematoxylin & eosin stained sections were reviewed together with a reticulin stain and at least one collagen stain (Sirius red stain, Masson`s trichrome, Elastica von Giesson stain).

At the start of the study we established clear and easily applicable definitions of the morphological abnormalities reported in association with INCIPH (2, 14-18).

At the level of the portal tract we evaluated: (1) phlebosclerosis (figure 1A); (2) presence of paraportal shunting vessels (figure 1B en 1C); (3) presence of portal tracts remnants (figure 1C); (4) thickened smooth muscle wall (arterialization) of the portal vein (figure 1D); (5) the presence of an enlarged branch of the portal vein (figure 2A) ; (6) presence of thin incomplete septa and (7) an increase in the number of thin-walled vessels, present in the portal tract (figure 2B). Phlebosclerosis was defined as a portal vein with reduced lumen in a fibrotic portal tract and graded according to the percentage

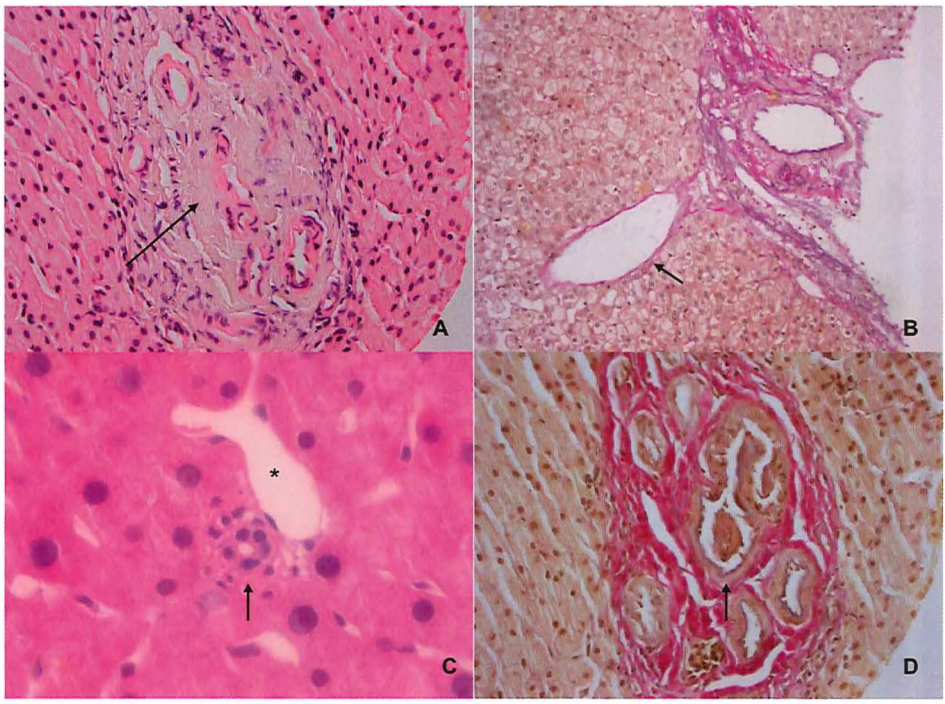

Figure 1 Phlebosclerosis; portal vein with reduced lumen in a fibrotic portal tract (arrow); haematoxylin and eosin staining (H&E), 20x. B: Paraportal shunting vessels with enlarged thin-walled vessels, outside, but in (close) contact to, the portal tract (arrow); Elastica von Giesson (EVG) staining, 10x. C: Portal vein remnants of which the total size was smaller than 2 times the diameter of the bile duct and the diameter of the bile duct being smaller than the size of the periportal surrounding hepatocytes (arrow) with also paraportal shunting vessel (*); haematoxylin and eosin (H&E) staining, 40x. D: Thickened smooth muscle wall (arterialization) of the portal vein (arrow); Elastica von Giesson (EVG) staining, 20x.

of the portal tracts affected (0, <50%, >50%). Paraportal shunting vessels were defined as enlarged thin-walled vessels, outside, but in (close) contact to, the portal tract. The size of these shunting vessels was assessed using a scale from 1 to 3, in which 1 was smaller, 2 comparable and 3 larger than the bile duct in the adjacent portal tract. The presence of portal tracts remnants was defined as a portal tract of which the total size was smaller than 2 times the diameter of the bile duct and the diameter of the bile duct being smaller than the size of the periportal surrounding hepatocytes. The presence of an enlarged portal vein branch in the portal tract (at least >3 times the size of the bile duct, or the hepatic artery branch (total diameter) in the absence of a bile duct) was defined as portal vein dilatation. Incomplete septa were defined as the presence of thin, blindly ending septa (18-19).

Histopathological changes in the parenchyma were scored as (1) nodular regeneration, (figure 2C); (2) increase in parenchymal draining vessels (figure 2D); (3) sinusoidal dilatation; (4) perisinusoidal fibrosis and (5) perivenular fibrosis. Nodular regeneration

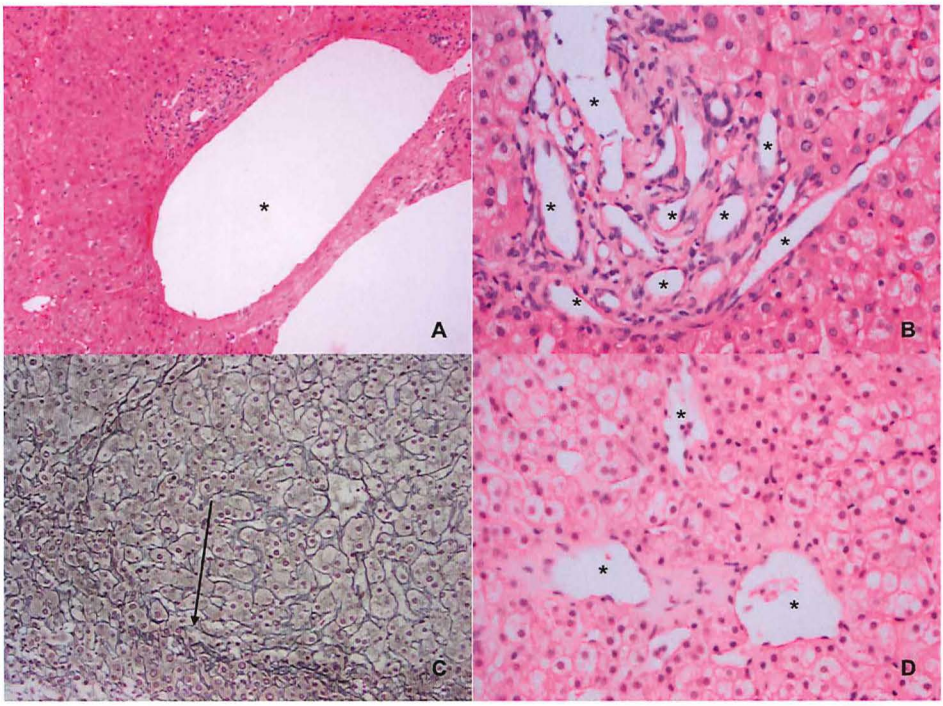

Figure 2 A: Portal vein dilatation with enlarged portal vein branch in the portal tract, at least >3 times the size of the bile duct (*); haematoxylin and eosin staining (H&E), 10x. B: Increase in the total number of thin-walled vessels (*), present in the portal tract; haematoxylin and eosin staining (H&E), 20x. C: Nodular regeneration with parenchymal micronodular transformation (arrow); reticulin staining, 10x. D: Increase in parenchymal draining veins (*);haematoxylin and eosin staining (H&E), 20x.

was defined as parenchymal micronodular transformation, with central hyperplasia and an atrophic rim in the absence of a fibrosis according to the description by Wanless (7). Nodular regeneration was graded as follows (7): 1, mild (focal nodularity with reticulin staining); 2, moderate (focal nodularity visible on H&E staining, and obvious with reticulin staining); 3, severe (clear nodularity both on H&E and reticulin staining). The dilatation of sinusoids and perisinusoidal fibrosis was graded as 0, 1/3, 2/3 and 3/3 according to the extent of the lobular area involved. Perivenular fibrosis was defined as an increase of fibrous tissue around the central vein and graded as present in less than 50% or in more than 50% of the central veins involved. Incomplete septa, periportal, perisinusoidal fibrosis and perivenular fibrosis were only assessed when a collagen stain was available.

Statistical analysis

Data are expressed as median and range for continuous variables, and as frequency with percentages for categorical variables. Continuous variables were compared using

an unpaired t test (for normally distributed ones) and a non-parametric test, such as the Wilcoxon test (for those with a skewed distribution). The categorical variables were analyzed by a Fisher exact test. P-values of <0.01 were considered statistically significant when 2 groups were compared. The association between nodular regeneration and phlebosclerosis was analyzed by a Kruskal-Wallis test. All statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS, Chicago, IL). The association between morphological features and the presence of portosytemic collaterals was studied using a t test. The association between morphological features and spleen size (cm) and platelet count (10^6/mm^3) was studied using a Fisher exact test. The association between the different morphological features and spleen size and platelet count was studied in a linear regression. The diagnostic performance of the morphological features for the diagnosis of INCPH was calculated as positive predictive value, negative predictive value and area under the curve.

At the end of our study, the different subgroups in terms of grades were too small and we decided to pool together all grades of the parameters, scored for comparison with patients without abnormalities for the specific parameter studied.

RESULTS

Clinical features of patients with INCPH and PVT

Liver specimens from 70 patients with INCPH and 23 patients with PVT were scored systematically. Associated disorders of INCPH are shown in table 1. Patient characteristics and presenting symptoms are shown in table 2. The vast majority of the patients with INCPH and PVT was of Caucasian origin (respectively 67 out of 70 and 22 out of 23). The remaining patients were of African descent. INCPH patients diagnosed after 2002 underwent complete thrombophilia screening, including bone marrow examination for the diagnosis of myeloproliferative disorders according to the previously defined work-up. Sixteen out of the 40 completely screened INCPH patients (40%) had an underlying thrombophilic condition (myeloproliferative disorder (n=3), protein S deficiency (n=5), protein C deficiency (n=4), factor V Leiden mutation (n=2), antiphospholipid syndrome (n=2)). Ten out of the 23 completely screened PVT patients (43%) had a thrombophilic underlying condition (myeloproliferative disorder (n=3), protein S deficiency (n=1), protein C deficiency (n=2), factor V Leiden mutation (n=2), antiphospholipid syndrome (n=2)). All HIV-infected patients had normal CD4 counts at the moment of INCPH diagnosis (median CD4 count (IQR): 230 (170-310). All HIV-infected patients were treated with didanosine. Only 10 were still receiving this drug at the moment of INCPH diagnosis. All HIV-associated INCPH patients underwent complete thrombophilia screening. In 4 out of these 15 patients, a thrombophilic condition could be demonstrated (Protein S deficiency (n=2) and Protein C deficiency (n=2)). No statistically significant differences

Table 1 Associated disorders upon diagnosis of 70 idiopathic noncirrhotic portal hypertension (INCPH) patients.

Associated disorder	n (%)
Azathioprine treatment	8 (11)
Hematological disorders	8 (11)
Malignancies*	4 (6)
Myeloproliferative disorders	3 (4)
Idiopatic thrombocytopenic purpura	1 (1)
HIV infection	15 (21)
Immunological disorders **	5 (7)
Genetic disorders ***	4 (6)
Arsenic treatment	4 (6)
Breast cancer (chemotherapy 7 years before diagnosis INCPH) ****	1 (1)
Crohn's disease (Azathioprine naïve)	1 (1)
No associated disorder	24 (34)

* B-acute lymfoblastic leukemia, non-Hodgkin lymphoma, multiple myeloma, rectal carcinoma
** Rheumatoid arthritis (n=2), systemic lupus erythematosus (n=2), hypogammaglobulinemia (n=1)
***Turner Syndrome (n=2), Schwachmann syndrome (n=1), Marfan syndrome (n=1)
****No oxaliplatin exposure

between the 2 groups were observed with regard to age, gender, spleen size, presence of associated disorders, presence of portosystemic collaterals, albumin, bilirubin and INR (table 2). Platelets were significantly higher in patients with PVT compared to patients with INCPH.

Tabel 2 Baseline characteristics of idiopathic noncirrhotic portal hypertension patients versus noncirrhotic PVT patients.

Baseline characteristics	INCPH (n=70)	PVT (n=23)	P
Male*	48 (69)	8 (35)	0.06
Age (years)	46 (7-80)	50 (20-83)	0.44
Associated disorder present*	46 (66)	17 (74)	0.38
Presence of esophageal varices	70 (100)	23 (100)	1
Ultrasound features			
Spleen size, cm	16 (13-21)	17 (13-24)	0.08
Presence of portovenous collaterals	69 (99)	22 (96)	0.94
Laboratory results			
Platelets, 10^6/mm^3	106 (27-454)	237 (10-593)	0.01
Albumin, g/L	38 (20-52)	38 (28-45)	0.41
Bilirubin, µmol/L	17 (5-100)	24 (10-66)	0.24
INR	1.1 (1.0-1.4)	1.0 (1.0-1.3)	0.28

Age, laboratory and spleen size data are expressed as the median and range (between brackets)
*Variables are represented as number of subjects and percentages (between brackets)

Pathological features of INCPH versus PVT liver specimens

The median biopsy length was 1.77 cm (range 0.9 to 5 cm) and the median number of portal tracts was 9.4 (range 6 to >10), which is considered valid. Table 3 shows the histological features observed in the 2 groups. The positive predictive value of portal tracts remnants, phlebosclerosis and NRH for the diagnosis of INCPH versus PVT is respectively 1, 0.8 and 0.9. The negative predictive value of portal tracts remnants, phlebosclerosis and NRH for the diagnosis of INCPH versus PVT is 0.38, 0.36 and 0.72, respectively. The area under the curve for the overall diagnostic performance of these three parameters for INCPH is 0.9 (95% CI 0.84-0.97).

Table 3. Comparison of histological characteristics of patients with idiopathic noncirrhotic portal hypertension (INCPH) (n=70) and noncirrhotic portal vein thrombosis (PVT).

Histological lesions	INCPH (n=70)	PVT (n=23)	P
Phlebosclerosis	67 (95)	15 (65)	<0.001
Paraportal shunt vessels	62 (89)	22 (96)	0.57
Portal vein remnants	32 (46)	0 (0)	<0.001
Portal vein arterialization	11 (16)	0 (0)	0.06
Portal vein dilatation	24 (34)	18 (78)	<0.001
Thin incomplete septa	7 (10)	0 (0)	0.18
Increased vascular channels in portal tracts	50 (71)	18 (78)	0.60
Nodular regeneration	39 (56)	5 (22)	0.003
Increased parenchymal draining vessels	29 (41)	6 (26)	0.22
Sinusoidal dilatation	66 (94)	23 (100)	0.61
Perisinusoidal fibrosis	64* (97)	23 (100)	0.95
Perivenular fibrosis	43* (65)	7 (30)	0.026
Steatosis (10-50%)	4 (6)	0	0.6

Variables are represented as number of subjects and percentages (between brackets)
* Based on 66 out of 70 biopsies

The grade of nodular regeneration (in INCPH and PVT specimens) was significantly associated with severity of phlebosclerosis (p<0.01) (figure 3).

The simultaneous occurrence of portal vein dilatation, portal tracts remnants, phlebosclerosis and nodular regeneration was different in liver specimens of INCPH patients compared to specimens of PVT patients (p<0.001). One of these features was found in 12%, two in 35%, three in 41% and four in 12% of the INCPH patients. None of these features were found in 23%, one in 41%, two in 32% and three in 5% of the PVT patients.

There was, if present, only minimal inflammation in patients with INCPH and PVT. Cirrhosis was excluded in all patients.

In patients with INCPH as well as in those with PVT, none of the histological features nor the combination of nodular regeneration, phlebosclerosis and portal tracts rem-

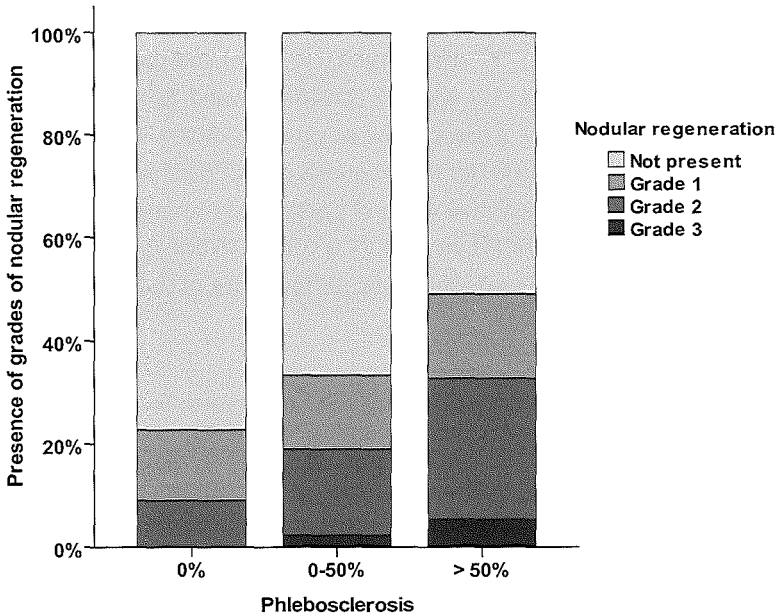

Figure 3 Association between grade of nodular regeneration and severity of phlebosclerosis (in patients with INCPH and portal vein thrombosis).
Out of the 22 patients without phlebosclerosis; 17 had no nodular regeneration, 3 had grade 1 nodular regeneration, 2 had grade 2 nodular regeneration and none had grade 3 nodular regeneration.
Out of the 42 patients with <50% phlebosclerosis; 28 had no nodular regeneration, 6 had grade 1 nodular regeneration, 7 had grade 2 nodular regeneration and 1 had grade 3 nodular regeneration.
Out of the 55 patients with >50% phlebosclerosis; 28 had no nodular regeneration, 9 had grade 1 nodular regeneration, 15 had grade 2 nodular regeneration and 3 had grade 3 nodular regeneration.

nants were positively correlated with clinical signs of portal hypertension (spleen size, platelet count and the presence of portovenous collaterals) ($p>0.5$).

Pathological features of INCPH versus HIV-associated INCPH

Significant differences between these two groups were found for nodular regeneration ($p=0.001$) and portal tracts remnants ($p<0.01$) (table 4).

The simultaneous occurrence of portal vein dilatation, portal tracts remnants, phlebosclerosis and nodular regeneration was not different in liver specimens of INCPH patients compared to specimens of HIV-associated INCPH ($p=0.1$). One of these features was found in 18%, two in 40%, three in 34% and four in 7% of the INCPH patients. Two of these features were found in 67% and three in 33% of the HIV-associated INCPH patients.

Tabel 4. Comparison of histological characteristics of patients with idiopathic noncirrhotic portal hypertension (INCPH) (n=55) and HIV-associated INCPH (n=15).

Histological lesions	INCPH (n=55)	HIV-INCPH (n=15)	P
Phlebosclerosis	52 (95)	15 (100)	1
Paraportal shunt vessels	50 (91)	12 (80)	0.57
Portal vein remnants	30 (53)	2 (13)	<0.01
Portal vein dilatation	21 (38)	3 (20)	0.23
Thin incomplete septa	6 (11)	1 (7)	1
Increased vascular channels in portal tracts	38 (69)	12 (80)	0.52
Nodular regeneration	25 (46)	14 (93)	0.001
Increased parenchymal draining vessels	24 (44)	5 (33)	0.56
Sinusoidal dilatation	52 (94)	14 (93)	1
Perisinusoidal fibrosis	50* (98)	15 (100)	1
Perivenular fibrosis	33* (65)	10 (67)	1
Steatosis (10-50%)	4 (6)	2 (13)	0.6

* based on 51 out of 55 biopsies

DISCUSSION

In this study, we demonstrated the presence of comparable histological abnormalities in Western INCPH patients, as described in Asian patients. Furthermore, we identified differences between liver specimens of all INCPH patients pooled together, and PVT patients as well as between INCPH patients with and without HIV infection.

Current data on histopathological features in INCPH liver specimens are derived from Eastern cohorts. We observed similar morphological features to those described in Asian INCPH patients (2, 20). Although the term hypoplastic portal tract is not used in Asian studies, we assume that the rudimentary or scarlike hyalinized portal tracts described by Okuda are equivalent to the hypoplastic portal tracts, portal tract remnants and micro- or minute portal tracts described in Western studies (21). Moreover, the ectopic portal veins described by Okuda et al. are likely the same as paraportal shunting vessels(7, 18, 22-23).

We established clear definitions of histological features associated with INCPH, which is of importance, since agreement on the morphological characteristics is an essential requirement for collaborative studies. Despite the fact that we did not take into account the interobserver variability, we believe these definitions are easily applicable. In addition, we think that our grading system may be valuable in larger studies because the presence of (discrete) microcirculatory disturbances is also described in 'normal' livers without any increase in portal pressure, and grading could increase the diagnostic performance (24).

First, we compared liver specimens of INCPH patients with liver specimens of patients with noncirrhotic PVT. A potential bias of this approach is the inclusion of INCPH patients in the PVT group in whom secondary extrahepatic portal vein thrombosis might have occurred. However, the clinical data of the patients with PVT did not demonstrate signs of portal hypertension (thrombocytopenia and splenomegaly) before the diagnosis of PVT and the patients included in the INCPH group all had patent portal veins upon radiological examination. Another potential limitation of the study is the fact that these 2 groups share etiological features, such as the presence of thrombophilia and myeloproliferative disorders characteristic of vascular liver disorders. Despite these potential limitations, significant differences between the two groups were observed. Portal vein remnants, phlebosclerosis, and nodular regeneration were significantly more frequent in INCPH and are assumed to be signs of INCPH (8). However, the specificity of these results needs to be confirmed in liver specimens of patients without portal hypertension. Since the presence of more than one feature occurred in different combinations, it is difficult to draw general conclusions based on these numbers. Other features, such as sinusoidal dilatation, paraportal shunting vessels and increased portal and parenchymal vessels, lack specificity for the diagnosis of INCPH and may as such be interpreted as pressure-related morphological signs of portal hypertension (21).

The observed differences in phlebosclerosis and nodular regeneration between INCPH and PVT are in line with the hypothesis of obstructive portal venopathy, proposed by Wanless, (25) which considers INCPH to be a microvascular disorder resulting from injury to the small portal vein branches, whereas PVT is by definition a macrovascular disorder (18, 21, 23, 26-28). Indeed, phlebosclerosis was observed in nearly all liver specimens of INCPH patients. According to Wanless' hypothesis, nodular regeneration results from intrahepatic circulation disturbances due to occlusion of the small portal veins (7). Corresponding to this hypothesis, we demonstrated a positive association between the degree of nodular regeneration and the severity of phlebosclerosis. The observation that portal vein dilation was more pronounced in the PVT group as compared to the INCPH group is in line with this hypothesis, considering that the small branches of the portal vein are affected in the latter group, compromising dilatation of the small portal vein branches. Moreover, portal vein remnants were completely absent in the PVT group and appeared to be a distinctive feature of INCPH, also pointing to microcirculatory disturbances of small portal vessels and thought to be the result of destruction of the most peripheral branches of the portal vein. We cannot exclude that, in line with the Wanless hypothesis, thrombosis in the PVT group may extend into the smaller branches of the portal vein and result over time in phlebosclerosis and/or the development of portal vein remnants (7). Since we included only PVT patients with liver biopsy performed in the first year after the diagnosis of PVT, this hypothesis cannot be assessed in this study.

Interestingly, despite similar occurrence of phlebosclerosis in patients with INCPH and HIV-associated INCPH, nodular regeneration was significantly more present in the latter. Pathophysiological differences between two groups have been suggested. INCPH is a disorder with a heterogeneous spectrum of associated disorders (5). On the contrary, the pathogenesis of HIV-INCIPH is considered to be more homogenous related to the antiviral therapy. Didanosine, a drug notorious for mitochondrial toxicity, plays an indispensable role in the development of this disorder (29-31). In particular, mitochondrial hepatocellular toxicity has been described with nucleoside reverse-transcriptase inhibitors (NRTIs) with associated steatosis, which we did not see in our biopsies (32-33). Considering the fact that mitochondria participate in many forms of liver injury, it can be hypothesized that this NRTI-induced mitochondrial toxicity specifically affects endothelial cells in HIV-infected patients with INCPH, resulting in a more uniform and extended vascular injury pattern and consequent nodular regeneration.

In our study, the observed histomorphological abnormalities did not correlate with clinical signs of portal hypertension, such as splenomegaly, ascites, esophageal varices and thrombopenia. This observation can be explained by the development of adaptative mechanisms, such as dilatation of the portal and splenic vein, formation of collaterals and spontaneous shunting in the disease course of portal hypertension (34).

In conclusion, portal vein remnants, phlebosclerosis and nodular regeneration are the most distinctive features of INCPH, supporting the obstructive portal vasculopathy theory. Pathologists should be aware of the association between these morphological features and INCPH, in order to suggest or support this clinical diagnosis in patients with noncirrhotic portal hypertension. Sinusoidal dilatation, paraportal shunting vessels and increased portal and parenchymal vessels may be interpreted as pressure-related morphological signs of portal hypertension. Finally, more severe nodular regeneration was observed in HIV-associated INCPH in comparison to other forms of INCPH.

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CHAPTER 4

IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION IS ASSOCIATED WITH POOR SURVIVAL: RESULTS OF A LONG-TERM COHORT STUDY

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ABSTRACT

Background

Idiopathic noncirrhotic portal hypertension (INCPH) is a rare disease in the Western world. As a result, little is known about the clinical characteristics and outcome of these patients. Survival in these patients is considered to be similar to the general population.

Aim

To investigate the clinical manifestations, pathophysiology, outcome and determinants of survival in Western INCPH patients.

Methods

Multicenter cohort study of INCPH patients.

Results

Sixty-two patients were followed for a median time of 90 months (range 24-310). Initial manifestations leading to the diagnosis of INCPH were related to portal hypertension in 82% of the patients. Histological signs of portal blood supply disturbances were present in nearly all patients. During follow-up, 12/62 patients developed liver decompensation, of which 4 were considered for liver transplantation. One patient died in the context of variceal bleeding. Hepatocellular carcinoma was not observed during follow-up. Twenty-three patients died during follow-up, only 4 of them due to liver related mortality. The Kaplan-Meier estimates for overall survival were 100% (95% CI 95-100%), 78% (95% CI 67-89%) and 56% (95% CI 40-72%) at 1, 5 and 10 years, respectively. Survival for INCPH was significantly decreased ($p<0.001$) compared to survival of the general population. Ascites was an independent predictor of poor outcome.

Conclusions

In comparison to the general population, survival in INCPH patients is poor. Mortality is related to associated disorders and medical conditions occurring at older age. Patients rarely die due to liver related complications. Patients with ascites have a poor prognosis.

INTRODUCTION

Liver cirrhosis is the most frequent cause of portal hypertension (1). However, a variety of disorders can cause portal hypertension in the absence of cirrhosis, referred to as non-cirrhotic portal hypertension. Schistosomiasis, congenital hepatic fibrosis, extrahepatic portal vein thrombosis, Budd-Chiari syndrome and chronic liver diseases such as non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis, alcoholic liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, are the most common causes of non-cirrhotic portal hypertension. If all these disorders have been ruled out, the diagnosis of idiopathic noncirrhotic portal hypertension (INCPH) can be made (2). In the past, this condition has been variably termed hepatoportal sclerosis, idiopathic portal hypertension, incomplete septal cirrhosis and nodular regenerative hyperplasia. However, since all these entities share histopathological characteristics (obliterative vascular lesions), it has been suggested that INCPH can be viewed as a distinct single entity with various pathological aspects, rather than different clinicopathological entities (2). In contrast to its high prevalence in India, INCPH is a rare disorder in the Western world (3). As a result, little is known about its pathophysiology, clinical characteristics and outcome. Thrombosis of the terminal portal vein branches causing alterations in portal blood supply has been suggested as a pathogenic mechanism. Based on expert opinion and the scarce Western cohort studies with limited follow-up (4-5) INCPH has been considered a disorder with a relatively benign disease course with a reported 5-year survival of nearly 100% (3). However, contrasting reports on outcome have been published. The development of non-cirrhotic portal hypertension has been associated with poor prognosis in several systemic disorders (6-7). Furthermore, liver failure requiring liver transplantation has been described in INCPH patients (4).

The aim of this study was to evaluate the clinical manifestations, pathophysiology, outcome and determinants of survival in a large cohort of Western INCPH patients.

PATIENTS AND METHODS

Patient selection and study design

All consecutive patients diagnosed with INCPH in 2 European tertiary referral centers (Erasmus MC University Hospital Rotterdam in The Netherlands and University Hospitals Leuven in Belgium) between January 1992 and December 2010 were included in the study. Patient identification was done by screening the computerised patient registration systems and liver pathology databases for clinical syndromes and lesions consistent with INCPH. The diagnosis of INCPH was considered when all of the following criteria were fulfilled: (1) evidence of portal hypertension (esophageal varices, hypersplenism or ascites); (2) radiological examination (abdominal CT or Doppler ultrasound) demon-

strating patent portal and hepatic veins at initial diagnosis; (3) liver biopsy showing no cirrhosis; (4) absence of liver disease known to cause portal hypertension in the absence of cirrhosis (chronic viral hepatitis, alcoholic liver disease, NAFLD, hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis and Wilson`s disease); (5) absence of exposure to copper sulphate, vinyl chloride monomer or Spanish toxic oil, and absence of past angiography with thorium sulphate. The presence or history of alcohol abuse was assessed by repeated interview of the patient and his or her relatives at the outpatient clinic and during admission in the hospital. Patients fulfilling the criteria of the metabolic syndrome were excluded because of the potential presence of NAFLD (8). Follow-up started at the diagnosis of INCPH and lasted until either December 2010 or death, whichever came first. For all patients, data on medical history, associated disorders, initial manifestations, performed examinations, treatment and outcome events were collected through an extensive and systemic review of the medical charts by one experienced investigator (JS). Ultrasound and computed tomography scans were repeated in all patients at least every 24 months. The study protocol was approved by the local medical ethical committees.

Histological evaluation

Systematic liver biopsy evaluation was performed by two experienced liver pathologists (JV and MK), blinded to the clinical context of the biopsy as well as the patient's outcome. Archived hematoxylin & eosin stained sections were reviewed as well as at least one collagen stain (Sirius red stain, Elastica van Giesson or Masson`s trichrome stain) on each case. Patients with liver specimens smaller than 1cm were excluded in order to avoid underscoring of severe fibrosis and cirrhosis. The following morphological features were scored: phlebosclerosis, thin incomplete septa (thin blindly ending septa), increased number of vascular channels in portal tracts, paraportal shunt vessels, hypoplastic portal tracts, portal vein dilatation (total diameter >3 times the size of bile duct, or hepatic artery branch– in the absence of bile duct-), nodular regeneration, increased parenchymal vessels, dilatation of sinusoids, perisinusoidal fibrosis and perivenular fibrosis (9-11). Incomplete septa were defined as the presence of thin blindly ending septa (11). Nodular regeneration was defined as parenchymal micronodular transformation, with central hyperplasia and an atrophic rim in the absence of a fibrosis (9). Perivenular fibrosis was defined as an increase of fibrous tissue around the central vein in relationship with the central vein lumen. Patients with histological features of alcoholic hepatitis (Mallory bodies) were considered as potential alcoholics and therefore excluded. Patients with histological features of non-alcoholic steatohepatitis and NAFLD (ballooning degeneration of hepatocytes and mixed lobular inflammatory infiltrates) were also excluded.

Statistical analysis

Continuous variables were expressed as median with range or mean ± Standard Deviation. Categorical variables were expressed as absolute and relative frequencies. Actuarial overall survival and transplantation-free survival were calculated by the Kaplan-meier method. Overall survival rates were measured from the date of diagnosis of INCPH. For transplantation-free survival, death or liver transplantation were considered events. Observed survival was compared with expected survival from matched gender-, age- and calendar time-specific death rates from the general Dutch population using life table method and Wilcoxon (Gehan) test. The potential contribution of clinical, biochemical or radiological variables to survival or transplant-free survival were estimated as a hazard ratio (HR) and corresponding 95% confidence interval (CI) using Cox regression univariable analysis. Occurrence of ascites and development of portal vein thrombosis during follow-up were analyzed as time-dependent factors. Only variables found to be statistically significant by univariate analysis were included in the multivariate backward stepwise Cox regression analysis. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS, Chicago, IL)

RESULTS

Patient characteristics

Out of 235 patients identified from the computerised patient registration systems (n=92) and liver pathology databases (n=143), 173 patients were excluded because of not fulfilling the definition of INCPH (absence of clinical signs of portal hypertension (n=59), presence of portal vein thrombosis (n=39), presence of Budd-Chiari syndrome (n=6) and presence of chronic liver disease (n=69)). In total, 62 patients fulfilled the diagnostic criteria of INCPH patients and were included in the study. The median duration of follow-up was 90 months (range 24 to 310 months). The demographic characteristics, results of laboratory testing and initial manifestations leading to the diagnosis of INCPH are summarized in table 1. Thirty-two patients (52%) were male and the majority (87%) were Caucasian. Initial manifestations leading to the diagnosis of INCPH were related to signs of portal hypertension in the majority of patients (82%). The most common presentation was variceal hemorrhage (44%) and ascites (after exclusion of ascites outside the setting of portal hypertension) was present in 21 patients (34%): transient in 12 and persistent in 9 patients. INCPH-associated disorders were present at initial diagnosis in 36 patients (58%) (table 1). The most common biochemical abnormality was alkaline phosphatase elevation. Baseline abdominal ultrasound revealed nodularity of the liver surface and heterogeneity of liver parenchyma, suggestive of cirrhosis, in 49 out of 62 patients (79%).

Table 1 Baseline characteristics of 62 patients with idiopathic noncirrhotic portal hypertension.

Characteristic	n (%)
Male	32 (52)
Age (years)	46 ± 18
Presenting signs at diagnosis	
Variceal hemorrhage	27 (44)
Splenomegaly	17 (28)
Signs of portal hypertension (endoscopy, ultrasound)*	9 (15)
Elevated liver enzymes	5 (8)
Pancytopenia	4 (6)
Laboratory results	
ASAT (× ULN)	1.1 ± 0.5
ALAT (× ULN)	0.9 ± 0.5
GGT (× ULN)	1.8 ± 1.5
AP (× ULN)	1.9 ± 1.5
Bilirubin level (μmol/L)	18.0 ± 15.0
Albumin level (g/L)	37.6 ± 5.6
INR	1.07 ± 0.1
Hemoglobin (g/L)	7.9 ± 1.6
Platelets (x10^6/ mm^3)	105 ± 77
White blood cells (x10^6/ mm^3)	4.8 ± 3.0
Associated disorders	
Azathioprine treatment**	8 (13)
Hematological disorders	8 (13)
Malignancies***	4 (7)
Myeloproliferative disorders	3 (5)
Idiopatic thrombocytopenic purpura	1 (2)
Chronic HIV infection	5 (8)
Immunological disorders #	5 (8)
Genetic disorders ##	4 (7)
Arsenicum treatment	4 (7)
Breast cancer (chemotherapy 7 years before diagnosis INCPH)	1 (1)
Crohn's disease (Azathioprine naive)	1 (2)
No associated disorder	26 (42)

Variables are represented as number of subjects and percentages (between brackets)
Age and laboratory data are expressed as the mean ± Standard Deviation
* Ascites and/or porto-venous collaterals
** Renal transplant patient (n=3), Crohn's disease (n=2), dermatomyositis (n=2), polyarteritis nodososa (n=1).
*** B-acute lymfoblastic leukemia, non-Hodgkin lymphoma, multiple myeloma
# Rheumatoid arthritis (n=2), systemic lupus erythematosus (n=1), hypogammaglobulinemia (n=1)
Turner Syndrome (n=2), Schwachmann syndrome (n=1), Marfan syndrome (n=1)

Histology

Liver specimens were obtained by needle biopsy in 58 patients, and at surgery in 4 patients. In 11 patients the original biopsy could not be re-assessed since biopsies could not be collected, however based on the original report severe fibrosis or cirrhosis was excluded in these patients. Therefore, 51 biopsies were available for standardized scoring. The median biopsy length was 1.6 cm (range 1 to 3.6 cm) and the median number of portal tracts was 9.2 (range 5 to >10) which is considered valid. The most prevalent histological features were phlebosclerosis, sinusoidal dilatation, paraportal shunt vessels and perisinusoidal fibrosis (table 2).

Table 2 Baseline histological features observed in liver biopsies of 51 patients with idiopathic noncirrhotic portal hypertension.

Histological lesion	n (%)
Phlebosclerosis	50 (98%)
Hypoplastic portal tracts	32 (63%)
Nodular regeneration	24 (47%)
Thin incomplete septa	7 (14%)
Circulation disturbances	
Dilated sinusoids	50 (98%)
Paraportal shunt vessels	49 (96%)
Increased vascular channels in portal tracts	35 (69%)
Increased parenchymal vessels	29 (57%)
Portal vein dilatation	18 (35%)
Fibrosis pattern *	
Perisinusoidal fibrosis	44 (94%)
Perivenular fibrosis	32 (68%)

Histological lesions are represented as number of subjects and percentages (between brackets)
* Based on 47 out of 51 biopsies

Liver-related outcome and treatment of complications

Variceal bleeding

Variceal haemorrhage occurred in 34 patients (55%): at presentation in 27 patients and during the follow up in 7 patients (figure 1). Failure of endoscopic treatment occurred in 4/34 (12%) and the global bleeding related mortality was 1/34 (3%).

Development of liver failure, ascites, portal vein thrombosis and hepatocellular carcinoma (HCC)

Twelve patients (19%) developed severe liver function impairment after a median of 71 months (range 12 to 156 months) (figure 2). Five out of these 12 patients underwent a second biopsy during follow-up. In none of these patients severe liver fibrosis could be

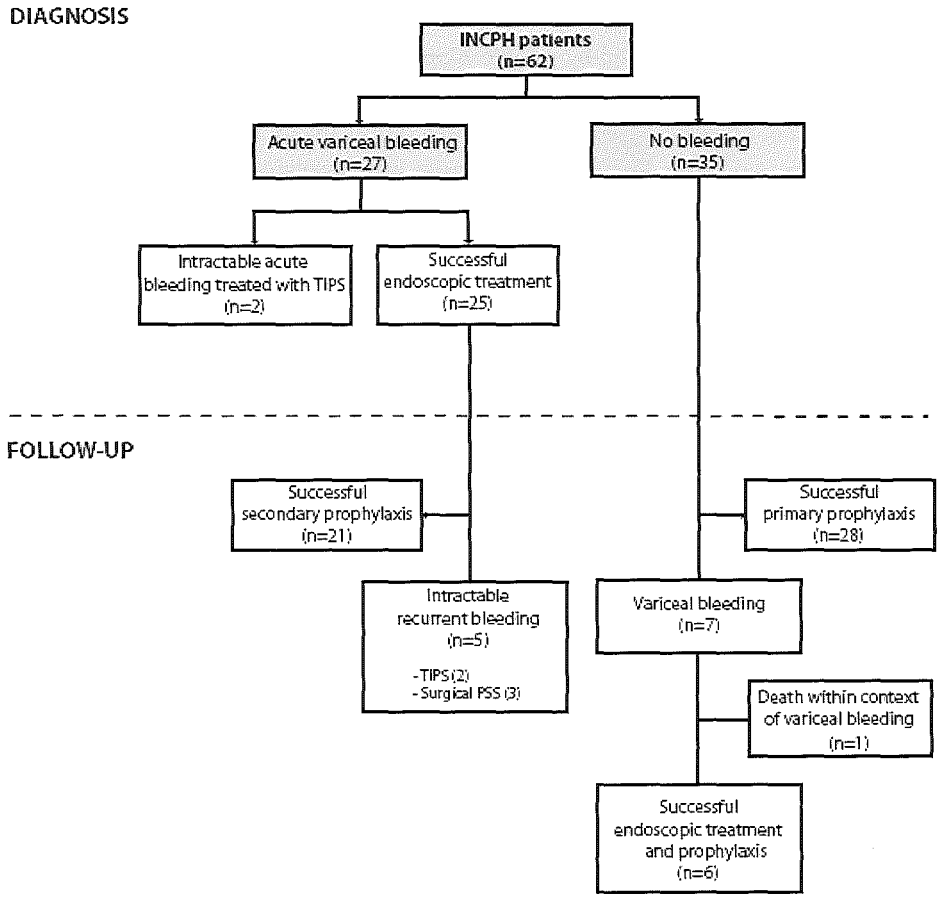


Figure 1 Clinical course of the idiopathic noncirrhotic portal hypertension patients presenting with acute variceal bleeding and patients with variceal bleeding during follow-up.

demonstrated. In 2 patients, no clear intercurrent condition could be attributed to the development of liver function impairment. These patients underwent liver transplantation. In 10 patients an intercurrent condition could be identified (portal vein thrombosis (n=2), infection (n=5), esophageal bleeding (n=1), heart failure (n=1) and development of the HELLP syndrome (n=1)). Liver function improved after controlling the intercurrent condition in 8 eight of these patients. The 2 patients with portal vein thrombosis and liver failure were listed for liver transplantation because of progressive function impairment, but died due to an infection before transplantation. Nineteen patients developed ascites after a median of 36 months (range 6 to 124 months). In 2 patients, no clear intercurrent condition could be attributed to the development of ascites. In 17 patients an intercurrent condition could be identified (portal vein thrombosis (n=4), infection (n=6), esophageal bleeding (n=4), renal failure (n=2) and development of the HELLP syndrome

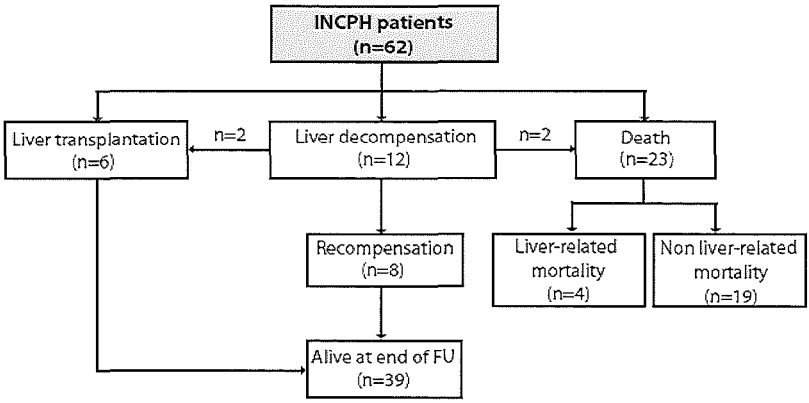


Figure 2 Flow diagram on outcome and survival for 62 patients with idiopathic noncirrhotic portal hypertension. Twelve patients developed severe liver function impairment during follow-up (in the context of portal vein thrombosis (n=2), infection (n=2), esophageal bleeding (n=2), development of malignancies (n=2), heart failure (n=1) and development of the HELLP syndrome (n=1)). In two out of these 12 patients liver transplantation was performed because of progressive liver failure. Liver function improved after controlling the underlying condition in 8 out of 12 patients that were still alive at the end of follow-up. The 2 patients with (non-hepatic) malignancy and liver decompensation died. Six patients underwent liver transplantation. (hepatopulmonary syndrome (n=1); progressive liver function deterioration (n=2); chronic hepatic encephalopathy (n=2) and pre-transplantation diagnosis of non-resectable (>8 cm) liver tumor (n=1)). Twenty-three patients died (liver related mortality (n=4) and non liver-related mortality (n=19).

(n=1)). In total, 8 patients (13%) developed portal vein thrombosis after a median of 82 months (range 9 to 132 months). Two of these patients developed severe liver function deterioration. Development of HCC did not occur during follow-up.

Portosystemic shunting

During follow-up, 18 patients (29%) were treated with surgical portosystemic shunting (1992-1999; n=7) or transjugular intrahepatic portosystemic shunting (TIPS) (1998-2010; n=11). Indications for these procedures were chronic ascites (n=7); failing secondary prophylaxis for variceal bleeding (n=5), refusal or inability to perform endoscopic secondary prophylaxis for variceal bleeding (n=4) and intractable acute bleeding (n=2). None of the patients treated with surgical portosystemic shunting developed encephalopathy over a median follow-up of 120 months (range 88 to 140 months). In 4 out of 11 patients, TIPS insertion was complicated by the development of hepatic encephalopathy. In 9 out of 11 patients undergoing TIPS, the wedge hepatic pressure gradient was measured at the moment of TIPS placement. In 7 patients (including the 4 patients developing hepatic encephalopathy) this gradient was normal (<5mmHg). In 2 patients this gradient was slightly increased (7mmHg and 8 mmHg). Renal insufficiency was present in 3 of these patients. In two patients hepatic encephalopathy could be managed conservatively. Two other patients underwent liver transplantation because of persistent severe hepatic encephalopathy.

Liver transplantation

Eight patients (13%) were listed and finally 6 patients underwent liver transplantation after a median of 86 months (range 50 to 122 months) (figure 2). Indications for liver transplantation were progressive liver failure (n=2); chronic hepatic encephalopathy (n=2); hepatopulmonary syndrome (n=1) and non-resectable liver tumor (>8cm) (n=1). In all of the explanted livers, histopathological examination confirmed the absence of severe fibrosis and liver cirrhosis. Pathological examination of the large liver tumor demonstrated the presence of malignant degeneration of an adenoma. None of these liver transplant patients died or developed clinical signs of portal hypertension during a median follow-up of 112 months (range 48 to 156 months).

Survival

Mortality

During follow-up, 23 patients (37%) died after a median of 82 months (range 24 to 240 months). Causes of death are shown in table 3. Four patients died due to liver related complications (figure 2). The majority of INCPH patients (83%) died due to non-liver related disorders, in particular malignancy.

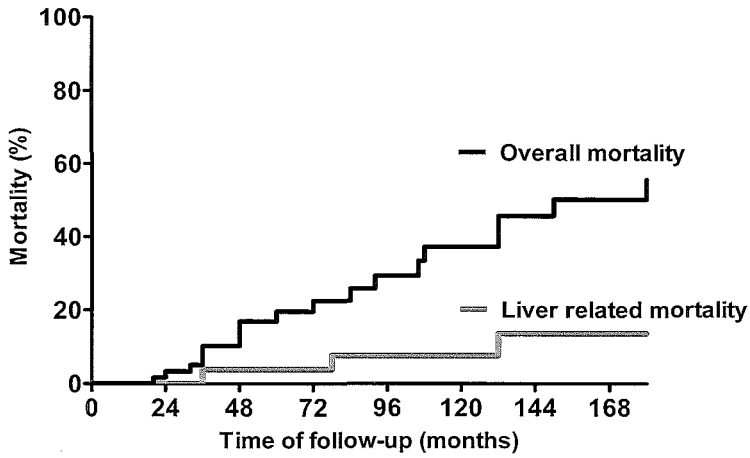
The Kaplan-Meier estimates for overall survival were 100% (95% CI 95-100%) at 1 year, 78% (95% CI 67-89%) at 5 years and 56% (95% CI 40-72%) at 10 years. The Kaplan-Meier estimates for transplantation-free survival were 100% (95% CI 95-100%) at 1 year, 72% (95% CI 59-85%) at 5 years and 40% (95% CI 23-57%) at 10 years. Cumulative overall and liver related mortality are depicted in figure 3. Overall survival in INCPH patients was significantly decreased compared with survival of a sample of the standardized Dutch population ($p<0.001$) (figure 4).

Table 3 Causes of death among 23 patients with idiopathic noncirrhotic portal hypertension.

Cause of death	n (%)
Liver related mortality	4 (17)
Liver failure	2 (9)
Complications of variceal bleeding (sepsis)	1 (4)
Hepatopulmonary syndrome	1 (4)
Non-liver related mortality	19 (83)
Malignancy *	10 (44)
Cardiac insufficiency	4 (17)
Neurological disorder	2 (9)
Renal insufficiency	2 (9)
Infection	1 (4)

Causes of death are represented as number of subjects and percentages (between brackets)

* Non-Hodgkin lymphoma (n=3), B-acute lymphoblastic leukemia (n=2), chronic myeloid leukemia (n=1), rectal carcinoma (n=1), prostate carcinoma (n=1), breast cancer (n=1), gastric carcinoma (n=1)



Patients at risk	62	58	39	26	19	15	12	10
Overall deaths	0	2	9	12	16	18	20	21
Liver related deaths	0	0	2	2	3	3	4	4

Figure 3 Cumulative overall and liver related mortality of 62 patients with idiopathic noncirrhotic portal hypertension. The number of patients at risk, overall and liver related deaths are shown along the x-axis.

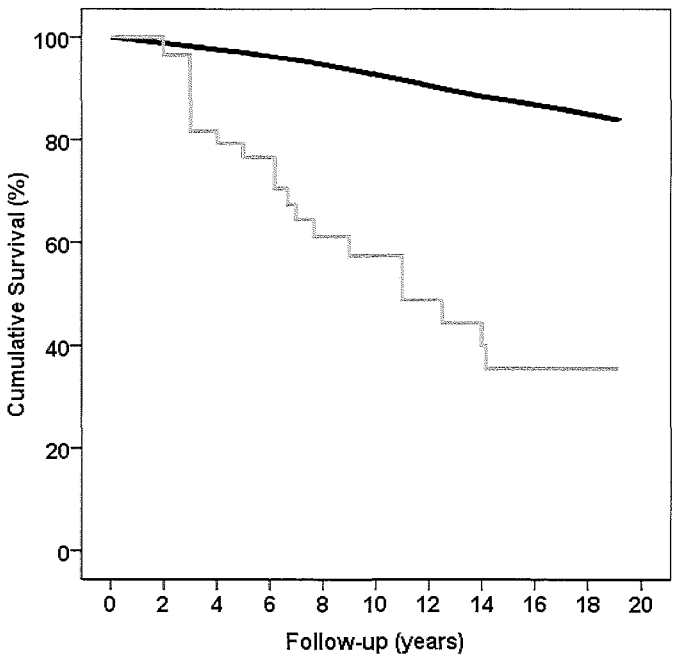


Figure 4 Overall survival of idiopathic noncirrhotic portal hypertension patients compared to a sample of the general population, matched for gender and age. Survival for idiopathic noncirrhotic portal hypertension patients was significantly worse ($p<0.001$) compared to survival in the general population

Determinants of survival

In the univariate analysis we found that age and ascites (at baseline and during follow-up) were significant predictors of survival (table 4). In the multivariate analysis, both covariates remained independent significant factors of survival (age: HR 1.04 p=0.01; ascites: HR 2.70 p=0.03). Neither the occurrence of variceal bleeding nor indicators of synthetic liver function at diagnosis influenced survival significantly. Age was the only independent significant factor which predicted transplantation-free survival.

Table 4 Univariate analysis of baseline variables associated with survival and OLT free survival.

Variable	Survival		OLT-free survival	
	HR (CI 95%)	p-value	HR (CI 95%)	p-value
Age (per 10 years)*	1.63 (1.22-1.63)	0.001	1.48 (1.10-1.79)	0.01
Female gender	0.98 (0.43-2.23)	0.96	1.15 (0.53-2.50)	0.72
Bleeding as initial symptom *	1.05 (0.46-2.40)	0.90	0.81 (0.38-1.76)	0.60
Presence of associated disorders*	0.70 (0.29-1.68)	0.41	0.97 (0.39-1.95)	0.73
Presence of medication associated with INCPH*	0.75 (0.27-2.06)	0.58	0.70 (0.26-1.90)	0.49
Spleen size*	1.00 (0.82-1.22)	0.99	0.98 (0.82-1.16)	0.98
Bilirubin level (per 10 µmol/L)*	1.10 (0.90-1.48)	0.27	1.22 (0.90-1.48)	0.13
Prothrombin Time (per 1 sec)*	0.98 (0.77-1.25)	0.87	0.96 (0.76-1.20)	0.95
Platelets (per 10 x10^6/mm^3)*	1.00 (0.90-1.10)	0.37	1.00 (0.90-1.10)	0.24
Ascites at baseline and during follow-up **	3.44 (1.35-8.80)	0.01	2.50 (1.08-5.77)	0.03
Development of portal vein thrombosis **	1.84 (0.53-6.40)	0.37	1.54 (0.45-5.25)	0.49

* Variable at baseline

**Analysed as time-dependent factor

DISCUSSION

The results of the current study demonstrate that long-term overall survival in Western INCPH patients is considerably shorter compared with survival of the general population and that mortality is mainly related to associated disorders and medical conditions occurring later in life, rather than complications of portal hypertension or liver failure.

So far, studies on prognosis and survival in INCPH patients were scarce and only reported on short term follow-up (2). The last two decades, no more than two cohort studies, each describing approximately 30 Western patients with INCPH, have been reported (4, 12). A recent study by Cazals-Hatem et al, consisting out of 59 patients with obliterative portal venopathy, focused on the clinical outcome of patients with this histological feature (13). Only 53% of these subjects had idiopathic non-cirrhotic portal hypertension at diagnosis. Our series of 62 well-characterized patients is the largest and most detailed

long-term follow-up study on the clinical aspects of INCPH in the Western world currently available. For the diagnosis of INCPH we applied the recently proposed strict clinical definition. In order to avoid misclassification of cirrhotics as INCPH patients, all patients with chronic liver disease and biopsies with an insufficient number of portal tracts were excluded.

INCPH in the Western world can be regarded a distinct entity compared to the Indian variant of the disorder. At initial diagnosis, patients in our study cohort were almost two decades older in comparison to the reported age in Indian patients. This discrepancy might be explained by etiological differences between the two entities. In our series, we demonstrated a wide variety of INCPH associated disorders such as the presence of a prothrombotic state and HIV infection. This finding raises the hypothesis that the development of INCPH results from the initiation of pathophysiological mechanisms or aggravation of an underlying susceptibility (e.g. thrombophilia) by an associated disorder occurring later in life. In contrast, the suspected pathophysiological mechanism of this disorder in Indian patients is abdominal infections during infancy, possibly explaining the clinical presentation at a younger age (14).

These study results are in line with previous studies suggesting an important role for thrombophilia in the pathophysiology of INCPH (4, 15). It has been hypothesised that this prothrombotic state leads to perisinusoidal fibrosis due to stellate cell activation and thrombosis of the terminal branches of the portal venules inducing phlebosclerosis. Indeed, in the majority of the INCPH patients we could demonstrate these histological features.

Our data confirm that the differential diagnosis of INCPH and liver cirrhosis remains a challenge. Before having histological specimens, 79% of our INCPH patients were radiologically and clinically misclassified as cirrhotics. This finding is in accordance with earlier reports (16). In order to avoid a misdiagnosis, INCPH should be considered in all patients with portal hypertension and/or isolated thrombopenia in the absence of liver disease regardless of the presence of radiological features of liver cirrhosis.

We found a relatively low incidence of portal vein thrombosis during follow-up, as compared with the previously reported 40-75% (17). We acknowledge that the retrospective character of the study may result in under-diagnosing of portal vein thrombosis. However, ultrasound or computed tomography scans were repeated regularly (at least every 2 years). Thus, based on our study results, we cannot recommend close monitoring of the portal vein in patients with INCPH. Whether it should be performed in subpopulations, such as HIV related INCPH remains to be elucidated.

Up to now, considering the preserved liver function in INCPH patients, hepatic encephalopathy has been assumed an exceptional complication of TIPS placement. Interestingly, in our study cohort, 4 out of 11 patients undergoing TIPS placement developed encephalopathy during follow-up. A potential explanation might be a deficient compensatory arterial change in INCPH, potentially sensitizing these patients to insufficient parenchymal blood flow (18).

So far, INCPH has generally been regarded a relative benign disorder with an almost similar survival to that of the general population (5-year survival of nearly 100% after eradication of varices) (3, 19). However, in our cohort, both overall and transplant-free ten years survival was around 50%. This substantial mortality is not primarily related to complications of liver disease. Firstly, none of the patients in our study cohort developed HCC during long-term follow-up. Due to the presumption that enhancement of hepatic carcinogenesis was provoked by liver regeneration in patients with parenchymal remodelling (20-21) and the observed high incidence of HCC in patients with Budd-Chiari syndrome (22), regular monitoring by liver ultrasound in INCPH patients remained a controversial issue up to now (23). Based on our findings, surveillance for HCC cannot be recommended. Secondly, in contrast to previous reports (24), mortality among INCPH patients was not related to manifestations of portal hypertension, such as variceal bleeding. Endoscopic treatment appears effective and safe in INCPH patients with variceal bleeding. Only 2 patients underwent TIPS placement because of intractable acute variceal bleeding despite endoscopic therapy. In our cohort, no patient died because of intractable bleeding and only 1 patient died in the context of variceal bleeding. Similar results have been demonstrated for variceal bleeding in patients with portal vein thrombosis (25). The significant better results in overall clinical outcome, in comparison with variceal bleeding in patients with liver cirrhosis, can be explained by intact liver function with better coagulation and probably less infections. Thirdly, liver function impairment occurs mainly in the context of intercurrent conditions with normalisation after stabilisation of these events. Only 2 patients underwent liver transplantation because of progressive liver failure. In agreement with previous short-term follow-up studies, post-transplantation outcome in these patients was good without recurrence of INCPH (16, 26-27). If mortality is not liver related, it is tempting to explain the observed poor long-term survival in these patients by the relative older age at presentation. Indeed, this translates in a higher incidence of additional disorders (malignancy and neurological disorders) influencing overall mortality in our cohort, but compared to survival rates in the general population of similar age and sex, mortality was still markedly higher suggesting that additional mechanisms influencing survival should be considered. Higher overall mortality in our cohort can be attributed to the natural history of the INCPH associated disorders. Furthermore, it can be postulated that

the presence of the hemodynamic alterations in these patients increases the chances of severe complications and subsequently mortality when additional medical conditions occur. Similarly, in patients with chronic granulomatous disease, the development of non-cirrhotic portal hypertension has been shown to be independently associated with poor survival outcome by increasing the rate of infection and sepsis (7). In multivariate analysis, only age and the presence or development of ascites were significantly associated with poor survival. Regarding the prognostic role of ascites, similar results have been reported in non-cirrhotic portal hypertension due to thrombosis in the splanchnic venous distribution (28-29). As a result, the presence or development of ascites in INCPH patients can be regarded an ominous sign. Regarding liver transplantation or liver failure, no significant risk factors could be identified due to the small amount of events.

Despite the extensive screening of computerised patient registration systems, the complete follow-up data and the strict data selection, this study has the limitations common to other retrospective surveys of rare disorders. Nevertheless, we believe that our findings may lead to the further understanding of this disease in Western patients, especially since prospective studies are hardly feasible given the rarity of INCPH.

We conclude that, INCPH in the Western world can be regarded a distinct entity compared to the disorder in Indian patients. The observed histological features suggest a thrombophilic factor in its pathophysiology. Overall survival in INCPH patients is much lower than generally considered. Mortality is mainly related to INCPH associated disorders and medical conditions accompanying older age (neurological disorders, heart failure) and not to complications of portal hypertension or the development of liver failure.

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CHAPTER 5

ETIOLOGY OF SPLENOMEGALY AND UPPER LIMIT OF NORMAL SPLEEN SIZE: RESULTS OF A LARGE POPULATION-BASED STUDY IN THE ELDERLY

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Submitted

ABSTRACT

Purpose

Currently, the generally accepted upper limit of normal (ULN) spleen size in adults ranges from 12-14cm. These standards cannot be extrapolated to an elderly population since autopsy studies demonstrated decreasing spleen size with increasing age. The aim of this study was to study the determinants and ULN spleen size in an elderly Caucasian population,

Materials and Methods

Reliable sonographic spleen size measurement was performed in 2582 participants of the Rotterdam Study. Determinants and ULN spleen size were assessed after exclusion of participants with conditions associated with splenomegaly (n=203).

Results

In univariable analysis, greater spleen size was associated with male sex ($p<0.001$), greater height (correlation coefficient (r)=0.42, $p<0.001$), higher weight (r=0.45, $p<0.001$), younger age (r=0.05, p=0.008), presence of liver steatosis ($p<0.001$), lower platelet count (r=-0.24, $p<.001$) and lower portal flow velocity (r=-0.81, $p<0.001$). In multivariable analysis only height (regression coefficient (β) = 0.027), weight (β=0.027), sex (β=0.38) and presence of steatosis (β=0.183) were independent determinants of spleen size. ULN spleen size was 12.2cm in men and 11.1cm in women. Spleen size larger than these ULN was significantly associated with higher prevalence of hematological disorders and liver cirrhosis.

Conclusion

Sex, height, weight and presence of liver steatosis are determinants of spleen size. In the elderly male population ULN spleen size (12cm) is at the lower end of the previously suggested range and in the elderly female population ULN spleen size is lower (11cm). Pathological disorders must be excluded in elderly with smaller spleen size than previously accepted.

INTRODUCTION

In clinical practice, accurate assessment of spleen enlargement is of considerable importance because splenomegaly may indicate serious underlying diseases (1-2). Since physical examination is unreliable to diagnose splenomegaly, ultrasonography is frequently performed for spleen size measurement (3-5). Simple sonographical measurement of splenic length, the applied assessment of spleen size in clinical practice, has been shown a good correlate of splenic volume (4, 6). Regarding the determinants of spleen size, discrepant data are available in current literature. Several authors have linked body height to spleen size. However, there is no consensus regarding the influence of age, sex and weight on spleen size. Currently, the generally accepted upper limit of normal (ULN) spleen size in adults ranges from 12-14cm (2, 7-9). However, these standards are based on studies performed more than 25 years ago, that included mainly younger participants. Extrapolation of these data to the elderly population is most probably incorrect since autopsy studies have suggested a decrease in spleen size with increasing age (10). Moreover, in view of the growing elderly population and the high incidence of disorders associated with spleen size enlargement in this age group, the knowledge of these standards is of significant importance. So far, large studies assessing normal spleen size standards in the elderly have not been performed. The lack of a reliable standard for a normal spleen size in this population may lead to unnecessary further work up or conversely underdiagnosis of several conditions (e.g. haematological disorders, malignancy) because of misinterpreted normal spleen sizes.

The aim of this study was to study the determinants and the ULN spleen size in an elderly Caucasian population.

METHODS AND MATERIALS

Study population

The Rotterdam Study is a large prospective population-based cohort study conducted among elderly inhabitants of Ommoord, a district of Rotterdam, The Netherlands. The rationale and study design have been described previously (11). The medical ethics committee at Erasmus University of Rotterdam approved the study, and written informed consent was obtained from all participants.

Abdominal ultrasonography was performed in the first two cohorts of the Rotterdam Study (February 2009-February 2012). Each participant completed an extensive interview and clinical examination that included, among others, anthropometric assessment and liver ultrasonography (12). Various parameters were assessed according to the generally accepted sonographic guidelines (13). Liver surface and parenchyma were assessed for the presence of nodularity (using a 3.5 and 5 MHz transducer), heterogene-

ity of liver parenchyma, liver steatosis and liver tumors. Portal vein diameter and flow velocity were determined along the hepatoduodenal ligament halfway between the venous confluens and the portal bifurcation. Hepatic vein diameter and flow pattern were determined 3cm from the inferior caval vein. Evaluation of portosystemic collaterals (paraumbilical vein, coronary vein, epigastric collaterals, splenorenal collaterals) was performed on the specific localizations. Spleen size determination was performed through the splenic hilum in an obique plane. The investigated subject was lying in the supine or lateral decubitus position. All ultrasound examinations were performed on a Hitachi HI VISION 900 with a curvilinear transducer by a sonographic certified nurse. All measurements were performed three times and the mean values were recorded. All images were recorded digitally and reviewed by a gastroenterologist with more than 10 years of ultrasound experience.

All participants were screened for the presence of the most prevalent conditions associated with splenomegaly. For the assessment of determinants and ULN spleen size, participants with the presence of these disorders were excluded. Liver cirrhosis and clinical significant portal hypertension (dilatation of portal vein (>15mm) and portosystemic collaterals) were assessed ultrasonographically. Liver cirrhosis was diagnosed if nodularity of the liver surface and parenchymal heterogeneity were present (14). Clinical significant portal hypertension was diagnosed when portosystemic collaterals could be identified. Data on acute viral infection, haematological diseases, chronic inflammatory disorders (systemic lupus erythematosus and rheumatoid arthritis) and chronic infectious disorders (endocarditis and malaria) were collected from records of general practitioners and medical specialists, and an automated Dutch pathology database (PALGA) by trained research nurses (12). Heart failure was defined as a combination of typical symptoms and signs of heart failure confirmed by objective evidence of cardiac dysfunction. Methods on event adjudication of prevalent heart failure for the Rotterdam Study have been described previously (12, 15-16).

Statistical analysis

In univariable analysis associations of covariables with spleen size were determined using the parametric Student T-tests and non-parametric Wilcoxon rank sum tests. In multiple linear regression analysis, regression coefficients (β) and significance of associations of different variables were determined, using spleen size as the dependent variable. The ULN spleen size is set to the 95th percentile, as is standard for distribution of a continuous variable in the normal population (17-18). A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Assessment of determinants and ULN of spleen size

Study population

A total of 3205 participants underwent abdominal ultrasonography. In 623 participants, reliable sonographic spleen size measurement could not be performed (incomplete visualisation of the splenic hilum (n=310), splenectomy (n=25) and lack of patient cooperation (mainly inability for deep inspiration) (n=298)). Consequently these participants were excluded from analysis, leaving 2582 for inclusion in the study. Distribution of spleen size by sex is illustrated in figure 1.

For the assessment of determinants and ULN of spleen size, 203 out of 2582 participants were excluded because of the presence of disorders associated with spleen enlargement: presence of liver cirrhosis (n=22), portal venous thrombosis with portosystemic collaterals (n=1), haematological disorder (n=28), a history of rheumatoid arthritis (n=64), and a history of moderate to severe heart failure (n=98). In total, 2379 participants were included for the remaining analyses. General characteristics of this study population are shown in table 1. Mean age of the participants was 75.6±5.9 years (range: 65-98 years), sixty percent were women and the vast majority (96.4%) was of Caucasian origin. Median spleen size in the general study population was 9.4cm (inter-

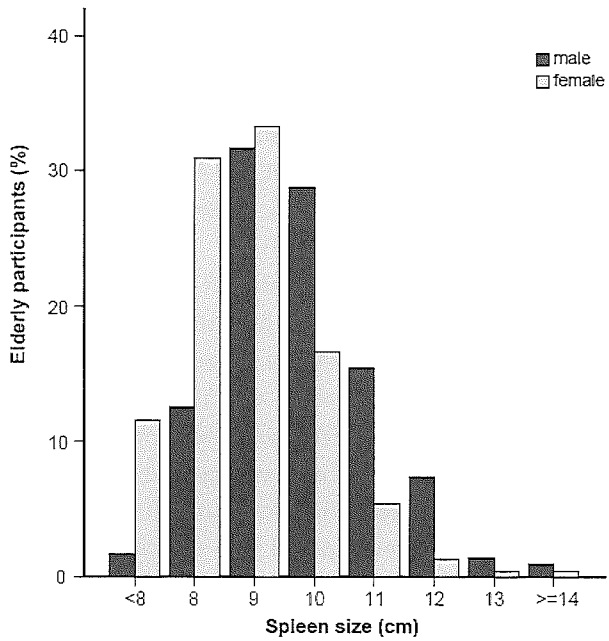

Figure 1 Distribution of spleen size (by sex) in elderly participants with complete spleen size measurement (n=2582).

Table 1 General characteristics of study population for the assessment of determinants and upper limits of normal spleen size (n=2379). Data are represented as mean (± standard deviation), median (25th-75th percentile) or percentages.

	Total (n=2379)	Men (n=951)	Women (n=1428)	p-value*
Age (years)	75.6 (±5.9)	75.4 (±5.7)	75.7 (±6.0)	0.1
Caucasian (%)	96.4	96.4	96.5	0.9
Alcohol intake (drinks/week)	5.0 (±5.9)	6.9 (±7.1)	3.8 (±4.6)	<.001
Diabetes Mellitus (%)	13.0	13.3	12.8	0.7
Length (cm)	166.5 (±9.1)	174.4 (±6.6)	161.2 (±6.4)	<.001
Weight (kg)	75.4 (±12.8)	81.9 (±11.0)	71.1 (±12.2)	<.001
BMI (kg/m2)	27.2 (±3.9)	26.9 (±3.1)	27.3 (±4.3)	0.009
Normal; BMI < 25 (%)	30.3	28.7	31.3	
Overweight; 25 ≤ BMI < 30 (%)	49.4	56.1	44.9	
Obese; BMI ≥ 30 (%)	20.4	15.2	23.8	
Liver steatosis (%)	34.8	34.9	34.8	0.9
Spleen size (cm)	9.4 (8.7-10.2)	10.0 (9.2-10.9)	9.0 (8.4-9.9)	<.001
Platelets (x10^9/L)	259 (219-303)	237 (198-271)	276 (238-320)	<.001

*Based on T-test, Wilcoxon rank sum test or Chi-square test.
Abbreviations: BMI, Body Mass Index; ALT, alanine aminotransferase; Hb, haemoglobin; WBC, white blood cell count.

quartile range (IQR) 8.7-10.2). Median spleen size was 10.0cm (9.3-11.0) in men versus 9.0cm (8.4-9.9) in women.

Spleen size determinants

In univariable analysis, greater spleen size was associated with male sex ($p<0.001$), greater height ($r=0.42$, $p<0.001$), heavier weight ($r=0.45$, $p<0.001$), younger age ($r=0.05$, $p=0.008$), presence of liver steatosis ($p<0.001$), lower platelet count ($r=-0.24$, $p<.001$) and lower portal flow velocity ($r=-0.81$, $p<0.001$).

Multiple linear regression analysis is illustrated in table 2. In the model adjusting for age, sex, height, weight, and presence of liver steatosis, all of these factors were associated with spleen size (p-values<0.04). Total explained variance (R^2) of the model including age, sex, height, weight and presence of liver steatosis was 26%. This same model was applied to calculate estimated marginal means (EMM) of spleen size for each variable. If the variable was continuous, it was recoded into categories.

Platelets also remained associated with spleen size after adjustment for age, sex, height and weight ($p<0.001$); however, portal flow velocity was not associated with spleen size ($p=0.8$). In multiple regression analysis, no interaction was demonstrated between presence of liver steatosis with age, sex, height, weight, or alcohol consumption, suggesting no independent association (p-values ≥ 0.05). Moreover, no interaction was demonstrated between age and sex with any of the other variables.

Table 2 Multiple linear regression model with spleen size (cm) as the dependent variable.

	B (SE)	p-value	Adjusted R^2
Model 1			0.14
Age (years)	-0.009 (0.004)	0.025	
Sex (male)	0.948 (0.048)	<.001	
Model 2			0.19
Age (years)	0.003 (0.004)	0.5	
Sex (male)	0.405 (0.067)	<.001	
Height (cm)	0.042 (0.004)	<.001	
Model 3			0.26
Age (years)	0.008 (0.004)	0.04	
Sex (male)	0.377 (0.064)	<.001	
Height (cm)	0.022 (0.004)	<.001	
Presence of liver steatosis	0.183 (0.051)	<.001	
Weight (kg)	0.027 (0.003)	<.001	

Table 3 Mean marginal spleen size for increments of body height, weight and presence of liver steatosis by linear regression method.

		Male				Female			
		70kg		80kg		70kg		80kg	
		No LS	LS	No LS	LS	No LS	LS	No LS	LS
Height	160cm	9.4	9.6	9.6	9.8	9.1	9.2	9.3	9.5
	170cm	9.7	9.8	9.9	10.1	9.3	9.5	9.5	9.7
	180cm	9.9	10.1	10.1	10.3	9.6	9.8	9.9	10.0

Abbreviations: LS: liver steatosis

In table 3 the marginal mean spleen size for increments of body height, weight and presence of liver steatosis are shown.

Assessment of the ULN spleen size

The ULN spleen size, or 95th percentile, was 11.8cm for the total population. ULN spleen size was 11.1cm in women and 12.2cm in men. Figure 2 demonstrates the median (50th percentile) and normal range (5th-95th percentile) for spleen size in the elderly, by sex and height. Due to the low number of women with height >180cm and men with height <160cm, no values are shown for these groups.

Assessment of clinical value of ULN spleen size

The clinical value of the determined ULN spleen size was assessed in all 2582 participants that underwent spleen size measurement (without exclusion of patients with disorders associated with spleen enlargement). Out of the 2582 participants, 97 participants had

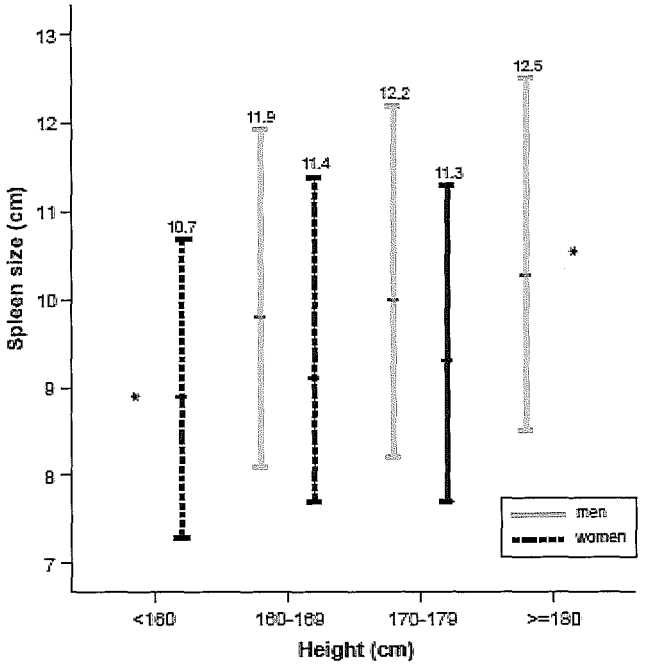

Figure 2 Fifth, 50th and 95th (upper limit of normal) percentile for spleen size by sex and height.

a spleen size of 12-12.9cm (3.8%) (figure 1). The etiology of spleen enlargement in these participants was haematological disorder (n=2), liver cirrhosis (n= 7), rheumatoid arthritis (n=2) and heart failure (n=5). In 81 out of these 97, no etiology could be retrieved. Out of the 2582 participants, 21 had a spleen size of 13.0-13.9cm (0.8%) (figure1). The etiology of spleen enlargement in these participants was liver cirrhosis (n=3) and heart failure (n=1). In 17 out of 21 participants, no etiology could be retrieved. Out of the 2582 participants, 17 had a spleen size of ≥14cm (0.7%) (figure 1). The etiology of spleen enlargement in these participants was haematological disorder (n=8), liver cirrhosis (n= 3), heart failure (n=3), and portal vein thrombosis (n=1). In 3 out of these 17, no etiology could be retrieved.

Applying the ULN spleen size according to gender, haematological disorders and liver cirrhosis were significantly more prevalent in the participants with spleen size above the ULN. Of the male participants with spleen size larger than 12cm, 9.7% had liver cirrhosis versus 0.4% of the participants with a lower spleen size ($p<0.001$) and 6.8% had haematological disorders versus 1.3% of the participants with a lower spleen size ($p=0.001$). Of the female participants with spleen size larger than 11cm, 4.4% had haematological disorders versus 0.3% of the participants with a lower spleen size ($p=0.001$) and 4.5% had liver cirrhosis versus 0.2% of the participants with a lower spleen size ($p=0.001$). No significant differences were observed between a history of heart failure or history

of rheumatoid arthritis for the ULN spleen size according to gender (p=0.4 and p=0.7 respectively).

DISCUSSION

This large cohort study demonstrates that sex, height, weight and the presence of liver steatosis are determinants of spleen size resulting in smaller ULN spleen size than the general expected 12-14cm in the elderly Western population characterized by predominately female gender and small length.

We identified length, weight, sex and the presence of liver steatosis as independent spleen size determinants. The observation that length and weight are associated with spleen size is in line with previously reported study results (5, 8, 19). Regarding the association between age and spleen size, discordant data have been reported (5, 17). Despite the significant correlation between age and spleen size in this study, we do believe that, taking into account the small correlation coefficient, this observation is clinically not relevant and that this observation is a result of the large cohort size. This is the first study demonstrating a link between the presence of liver steatosis and spleen size. Since an increase in spleen size is the first sign of increasing portal pressure, this observation might be explained by the presence a small increase in portal venous pressure in patients with liver steatosis as demonstrated by Francque et al (20).

Based on studies performed more than 25 years ago, the generally accepted ULN spleen size in adults ranges between 12 and 14cm (2, 7-9). However, these data cannot be extrapolated to elderly persons since these studies included mainly younger participants and decreasing, spleen size has been reported with increasing age (10). Up to now, this is the first large study assessing ULN spleen size in elderly. Subject selection is an essential issue in studies determining reference values. By using a well described elderly population in which all disorders associated with spleen size enlargement were elaborately excluded, we applied the most accepted and reliable method for defining ULN (17-18). At first sight, our results confirm the autopsy study suggesting lower spleen size in elderly persons. In the male participants, the ULN spleen size was at the lower end of the recommended range of 12-14cm. In female participants, the median spleen size was lower (11.1cm) than this range. However, in multivariable analysis, age was only marginally associated with larger spleen size. The decrease in median spleen size with age can be explained by the generally smaller body size of the elderly population and the distinct association between length and spleen size. In addition we validated the ULN spleen size in all the participants with a reliable spleen size measurement according to gender (females 11cm and males 12cm). These ULN spleen size were significantly associated

with haematological disorders and liver cirrhosis, indicating that pathological disorders must be excluded in persons with larger spleens than the determined ULN spleen size. The prevalence of pathological disorders increases with increasing spleen size. In 82% of the participants with spleen size above 14 cm severe pathological disorders could be diagnosed and should as such be regarded an alarm symptom in the elderly population and not the upper end of the recommended normal range.

The strengths of this study are the large number of investigated participants, the wide age distribution (range: 65 to 98 years) and its population-based character, which prevents the occurrence of recall or selection bias. Furthermore, we elaborately excluded all participants possibly suffering from conditions associated with spleen enlargement, which may have erroneously increased the ULN spleen size. The fact that all ultrasound examinations were performed by a single nurse practitioner may be regarded a shortcoming of the study. However, the nurse was certified in abdominal ultrasound and all examinations were digitally stored and reviewed by a gastroenterologist with more than 10 years sonographic experience. Furthermore, the strong correlation between platelet count and spleen size supports our conclusion that the spleen size measurements were performed accurately. Finally, we only measured splenic length and not splenic volume. However, splenic length measurement is generally regarded the most accurate and reasonable assessment of spleen size in daily clinical practice and correlates well with 3-dimensional computed tomography volume assessment (4-6).

In conclusion, due to the influence of body height on spleen size and the smaller body size in elderly, the median ULN spleen size in the elderly population is smaller than the generally considered 12-14cm. The clinical importance of these results is that physicians should exclude pathological conditions at a lower spleen size in elderly patients (in female persons >11cm and in male persons >12cm).

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CHAPTER 6

HIV-ASSOCIATED IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION: CLINICAL FEATURES, PREVALENCE AND RISK FACTORS

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ABSTRACT

Background

Idiopathic noncirrhotic portal hypertension (INCPH) has been reported increasingly in patients with HIV infection.

Aim

To evaluate the number of nationwide diagnosed HIV-associated INCPH cases and to assess its clinical features, risk factors and outcome.

Methods

All HIV centers in The Netherlands were contacted and requested to notify INCPH cases diagnosed in their population. A case-control study was performed to identify the risk factors of INCPH. The cases were group-matched for duration of follow-up after HIV diagnosis to controls. Controls were selected from a database of HIV patients with negative screening for signs of portal hypertension on abdominal ultrasound. Univariate and multivariate conditional logistic regression analyses were performed.

Results

On 1st of July 2011, 18.085 individuals were infected with HIV in The Netherlands. Within this population sixteen patients with clinically overt INCPH were identified. At the time of INCPH diagnosis, cases had a lower platelet count, a higher ALT level. In uni- and multivariate analysis, didanosine (OR:1.9 (1.3-2.8)), concomitant didanosine and stavudine treatment (OR:6.3 (2.1-19.1)) and concomitant didanosine and tenofovir treatment (OR:5.1 (1.2-22.6)) were independently associated INCPH. During follow-up, 4 patients died (malignancy (n=3), liver failure (n=1)). A significant decline in platelets was observed after didanosine discontinuation (p=0.003).

Conclusions

HIV-associated clinically relevant INCPH appears to be a rarely diagnosed disease. Long-term exposure to didanosine and short-term combination of didanosine and stavudine or tenofovir exposure are associated with INCPH. Mortality in HIV-associated INCPH is mainly related to HIV associated disorders. Portal hypertension sustains despite didanosine discontinuation.

INTRODUCTION

Idiopathic noncirrhotic portal hypertension (INCPH) is a clinical entity consisting of intrahepatic portal hypertension in the absence of cirrhosis and other causes of liver disease (1). In contrast to the high prevalence of this disorder in India and Japan, it is considered to be rare in the Western world. Although no pathognomonic histological findings exist, obliterative portal venopathy is a frequently observed morphological feature in liver specimens from INCPH patients (2). The etiology of INCPH can theoretically be divided in five categories: chronic infections, exposure to medication or toxins, thrombophilia, immunological disorders and genetic disorders (1).

Several reports have described the occurrence of INCPH in patients with human immunodeficiency virus (HIV) infection (3-9). Opinions regarding the prevalence of this disorder are discordant. According to some experts, the prevalence of INCPH in HIV-infected patients is assumed to be substantial (10), whereas others agree that it is a rare disease (8-9). Furthermore, clinical outcome and survival in HIV-associated INCPH patients remains to be elucidated (5, 11-13).

Currently available data regarding HIV-associated INCPH are derived from studies focusing on the histological entity obliterative portal venopathy or from small case series with limitations in study design (11, 14). Most cases were recruited in university hospital cohorts possibly inducing selection bias. Putative differential information bias is present in the reported case-control studies since controls were selected from databases in which the presence of INCPH or its histological features was not excluded. In addition, patients with chronic viral hepatitis were not excluded in most studies, potentially leading to misclassification of patients with severe fibrosis, underscored on liver biopsy, as INCPH patients. Finally, extrapolation of the study results obtained from obliterative portal venopathy studies could be scientifically questioned, since these histopathological lesions can occur in the absence of portal hypertension (2, 15).

The objective of this study was to evaluate the number of nationwide diagnosed patients with HIV-associated INCPH and to assess the clinical features, risk factors and outcome of this disorder.

PATIENTS AND METHODS

Case identification

Case identification was done by a systematic survey. All 25 designated HIV treatment centres in the Netherlands were contacted and requested to notify INCPH cases diagnosed in their population of HIV-infected patients. The diagnosis of INCPH was considered when all of the following criteria were fulfilled: (1) clinical signs of portal hypertension (esophageal varices, hypersplenism or ascites); (2) radiological examination demon-

strating patent portal and hepatic veins at initial diagnosis; (3) liver biopsy showing no cirrhosis; (4) absence of noncirrhotic liver disease known to cause portal hypertension (chronic viral hepatitis B or C, alcoholic liver disease, NAFLD, hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, primary sclerosing cholangitis (PSC) and Wilson`s disease); (5) absence of exposure to copper sulphate, vinyl chloride monomer or Spanish toxic oil, and absence of past angiography with thorium sulphate (1). Follow-up started at the diagnosis of HIV infection and lasted until either July 2011 or death, whichever came first. For all patients data on clinical characteristics, medical history, initial manifestations, examination reports, antiretroviral treatment, clinical events during follow-up and outcome were collected through an extensive and systemic review of the medical charts.

Thrombophilia assessment was performed by screening for protein S and protein C deficiency. In view of the possibility of an acquired deficiency due to hepatocellular dysfunction a deficiency was only considered if only one of the two proteins was deficient (isolated deficiency).

Control patients selection

All HIV-infected patients that visited the outpatient clinic in our hospital between February 1st and March 31st 2011 underwent spleen size determination and visualisation of the falciform ligament by abdominal ultrasound. From this cohort (n=300), control patients with normal spleen size (<12cm) and no repermeabilization of the paraumbilical vein were group matched for duration of HIV-infection to INCPH cases in a 1:4 ratio.

Data on laboratory parameters and highly active antiretroviral therapy (HAART) exposure for controls were collected on the latest outpatient clinic visit. No liver biopsies were performed in these patients.

Data on medication intake and prevalence of HIV infection were derived from a central database that is maintained by the Dutch HIV monitoring foundation (report of 2011) (16). The study protocol was approved by the local medical ethical committees.

Histological evaluation

Liver biopsy was available for all patients with INCPH. Systematic liver biopsy evaluation was performed by two experienced liver pathologists (JV and MK), blinded to the clinical context of the biopsy. Archived hematoxylin & eosin, reticuline and collagen (Sirius red stain, Elastica van Giesson or Masson`s trichrome stain) were reviewed on each case. The following morphological features were assessed: phlebosclerosis, nodular regeneration, thin incomplete septa (thin blindly ending septa), hypoplastic portal tracts, increased vascular channels in portal tract, paraportal shunt vessels, portal vein dilatation (total diameter >3 times the size of bile duct, or hepatic artery branch– in the absence of bile

duct-), increased parenchymal draining vessels, dilatation of sinusoids, perisinusoidal fibrosis, perivenular fibrosis and liver steatosis (micro- and macrovesicular) (15, 17-18).

Statistical analysis

Each covariate was described as number and percentage for discrete variables, median and interquartile range (IQR) for continuous variables. The X^2 test or Fisher's exact test were used to compare proportions. The Mann-Whitney U test was used to compare continuous variables. Associations between HIV-associated INCPH and the following factors were first assessed by univariate analysis: age, sex, mode of HIV acquisition, race, current and nadir CD4 count, current and maximum HIV-1 viral load and total duration of HAART. The odds ratio of HIV-associated INCPH and the controls were calculated to study the association of exposure to specific combinations of drugs, cumulative exposure to individual antiretroviral drugs, concurrent use of specific combinations and exposure to different drug classes, all expressed as number of months. The number of cases is low and in order to avoid overfitting, several multivariable models were studied with a maximum of 3 variables. Best model fit was achieved by comparing the log likelihood and Akaike's Information Criterion. Analysis was repeated after one by one adjusting for ALT, CD4 and platelet count.

The probability of the development of HIV-associated INCPH given the duration of antiviral drug exposure was estimated using the log odds ratio between INCPH and control patients adjusted by the prevalence of HIV-associated INCPH in the Dutch HIV population. The following formula applies: the population log odds ratio = the log odds ratio between cases and controls – ln (number of cases / number of controls) + ln (prevalence/ (1-prevalence)).

Especially for the INCPH cases a repeated measurement analysis, with random intercept and slope, was applied to study the behaviour of the platelet count after diagnosis until end of follow-up. The linearity assumption was tested by extending the model with a quadratic time-effect.

The level of statistical significance was set at $p<0.05$.

RESULTS

Characteristics of INCPH and control patients at HIV and INCPH diagnosis

A total of 16 well-characterized INCPH patients were identified. On 1st of July 2011, the prevalence of HIV infection in the Netherlands was 18.085 signifying that 0.1% of the Dutch HIV population is diagnosed with clinically overt INCPH. Characteristics of INCPH cases at baseline and at the time of INCPH diagnosis are summarized in table 1.

Patient characteristics for cases at the time of HIV and INCPH diagnosis are shown in table 2. Patient characteristics for controls at the time of HIV and at the time of ultra-

Table 1 Characteristics of 16 HIV-infected patients at baseline and at the time of idiopathic noncirrhotic portal hypertension diagnosis.

Case no.	Sex	HIV transmission	Age at HIV diagnosis (yr)	CD4 count, cells/mm3	Age at INCPH diagnosis (yr)	INCPH presenting symptom	ALT at INCPH	Platelets at INCPH diagnosis	Duration of HAART (years)	HAART at INCPH diagnosis	DDI exposure (years)	Coagulation abnormalities
1	M	MSM	43	80	53.1	EVB	76	118	5	DDI+D4T	10	PCD
2	F	Blood	20	120	33.7	LTD	132	148	12	NVP+FTC	7	no
3	M	IVD	29	300	38.4	pancytopenia	58	92	11	DDI+EFV+ZDV	15	no
4	M	MSM	54	130	64.0	EVB	44	82	8	ABC+LAM+ZDV	4	PSC
5	M	IVD	39.0	10	47.4	EVB	42	150	9	LAM+ LPV/r+TEN	10	no
6	F	Hetero	26	130	38.9	EVB	20	90	9	DDI+LAM+D4T	5	ND
7	M	unknown	40	370	55.6	LTD	47	125	11	TEN+LPV/r+FTC	5	no
8	M	MSM	31	140	43.6	LTD	38	180	6	DDI+LPV/r+LAM+ZDV	14	PSD
9	M	MSM	45	520	51.7	EVB	20	205	7	DDI+LAM+IDV	1	ND
10	F	IVD	33	130	37.7	EVB	44	180	4	DDI+D4T+EFV	3	no
11	M	MSM	28	400	38.5	LTD	178	140	9	ATA+ABC+LAM	6	ND
12	M	MSM	35	20	49.3	EVB	145	148	13	DDI+LAM+TEN+NVP	9	ND
13	F	Hetero	27	80	37.5	abd pain	28	88	10	no	3	no
14	F	Hetero	21	130	42.8	confusion	60	158	11	DDI+LAM+NVP	7	no
15	M	MSM	36	560	47.3	EVB	78	73	9	LAM+DDI+ATA	6	no
16	M	MSM	36	120	45.1	EVB	31	67	9	FTC+fAPV+r+TEN	3	ND

Abbreviations: ABC, abacavir; ATA, atazanavir; EBV, esophageal variceal bleeding; LTD, liver test disturbances; FTC, emtricitabine; fAPV, fosamprenavir; DDI, didanosine; D4T, stavudine; IDV, indinavir; IVD, intravenous drug use; LAM, lamivudine; LPV/r, lopinavir/ritonavir; MSM, men who have sex with men; ND, not done; NVP, nevirapine; PCD, protein C deficiency; PSD, protein S deficiency; TEN, tenofovir; ZVD, zidovudine

sound examination are shown in table 2. Six out of 64 controls (11%) were exposed to didanosine and stavudine concomitant treatment, 27 (42%) were exposed to stavudine monotherapy and 21 (33%) were exposed to didanosine monotherapy. HIV-infected patients with INCPH and control patients were similar regarding age, gender, race, HIV transmission category and duration of follow-up after HIV diagnosis. At the time of INCPH diagnosis, patients with INCPH had a median age of 44 years (range 34 to 64 years) and the majority of these patients (88%) was of Caucasian descent. At INCPH diagnosis, INCPH patients had a platelet count and CD4 count in comparison to control patients. INCPH patients had higher serum levels of alanine aminotransferase (ALAT), alkaline phosphatase (AP) and gamma glutamyl transferase (GGT) in comparison to control patients.

Table 2 Demographics and biological characteristics of 16 idiopathic noncirrhotic portal hypertension and 64 control patients at HIV diagnosis and at the time of INCPH diagnosis.

Patients characteristics	**INCPH cases (n=16)**	**Control patients (n=64)**	**p**
Male gender	11 (69)	44 (69)	0.61
MSM / Heterosexual / Other *	8 (50) / 3 (19) / 5 (31)	28 (44) / 23 (36) / 13 (20)	0.29
Caucasian race	14 (88)	45 (70)	0.16
Time of known HIV infection, years	15.6 (14.9-19.2)	14.9 (13.1-18.6)	0.35
Characteristics at HIV diagnosis	**INCPH cases**	**Control patients**	**p**
Age, years	34.4 (28.0-40.0)	39.2 (30.9-45.1)	0.67
Body mass index, kg/m^2	22.4 (20.3-26.1)	22.1 (20.2-26.2)	0.72
CD4 count, cells/mm^3	130 (90-351)	220 (70-290)	0.33
Platelets, 10^6/L	175 (133-267)	196 (136-244)	0.13
ALT, IU/ml	32 (19-39)	23 (15-44)	0.16
Alkaline phosphatases, IU/L	65 (51-80)	65 (53-85)	0.51
Gamma GT, IU/L	29 (17-119)	25 (16-81)	0.97
Body mass index, kg/m^2	22.5 (21.1-23.8)	23.4 (21.4-26.3)	0.21
Creatinine, µmol/L	78 (74-91)	73 (63-80)	0.08
Characteristics at INCPH diagnosis	**INCPH cases**	**Control patients****	**p**
Age, years	44.2 (38.4-51.9)	52.5 (46.6-61.1)	0.06
Body mass index, kg/m^2	21.8 (20.3-24.6)	23.6 (21.7-26.5)	0.13
CD4 count, cells/mm^3	225 (165-310)	440 (360-630)	<0.001
HIV RNA <50, copies/ml	10 (63)	53 (83)	0.33
Hemoglobin, g/L	7.8 (5.9-8.7)	8.5 (7.9-9.1)	0.03
Platelets, 10^6/L	137 (88-156)	230 (166-240)	<0.001
ALT, IU/ml	46 (33-78)	24 (20-35)	<0.001
Alkaline phosphatases, IU/L	145 (135-252)	84 (61-101)	<0.001
Bilirubin, (µmol/L)	19 (10-30)	15 (10-25)	0.45
Gamma GT, IU/L	221 (133-445)	75 (32-109)	<0.001
INR	1.2 (1.0-1.3)	1.1 (1.0-1.2)	0.59
Creatinine, µmol/L	76 (54-87)	79 (66-91)	0.19

Data are presented as number of patients and percentages (between brackets) for discrete variables and median and IQR (between brackets) for continuous variables.

* HIV transmission category

** Data were collected at the latest outpatient clinic visit

Initial manifestations leading to the diagnosis of INCPH were related to signs or complications of portal hypertension in the majority of patients (88%). Nine patients presented with variceal bleeding, five with liver test disturbances and splenomegaly, one with abdominal pain and one with neurological symptoms unrelated to portal hypertension. Ultrasound examination at the moment of INCPH diagnosis was performed in all patients. In none of the patients sonographic features of PSC or HIV-cholangiopathy were present (19).

Liver histology of INCPH patients

All HIV-infected patients with INCPH underwent needle biopsies in the work-up of suspected liver disease in patients with portal hypertension. In one patient the original biopsy could not be collected, however based on the original report severe fibrosis or cirrhosis was excluded in this patient. Therefore, 16 biopsies were available for standardized scoring. The median biopsy length was 1.5 cm (range 0.8 cm to 2.0 cm) and the median number of portal tracts was 9.5 (range 8 to>10) which is considered valid. Severe fibrosis or liver cirrhosis was not observed. The most prevalent histological features were phlebosclerosis (n=16), nodular regeneration (n=15), dilated sinusoids (n=15), paraportal shunting vessels (n=13), increased vascular channels in portal tract (n=13), perisinusoidal fibrosis (n=16) and perivenular fibrosis (n=11). Only a minority of the specimens demonstrated increased parenchymal drainage vessels (n=6) and portal vein dilatation (n=4). Thin incomplete septa and liver steatosis were not observed.

Association between antiretroviral treatment and INCPH

Cumulative exposure to antiretroviral drugs and its combinations in INCPH and control patients, as well as odds ratio for INCPH diagnosis by years of exposure to antiretroviral drugs were analysed (table 3). Tested in several multivariate models; cumulative exposure to zalcitabine, efavirenz, PI, saquinavir, ritonavir, lopinavir and fosamprenavir

Table 3 Exposure to antiretroviral treatment (months) in 16 idiopathic noncirrhotic portal hypertension and 64 control patients.

Duration of antiretroviral treatment	INCPH cases (n = 16)		Control patients (n = 64)		Odds ratio (95% CI)	P
	Mean	Median (IQR)	Mean	Median (IQR)		
NRTI	119	120 (91-155)	114	126 (84-156)	1.2 (1.0-1.5)	0.03
D drugs	114	120 (72-153)	19	0 (0-24)	2.1 (1.4-3.2)	<0.001
Didanosine	81	76 (45-120)	17	0 (0-15)	2.0 (1.4-2.8)	<0.001
Stavudine	28	36 (0-51)	8	0 (0-14)	2.5 (1.5-4.1)	<0.001
Zalcitabine	5	0 (0-3)	1	0 (0-0)	5.8 (1.0-32.8)	0.02
Non-D drugs	130	132 (74-155)	197	216 (130-264)	0.9 (0.9-1.0)	0.19
Lamivudine	50	48 (21-87)	91	102 (60-120)	0.8 (0.7-1.0)	0.07
Zidovudine	39	28 (17-48)	45	24 (0-93)	1.0 (0.9-1.2)	0.92
Abacavir	5	0 (0-2)	17	0 (0-24)	0.8 (0.5-1.2)	0.18
Tenofovir	29	27 (0-53)	35	24 (0-72)	1.0 (0.8-1.2)	0.97
Emtricitabine	7	0 (0-3)	8	0 (0-12)	0.9 (0.5-1.5)	0.65
NRTI combinations	35	33 (0-69)	2	0 (0-0)	2.6 (1.4-4.7)	<0.001
Didanosine + Stavudine	23	33 (0-42)	1	0 (0-0)	3.1 (1.6-6.2)	<0.001
Didanosine + Tenofovir	12	0 (0-37)	1	0 (0-0)	3.2 (1.2-8.6)	0.002

Table 3 Exposure to antiretroviral treatment (months) in 16 idiopathic noncirrhotic portal hypertension and 64 control patients. (continued)

Duration of antiretroviral treatment	INCPH cases (n = 16)		Control patients (n = 64)		Odds ratio (95% CI)	P
	Mean	Median (IQR)	Mean	Median (IQR)		
NNRTI	58	56 (14-92)	74	84 (24-120)	1.0 (0.8-1.1)	0.71
Nevirapine	38	30 (2-71)	59	45 (0-120)	1.0 (0.8-1.1)	0.59
Efavirenz	34	0 (0-60)	12	0 (0-12)	1.1 (0.9-1.3)	0.29
Etravirine	0	0 (0-0)	0	0 (0-0)		
II						
Raltegravir	1	0 (0-0)	2	0 (0-0)	0.6 (0.1-4.5)	0.62
PI	75	62 (36-120)	35	12 (0-60)	1.3 (1.1-1.6)	0.001
Saquinavir	16	12 (0-36)	6	0 (0-0)	1.5 (1.0-2.1)	0.02
Indinavir	14	6 (0-22)	9	0 (0-3)	1.4 (1.0-2.0)	0.10
Ritonavir	71	82 (28-117)	24	5 (0-38)	1.4 (1.1-1.6)	<0.001
Nelfinavir	4	0 (0-0)	2	0 (0-0)	1.4 (0.8-2.3)	0.31
Lopinavir	45	45 (0-99)	8	0 (0-0)	1.5 (1.2-1.9)	<0.001
Atazanavir	15	0 (0-27)	8	0 (0-0)	1.2 (0.9-1.6)	0.21
Fosamprenavir	14	0 (0-0)	1	0 (0-0)	1.9 (0.3.-13.7)	0.09
Tipranavir	0	0 (0-0)	0	0 (0-0)		
Darunavir	0	0 (0-0)	2	0 (0-0)	0.3 (0.1-4.6)	0.20

Data are presented as mean, median and IQR (between brackets) time of exposure to a given antiretroviral drug or combination in months.
Odds ratio (per year of exposure), 95% confidence intervals and p value from univariate conditional logistic regression.

did not achieve significance. In multivariate analysis adjusted for years of HIV follow-up, cumulative exposure to didanosine, stavudine, D drugs, concomitant didanosine and stavudine and concomitant didanosine and tenofovir remained significant (table 4). Similar results were obtained after one by one adjustment for serum levels of ALT, CD4 and for platelet count.

All INCPH cases had been exposed to didanosine and 11 to stavudine. Eight cases had been treated with a combination of didanosine and stavudine. The median duration of single use for didanosine and stavudine treatment until INCPH diagnosis was 70.2 months (range 15 to 180 months) and 36 months (range 26 to 60 months), respectively. The median duration of combination therapy with didanosine and stavudine was 15 months (range 0 to 36 months).

Of the 18,085 Dutch HIV-infected patients, 7132 have been exposed to didanosine signifying that 2‰ of the HIV-infected patients exposed to didanosine are currently diagnosed with INCPH.

The estimated probabilities of the development of overt INCPH given the duration of exposure to didanosine and concomitant use of both drugs are demonstrated in

Table 4 Association between antiretroviral treatment and idiopathic noncirrhotic portal hypertension in multivariate analysis.

Antiretroviral treatment	Odds ratio (95% CI)	p
Model #1		
Didanosine	2.5 (1.3-4.8)	<0.001
Stavudine	3.4 (1.3-9.1)	0.003
Zalcitabine	70 (0.9-5175)	0.09
Model #2		
NRTI	0.5 (0.2-1.4)	0.18
D drugs	3.3 (1.3-7.9)	<0.001
Non-D drugs	1.2 (0.7-1.9)	0.55
Model #3		
Didanosine (mono)	2.0 (1.3-2.0)	<0.001
Stavudine (mono)	3.0 (0.9-9.7)	0.10
Didanosine + Stavudine	6.3 (2.1-19.1)	<0.001
Model #4		
Didanosine (mono)	2.0 (1.3-2.9)	<0.001
Tenofovir (mono)	1.3 (0.9-1.7)	0.22
Didanosine + Tenofovir	5.1 (1.2-22.6)	0.01

All variables are continuous
In model 3 and 4, didanosine (mono), stavudine (mono) and tenofovir (mono) are defined as the time of exposure (years) outside the setting of the combination of these drugs.
p-value and adjusted odds ratio per year of exposure.

figure 1. The estimated probability of INCPH increases substantially after 11 years of treatment with didanosine and after 4 years of combination treatment with didanosine and stavudine.

Clinical outcome of HIV-infected INCPH cases

During a median follow-up of 72 months (range 22 to 141 months), 4 out of 16 INCPH patients died after a median of 34 months (range 22 to 52 months). Causes of death were non-Hodgkin lymphoma (n=2), rectal carcinoma (n=1) and liver failure (n=1). The Kaplan-Meier estimates for overall survival were 100% (95% CI 80-100%) at 1 year and 75% (95% CI 50-90%). None of the patients developed clinical or radiological signs of PSC or HIV/AIDS cholangiopathy during follow-up. One patient developed transient liver failure in the context of variceal bleeding. Variceal bleeding occurred in 5 patients during follow-up, but could be managed successfully by primary and secondary prophylaxis in all of them. Autopsy was performed in the patient that died of liver failure. Pathological examination of this liver specimen did not demonstrate severe fibrosis.

During follow-up, 4 out of 16 INCPH patients (25%) developed portal vein thrombosis after a median of 30 months (range 24 to 56 months).

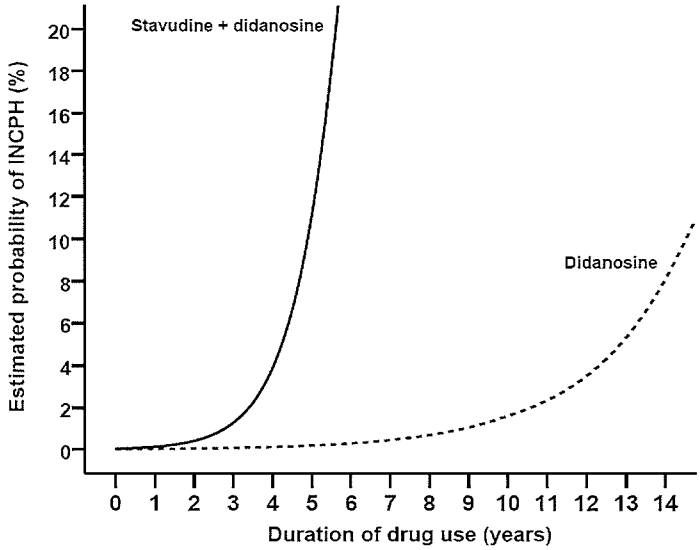

Figure 1 Estimated probability of idiopathic noncirrhotic portal hypertension according to the duration (in years) of didanosine and concomitant stavudine and didanosine use.
For the calculation of the estimated probability of idiopathic noncirrhotic portal hypertension, the prevalence was assumed to be 1‰ and the estimated OR obtained from the univariate analysis of didanosine, stavudine and concomitant treatment were used.
The estimated probability of INCPH increases substantially after 11 years of treatment with didanosine and after 4 years of combination treatment with didanosine and stavudine.

At the moment of INCPH diagnosis, didanosine and stavudine treatment was discontinued in 6 out of 9 cases that still were treated with these drugs. In 3 out of 9 cases the drugs were discontinued within the time course of 3 years. During follow-up, clinical signs of portal hypertension (splenomegaly and esophageal varices) sustained. The evolution of platelet count during follow-up is depicted in figure 2. A statistical significant mean decline was observed (p=0.003; slope: -4.2/year (CI:-6.7-(-1.8)). During follow-up, transient elastography was performed in 12 out of 16 cases. Median liver stiffness in these patients was 7.8 kPa (range 5 to 11 kPa).

DISCUSSION

This study highlights several important features of HIV-associated INCPH. We demonstrated that HIV-associated INCPH is a rarely diagnosed disorder in the general HIV population. Furthermore, we showed that prolonged didanosine exposure and short-term combination treatment with didanosine plus either stavudine or tenofovir are important risk factors for the development of this disorder and that portal hypertension sustains despite D drugs discontinuation.

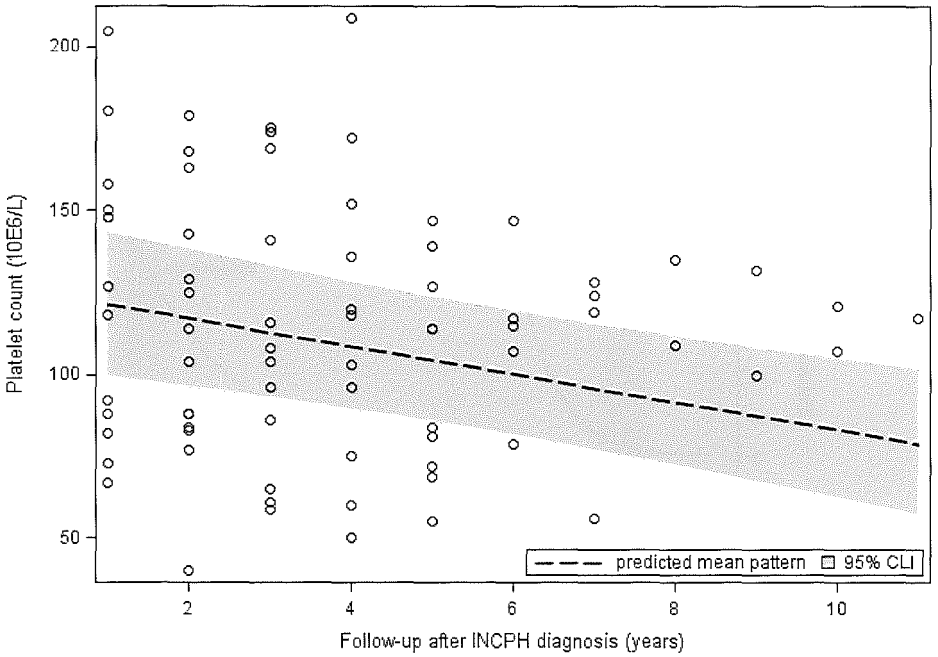

Figure 2 Evolution of the platelet count after idiopathic noncirrhotic portal hypertension diagnosis and didanosine discontinuation.
A repeated measurement analysis, with random intercept and slope, was applied to study the behaviour of the platelet count after diagnosis until end of follow-up.
During follow-up, a statistical significant mean decline in platelet count was observed (p=0.003; slope: -4.2/year (CI:-6.7-(-1.8)).

By performing a nationwide survey in all designated HIV treatment centers, we assessed the nationwide number of diagnosed HIV-associated INCPH patients. Since currently, 0.1% of the general HIV population in the Netherlands is diagnosed with this disorder, it can be regarded a rarely diagnosed disorder. However, these data can be an underestimation of its actual prevalence since patients without clinical complications of portal hypertension might remain undiagnosed.

For case selection in this case-control study we applied the recently proposed, strict INCPH definition (1). All INCPH patients included in this study had clinical signs of portal hypertension and exclusion of severe fibrosis and cirrhosis was not only based on pathological examination of biopsy specimens, but also on the exclusion of all its potential causes (chronic viral hepatitis B and C, chronic vitamin A use, presence of the metabolic syndrome and alcohol abuse, ...) in order to avoid biopsy misclassification. By performing a nationwide systematic survey in secondary and tertiary hospitals we tried to avoid overrepresentation of the most severe cases. Regarding control patients,

selection bias was avoided by selecting them out of HIV-infected patients without radiological signs of portal hypertension. Control patients were group matched for duration of follow-up after HIV-infection. INCPH and control patients were similar in age at HIV and INCPH diagnosis, gender, race, risk factor for transmission, BMI and serum creatinine level. Prolonged didanosine monotherapy and short-term combination treatment with didanosine and stavudine or tenofovir were identified as strong and independent risk factors for INCPH. Despite the frequently reported association between didanosine and INCPH, its etiological role remained a matter of debate as the drug was widely used in HIV treatment in the past and reports on INCPH were scarce. As all INCPH patients in our cohort had been exposed to didanosine and its cumulative exposure was associated with high odds ratios, our study results support this association (7, 12-14). Among the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), the so called "D drugs" (didanosine, stavudine and zalcitabine) are the strongest inhibitors of mitochondrial DNA polymerase in comparison to the other NRTIs (zidovudine, lamivudine and abacavir) (20-21) and subsequently are notorious for their mitochondrial toxicity. This drug-induced depletion of mitochondrial DNA is the presumed underlying mechanism of lactic acidosis associated with steatosis, steatohepatitis and liver failure in HIV infected individuals (22-23). Based on our study results, we hypothesize a link between mitochondrial hepatotoxicity and INCPH. First, this disorder is significantly associated with long-term exposure to antiretroviral drugs with the most potent ability to inhibit mitochondrial DNA polymerase-γ. INCPH was not associated with the NRTIs with lower ability to inhibit mitochondrial DNA polymerase or other antiretroviral drugs. Second, the observed synergistic risk profile of concomitant exposure to didanosine and stavudine has been reported for other mitochondrial toxicity related adverse effects in vitro and in vivo (21). Walker et al demonstrated lower liver mitochondrial DNA content in HIV-infected patients receiving concomitant didanosine and stavudine treatment in comparison to patients receiving only one drug (22). Furthermore, an increased risk of neuropathy and pancreatitis has been demonstrated in patients with concomitant D drug treatment (24-25). Third, we demonstrated an association between INCPH and concomitant treatment with tenofovir and didanosine. This observation can be explained by an increase of serum concentration and area under the curve of didanosine due to drug-drug interaction potentially increasing mitochondrial toxicity (26-27). On the other hand, some of our observations contradict this link. First, previous reports on mitochondrial liver toxicity, describe liver steatosis with liver failure, where patients with INCPH exhibit normal liver function and histopathological examination did not demonstrate the presence of steatosis (23, 28). Furthermore, portal hypertension does not decrease after D drug cessation, whereas mitochondrial toxicity improves after drug withdrawal. As a result, more research is warranted to verify this hypothesized association between mitochondrial toxicity and HIV-associated INCPH.

As most patients with HIV-associated INCPH present with variceal bleeding (in our study, 9 out of 16 patients), early diagnosis of INCPH remains a challenge (12). Notwithstanding the fact that prolonged didanosine treatment and combination therapy with didanosine and stavudine is currently avoided, many HIV-infected patients have been exposed to these drugs in the past. Our study results encourage screening these patients by means of liver tests and blood platelets in order to prevent bleeding associated morbidity. In patients with liver test disturbances and thrombocytopenia efforts should be undertaken to demonstrate portal hypertension by Doppler ultrasound and endoscopy.

In comparison to INCPH patients outside the setting of HIV infection, outcome in HIV-associated INCPH is mainly to HIV-associated disorders (29). In the present study, one INCPH patient died because of a liver-related disorder. No patient died because of intractable variceal bleeding. Because of normal synthetic function, survival in patients with noncirrhotic portal hypertension has been considered to be better than survival in patients with cirrhosis (1). Indeed, in this small cohort 5-year survival for HIV-associated INCPH was 75% whereas decompensation of liver disease in patients with HCV/HIV coinfection has been demonstrated to decrease survival significantly (2-year survival of 50%) (30-31). As a result, it can be speculated that patients with HIV-associated INCPH have a better prognosis than HIV-infected patients with cirrhotic portal hypertension. However, since the presence of chronic viral hepatitis is an exclusion criterion for the diagnosis of INCPH, these differences in survival cannot be compared without bias. Considering the rarity of the disorder, large international multicenter trials are required to achieve sufficient power to demonstrate significant differences in survival between cirrhotic and noncirrhotic HIV-infected patients with portal hypertension.

In contrast to the usually observed reversibility of NRTIs related adverse effects, our study results demonstrate that INCPH is not reversible after discontinuation of these drugs (24). Thrombocytopenia and clinical signs of portal hypertension sustained during follow-up (despite immediate interruption at INCPH diagnosis of didanosine and stavudine). Taking into account the histological features observed in liver specimens retrieved at INCPH diagnosis of these patients, this clinical observation is reasonable. Severe phlebosclerosis and nodular regeneration was observed in all patients. This obliteration of small portal veins and subsequent regeneration of the liver parenchyma is unlikely to reverse. It can be suggested that misclassification of patients with liver cirrhosis as INCPH patients explains the observation that thrombocytopenia worsens during follow-up. However, in order to avoid sampling error we excluded all patients with insufficient number of portal tracts or presence of known liver disease. Furthermore, biopsies were reviewed by 2 experienced liver pathologists blinded for the clinical context. Finally, transient elastography during follow-up demonstrated values lower than those observed in patients with liver cirrhosis (32).

Despite the strict definition of INCPH cases, the participation of all Dutch designated HIV treatment centers, the identification of controls selected out of a database of HIV-infected patients without radiological signs of portal hypertension and the complete follow-up data, several limitations of the study must be addressed. First, as didanosine has been linked with INCPH since the first description of this disorder in HIV-infected patients, clinicians might have pursued this diagnosis more readily in patients receiving this drug. Therefore, our case control study might be subject to detection bias. Second, the fact that the cases were identified in the Netherlands and the controls only in centre might induce bias to the study. However, Based on the Dutch HIV monitoring foundation report of 2011, this control group is representative for the Dutch population of HIV patients; the majority being male, from European descent, infected on average > 8 years and comparable regarding HAART (30% exposure to didanosine, 40% exposure to stavudine and 10% exposure to combination therapy of didanosine and stavudine) (16). Third, false positive associations could result from the limited number of patients compared with the number of analyzed variables and therefore caution must be taken when interpreting associations. However, this shortcoming is typical for studies of rare disorders such as INCPH and since the observed associations are very strong and in line with previous studies we believe that the link between exposure to these drugs and INCPH is very likely to be real. On the other hand, the limited number of cases might lead to a difficulty to detect other differences.

We conclude that INCPH is a rarely diagnosed disorder in the general HIV population. Prolonged didanosine exposure or short-term combination exposure with didanosine plus either stavudine or tenofovir are independent risk factors for the development of this disorder. Mortality in HIV-associated INCPH appears to be related to HIV associated disorders, but liver failure also occurs. Finally, portal hypertension sustains after D drugs discontinuation.

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CHAPTER 7

IDIOPATHIC NONCIRRHOTIC PORTAL HYPERTENSION IS NOT A RARE CAUSE OF PORTAL HYPERTENSION IN HIV-INFECTED PATIENTS

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Submitted

ABSTRACT

Background

Idiopathic noncirrhotic portal hypertension (INCPH) has been reported increasingly in HIV patients. The aim of our study was to assess the prevalence of portal hypertension in HIV-infected persons, role of didanosine in INCPH and risk factors for INCPH in HIV-positive patients exposed to didanosine.

Methods

All adult HIV patients seen on our outpatient clinic between February and October 2011 underwent sonographical spleen size determination and assessment of portosystemic collaterals. Patients with splenomegaly underwent an extensive ultrasound examination. A case-control study was performed in order to identify differences between HIV-infected INCPH patients (identified from a national registry) and HIV patients treated with didanosine without portal hypertension (identified by screening).

Results

Portal hypertension could be diagnosed in 19 out of 1019 screened HIV-infected patients of which 15 had liver cirrhosis. Four out of 1019 screened HIV-infected patients (95%CI 0.2-1.0%) were diagnosed with INCPH. All INCPH patients were treated with didanosine. INCPH was not found in any of the 910 HIV-infected patients that had never been exposed to didanosine (95%CI 0-0.4%). In comparison to didanosine treated patients without portal hypertension (n=96), INCPH patients (n=18) were treated longer with either didanosine (86 vs. 51months, $p<0.01$) or didanosine and stavudine combination treatment (21 vs. 4months, $p<0.001$).

Conclusion

INCPH is a frequent cause of portal hypertension in HIV-infected persons in low prevalence areas of viral hepatitis. Didanosine has an essential role in the development of HIV-associated INCPH. Risk factors for INCPH are long-term treatment with didanosine and short-term combination treatment with didanosine and stavudine.

INTRODUCTION

In the Western world, the introduction of highly active antiretroviral therapy (HAART) has significantly reduced acquired immunodeficiency syndrome- (AIDS-) related mortality and subsequently changed the clinical profile of HIV-infection from a subacute lethal disease to a chronic ambulatory disorder (1-2). Since the mortality of HIV-infected patients on HAART due to opportunistic disease has decreased, liver disease has emerged as one of the main causes of morbidity and mortality in these patients (3-4). Main causes of chronic liver disease are chronic hepatitis B and C infection, alcohol abuse, nonalcoholic fatty liver disease and HAART related toxicity (5). As a result of the higher prevalence of these risk factors for liver disease in patients with human immunodeficiency virus (HIV) infection, liver cirrhosis is present in 2-8% of these patients of which 60% have clinical signs of portal hypertension (6). Subsequently, liver cirrhosis is regarded the most frequent cause of portal hypertension in HIV-infected patients in the Western world (7). However, over the last decade portal hypertension in the absence of cirrhosis, chronic liver diseases, extrahepatic portal vein thrombosis and Budd-Chiari syndrome (referred to as idiopathic noncirrhotic portal hypertension) (INCPH) has been reported increasingly in HIV-infected persons (8-14). Up to now, the clinical impact of this disorder in HIV-infected patients remains unclear. The prevalence of this disorder is a subject of debate. Based on national registry data it can be regarded a rare disorder (15). However, these reports can underestimate the real burden of this disorder because of physician unawareness and underdiagnosis of the disorder in patients without complications of portal hypertension. As a result, according to expert opinions, the reported HIV-associated INCPH cases are regarded the tip of the iceberg (16). Large scale, systematic screening for portal hypertension in HIV patients in order to identify the real clinical impact of the disorder has not been performed so far. Furthermore, INCPH has only been reported in HIV-infected patients exposed to didanosine treatment. Nevertheless, this unique role of didanosine in the pathophysiology of INCPH remains controversial as the drug was widely used in HIV treatment in the past and reports on INCPH remain scarce. In addition, the observation that INCPH has not been reported outside the setting of this drug could be a result of detection bias. Finally, the reason why only a minority of patients exposed to didanosine develops INCPH remains to be elucidated.

The aim of this study was threefold. First, to determine the prevalence and etiology of clinically significant portal hypertension (cirrhotic and noncirrhotic) by systematically screening a large cohort of HIV-infected patients. Second, to assess the association between HIV-associated INCPH and didanosine. Third, to identify risk factors for INCPH in didanosine treated HIV patients.

PATIENTS AND METHODS

Prevalence and etiology of portal hypertension

In order to determine the prevalence and etiology of clinically significant portal hypertension (cirrhotic and noncirrhotic) we screened all adult HIV-infected patients seen at the outpatient clinic between February 2011 and September 2011 for signs of portal hypertension in 2 phases. In the first phase, all HIV patients were invited to undergo sonographical spleen size determination and visualization of the splenic hilum, the falciform ligament and the epigastric region for the determination of portosystemic collaterals. This examination was performed by 2 clinical investigators trained in abdominal ultrasound (TK and MR). In the second phase, HIV-infected patients with splenomegaly (>12 cm) and/or presence of portosystemic collaterals (splenic collaterals, repermeabilization of the paraumbilical vein and epigastric collaterals) were invited to undergo transient elastography and extensive abdominal ultrasound examination (on Hitachi Hi Vision Preirus) performed by 3 experienced gastroenterologists (JS, RDK and PT) with at least 8 years of ultrasound experience according to the generally accepted ultrasound guidelines (17).

The presence of portosystemic collaterals or hepatofugal flow in the portal vein was considered a diagnostic sign of portal hypertension. The following sonographic features were considered suggestive signs of portal hypertension: portal vein diameter > 12.5 mm, portal vein flow velocity < 15 cm/s, congestion index > 0.1 and splenic vein dilatation > 1 cm (18-19). When one of the suggestive sonographic signs for portal hypertension, portosystemic collaterals or an unexplained spleen size enlargement over 15 cm were present, an upper endoscopy was performed for endoscopic signs of portal hypertension. Patients with liver stiffness above 9.5 kPa on transient elastography were considered to have significant fibrosis and underwent liver biopsy (20). In order to determine the etiology of portal hypertension; patient files were reviewed for the presence of risk factors for chronic liver disorders and if needed interviews with patients were repeated. The presence or history of alcohol abuse was assessed by repeated interview of the patient and his or her relatives at the outpatient clinic and during admission in the hospital. Patients fulfilling the criteria of the metabolic syndrome were considered as NAFLD (21). Chronic viral hepatitis, autoimmune hepatitis, cholestatic liver disease and hereditary hemochromatosis were diagnosed according to the recommended laboratory methods. The diagnosis of INCPH was considered when all of the following criteria were fulfilled: (1) clinical signs of portal hypertension; (2) radiological examination demonstrating patent portal and hepatic veins at initial diagnosis; (3) liver biopsy showing no cirrhosis; (4) absence of noncirrhotic liver disease known to cause portal hypertension; (5) absence of exposure to copper sulphate, vinyl chloride monomer or Spanish toxic oil, and absence of past angiography with thorium sulphate (22).

In order to evaluate the association between HIV-associated INCPH and didanosine exposure we compared the presence of INCPH between HIV-infected patients with and without didanosine exposure.

Risk factors for INCPH in patients exposed to didanosine

In order to assess the risk factors for the development of INCPH in patients treated with didanosine we performed a case-control study. Cases were identified by screening the large cohort of HIV-infected patients and from the Dutch HIV monitoring foundation (23). Control population comprised all HIV-infected patients exposed to didanosine but without sonographical signs of portal hypertension.

Data on clinical characteristics, medical history and laboratory parameters were collected through an extensive and systematic review of the medical charts in cases and controls. These data were assessed at HIV diagnosis, at the start of didanosine and at INCPH diagnosis or at the moment of the spleen size determination for the patients treated with didanosine without signs of portal hypertension. Data on medication intake of the cases and controls were derived from the Dutch HIV monitoring foundation report of 2011 (23).

Statistical analysis

Each covariate was described as number and percentage for discrete variables and median and interquartile range (IQR) for continuous variables. The Fisher's exact test was used to compare proportions. The Mann-Withney U test was used to compare continuous variables. The exact Wilcoxon test was used to compare non-parametric data. Exact 95% confidence intervals (CI) were reported. The association between patient characteristics at HIV diagnosis and at the start of didanosine treatment were studied in logistic regression analyses. Cumulative exposure to antiretroviral drugs and its combinations in INCPH patients and control patients, as well as odds ratio for INCPH diagnosis by months of exposure to antiretroviral drugs were analysed at the moment of INCPH diagnosis for cases and at the moment of ultrasound examination for controls. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed with the Statistical Package for Social Sciences for Windows, version 17.0 (SPSS, Chicago, IL) and SAS.

RESULTS

Prevalence and etiology of portal hypertension

Sonographical screening for the presence of splenomegaly and portosystemic collaterals was performed in 1019 out of 1061 adult HIV-infected patients seen at the outpatient clinic between February 2011 and September 2011. Patient characteristics are listed in table 1 and the screening flowchart is depicted in figure 1.

Table 1. Baseline characteristics of the 1019 HIV-infected patients screened for splenomegaly and portosystemic collaterals.

Variable at the moment of ultrasound examination	HIV-infected patients (n=1019)
Age at time of visit, years	45 (19-84)
Time after HIV diagnosis, years	7.5 (0-30)
Male gender*	742 (73)
Mean spleen size, cm	10.6 (6-23)
Exposure to any HAART treatment*	822 (81)
Exposure to didanosine treatment	100 (10)
Exposure to stavudine treatment	129 (16)
Exposure to combination treatment with didanosine and stavudine	19 (2)
Risk factor for HIV transmission*	
Unknown exposure	48 (5)
Sex between males-females	369 (36)
Sex between males-males	423 (42)
Bisexual	43 (4.3)
Injection drug use	30 (3)
Blood transfusion	19 (2)
Perinatal transmission	0 (0)
Ethnic group*	
European	618 (61)
American	168 (16)
Asian	38 (4)
Australian	1 (0)
African	194 (19)
Co-infections*	86 (9)
Acute hepatitis B infection	1 (0)
Chronic hepatitis B infection	38 (4)
Acute hepatitis C infection	3 (0)
Chronic hepatitis C infection	47 (5)
Chronic hepatitis B and C infection	1 (0)

*Variables are represented as number of subjects and percentages (between brackets)
Age, spleen size and time after HIV diagnosis are expressed as median and range (between brackets)

Clinical significant portal hypertension could be diagnosed in 19 out of 1019 screened HIV-infected patients (prevalence 1.9% (95% CI 1.2-2.9%). In 15 out of these 19 patients, liver cirrhosis could be diagnosed (chronic hepatitis C virus (n=8), chronic hepatitis B virus (n=2), coinfection with chronic hepatitis C and B virus (n=1), alcohol abuse (n=2), non-alcoholic fatty liver disease related liver cirrhosis (n=2)). In 4 out of the 19 patients with sonographic signs of portal hypertension, INCPH could be diagnosed (overall

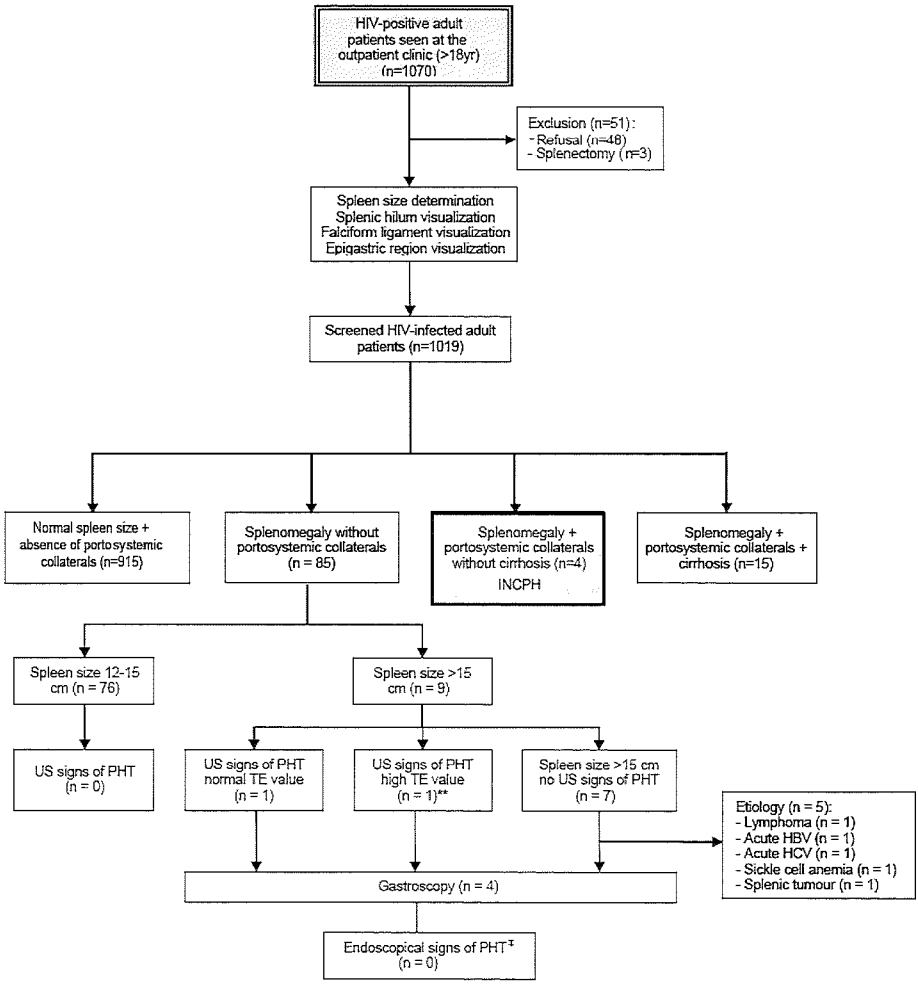

Figure 1 Study algorithm. Screening for splenomegaly and portosystemic collaterals was performed in 1019 HIV-infected patients. In 915 out of the 1019 patients, no splenomegaly or portovenous collaterals could be demonstrated. In 85 out of 1019 patients, splenomegaly (without portovenous collaterals) was present. These patients underwent an additional extensive abdominal ultrasound examination. In 76 out of the 85 patients with splenomegaly, spleen size was between 12 and 15 cm. In none of these patients suggestive sonographic signs of portal hypertension, liver cirrhosis or high transient elastography values were observed. In 9 of these 85 patients with splenomegaly, spleen enlargement was > 15 cm. In 7 of these 9 patients, a cause for this spleen enlargement could be identified. In 2 of these 9 patients, despite lack of suggestive sonographic signs for portal hypertension, gastroscopy was performed. No endoscopical signs of portal hypertension were observed. Clinical significant portal hypertension could be diagnosed in 19 out of 1019 screened HIV-infected patients. In 15 out of these 19 patients, liver cirrhosis could be diagnosed (chronic hepatitis C virus (n=8), chronic hepatitis B virus (n=2), coinfection with chronic hepatitis C and B virus (n=1), alcohol abuse (n=2), non-alcoholic fatty liver disease related liver cirrhosis (n=2)). In 4 out of the 19 patients with sonographic signs of portal hypertension, INCPH could be diagnosed.

* Identified HIV-positive INCPH patients

** Histologically diagnosed HIV-infected patient with liver cirrhosis (without sonographic or endoscopic signs of portal hypertension).

Abbreviations: PHT, portal hypertension; TE, transient elastography; US, sonographic.

INCPH prevalence 0.4% (95% CI 0.2-1.0%)). All INCPH patients had been exposed to didanosine. One of these 4 patients was still being treated with this drug at the moment of INCPH diagnosis. In 3 of these 4 patients didanosine treatment was stopped before INCPH diagnosis. Since 100 out of 1019 HIV-infected patients were exposed to didanosine, the prevalence of INCPH in HIV-infected patients exposed to didanosine was 4% (95% CI 1.6-9.8%). None of the other 919 screened HIV-infected patients (never exposed to didanosine) had INCPH (95% CI 0-0.4%).

Risk factors for INCPH in patients exposed to didanosine

In order to assess the risk factors for the development of INCPH in patients treated with didanosine we performed a case-control study. In total 18 HIV-infected patients could be identified as HIV-associated INCPH cases. Four HIV-associated INCPH patients were found by screening the large cohort of HIV-infected patients and another 14 HIV-associated INCPH patients were identified in the Dutch HIV monitoring foundation (23). Cases from both groups had similar median duration of didanosine exposure (76 months (IQR 44-129) and 78 months (IQR 45-120) respectively (p=0.96), age (p=0.65) and gender (p=0.85). The 96 screening patients exposed to didanosine but without sonographical signs of portal hypertension served as controls.

HIV-infected patients diagnosed with INCPH and patients exposed to didanosine without signs of portal hypertension were similar regarding gender, risk for HIV transmission and duration of HIV infection (table 2). Patients with INCPH were more frequently of European descent in comparison to patients exposed to didanosine without signs of portal hypertension (p=0.03). INCPH was not associated with age, BMI, CD4 count, platelets, alanine aminotransferase (ALT), alkaline phosphatase (AP), creatinine and bilirubin at HIV diagnosis (table 2). INCPH was not associated with ALT, creatinine and CD4 count at the start of didanosine treatment (table 2).

At the moment of INCPH diagnosis, patients with INCPH had a lower platelet count and CD4 count in comparison to patients treated with didanosine without signs of portal hypertension (table 2). Patients with INCPH had at that time higher serum levels of ALAT and AP in comparison to patients exposed to didanosine without signs of portal hypertension. INCPH was not associated with BMI, dosage didanosine treatment, HIV RNA, creatinine and bilirubin at INCPH diagnosis (table 2).

In univariate analyses only cumulative exposure to didanosine, D drugs (didanosine, stavudine and/or zalcitabine) and combination therapy of didanosine and stavudine were significantly associated with INCPH (OR 1.23 (1.1-1.4); OR 2.08 (1.4-2.8) and OR 1.37 (1.1-2.7) respectively) (table 3). At the moment of INCPH diagnosis, HIV-infected

Table 2. Characteristics of 18 patients with HIV-associated idiopathic noncirrhotic portal hypertension (INCPH) and 96 HIV-infected patients treated with didanosine without sonographic signs of portal hypertension (didanosine group): at HIV diagnosis, at the start of didanosine treatment and at INCPH diagnosis or moment of ultrasound examination.

Patients characteristics	**INCPH patients (n=18)**	**Didanosine group (n=96)**	**p**	
Male gender	13 (72)	68 (71)	0.81	
Caucasian race	15 (83)	52 (54)	0.03	
MSM / Heterosexual / Other*	9 (50) / 4 (22) / 5 (28)	32 (33) / 40 (42) / 24 (25)	0.14	
Duration of HIV infection, years	16.3 (15.2-19.1)	14.8 (10.7-18.4)	0.19	
Characteristics at HIV diagnosis	**INCPH patients (n=18)**	**Didanosine group (n=96)**	**p**	**p-adj****
Age, years	39.1 (30.9-45.1)	36.9 (29.0-46.2)	0.42	0.86
BMI, kg/m^2	23.5 (21.9-26.4)	22.9 (20.7-24.7)	0.93	0.77
CD4 count, cells/mm^3	160 (13-465)	180 (80-290)	0.85	0.08
Platelets, giga/L	238 (162-275)	220 (161-264)	0.26	0.54
ALT, IU/ml	71 (28-117)	21 (14-37)	0.06	0.11
Alkaline phosphatase, IU/L	68 (54-82)	65 (50-97)	0.53	0.95
Creatinine, µM	91 (80-110)	69 (74-90)	0.07	0.56
Bilirubine, µmol/L	10 (4-14)	8 (6-10)	0.31	0.33
Characteristics at the start of didanosine	**INCPH patients (n=18)**	**Didanosine group (n=96)**	**p**	**p-adj****
ALT, IU/ml	51 (10-74)	27 (19-31)	0.18	0.21
Creatinine, µM	54 (48-77)	67 (60-91)	0.36	0.23
CD4count, cells/mm^3	80 (60-415)	280 (150-675)	0.71	0.92
Characteristics at INCPH diagnosis	**INCPH patients (n=18)**	**Didanosine group*** (n=96)**	**p**	
Age, years	50.5 (41.2-58.3)	50.6 (46.3-61.7)	0.49	
BMI, kg/m^2	21.1 (19.3-24.1)	24.5 (21.6-27.3)	0.31	
Didanosine dosage, mg/day	400 (400-400)	400 (250-400)	0.17	
CD4 count, cells/mm3	205 (105-275)	480 (340-700)	<0.001	
HIV RNA <50, copies/ml	12 (67)	81 (84)	0.26	
Platelets, giga/L	167 (106-200)	199 (161-244)	<0.001	
ALT, IU/ml	52 (27-123)	28 (21-39)	<0.001	
Alkaline phosphatase, IU/L	113 (61-301)	89 (67-102)	<0.001	
Creatinine, µM	104 (83-163)	72 (66-83)	0.61	
Bilirubine, µmol/L	7 (5-19)	7 (5-11)	0.09	

Data are presented as number of patients and percentages between brackets for discrete variables and median (IQR) for continuous variables.

* Risk factors for HIV transmission

**Adjusted for duration of didanosine treatment and time to event

*** Data assessed at the moment of sonographical spleen size determination

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; BMI, body mass index; MSM, men who have sex with men; p-adj, p-adjusted.

Table 3. Exposure to antiretroviral treatment (months) in 18 patients with idiopathic noncirrhotic portal hypertension (INCPH) and 96 HIV-infected patients with didanosine exposure without sonographical sings of portal hypertension.

Duration of antiretroviral treatment	INCPH cases (n = 18)		Control patients (n = 96)		Odds ratio per year (95 % CI)	P
	Mean	Median (IQR)	Mean	Median (IQR)		
NRTI	132	128 (96-168)	139	144 (108-174)	1.2 (1.0-1.5)	0.1
D drugs	123	124 (72-156)	72	65 (26-114)	1.37 (1.1-2.7)	<0.001
Didanosine	88	84 (48-120)	51	30 (12-96)	1.23 (1.1-1.4)	0.002
Stavudine	30	36 (0-48)	18	3 (0-29)	1.13 (0.9-1.3)	0.3
Zalcitabine	5	0 (0-0)	3	0 (0-0)	1.56 (1.0-20.0)	0.2
Non-D drugs	154	132 (81-216)	187	181 (131-240)	0.9 (0.9-1.2)	0.4
Lamivudine	57	48 (21-96)	93	100 (60-120)	0.7 (0.6-1.0)	0.1
Zidovudine	40	30 (18-48)	30	16 (2-36)	1.24 (0.9-1.4)	0.1
Abacavir	10	0 (0-2)	16	0 (0-21)	0.9 (0.5-1.2)	0.3
Tenofovir	36	36 (0-66)	31	21 (0-56)	1.0 (0.8-1.2)	0.8
Emtricitabine	11	0 (0-12)	11	0 (0-23)	0.9 (0.5-1.5)	0.6
NRTI combinations	35	33 (0-63)	10	0 (0-12)	1.49 (1.2-4.0)	<0.001
Didanosine + Stavudine	25	30 (0-36)	4	0 (0-0)	2.08 (1.4-2.8)	<0.001
Didanosine + Tenofovir	16	0 (0-39)	6	0 (0-0)	1.23 (1.1-6.0)	0.1
NNRTI	60	60 (22-78)	72	81 (12-124)	1.0 (0.8-1.2)	0.7
Nevirapine	41	24 (0-66)	43	12 (0-71)	1.0 (0.9-1.1)	0.7
Efavirenz	35	0 (0-60)	26	0 (0-46)	1.0 (0.8-1.2)	0.7
Etravirine	0	0 (0-0)	1	0 (0-0)		
II						
Raltegravir	2	0 (0-0)	2	0 (0-0)	0.6 (0.1-4.5)	0.2
PI	83	68 (48-120)	66	60 (8-120)	1.1 (1.1-1.3)	0.1
Saquinavir	21	12 (0-36)	14	0 (0-10)	1.2 (0.9-1.6)	0.1
Indinavir	11	2 (0-22)	11	0 (0-11)	1.2 (1.0-1.8)	0.2
Ritonavir	75	76 (28-117)	52	33 (1-90)	1.1 (1.1-1.6)	0.1
Nelfinavir	3	0 (0-0)	6	0 (0-0)	0.9 (0.7-2.3)	0.7
Lopinavir	47	36 (0-96)	30	1 (0-41)	1.2 (0.9-1.9)	0.1
Atazanavir	14	0 (0-27)	8	0 (0-0)	1.2 (0.9-1.6)	0.2
Fosamprenavir	14	0 (0-0)	1	0 (0-0)	1.45 (0.8.-13.)	0.1
Tipranavir	0	0 (0-0)	0	0 (0-0)		
Darunavir	0	0 (0-0)	3	0 (0-0)	0.3 (0.1-4.6)	0.1

Data are presented as mean, median and IQR (between brackets) time of exposure to a given antiretroviral drug or combination in months.

Odds ratio (per year of exposure), 95% confidence intervals and p-value from univariate conditional logistic regression.

Abbreviations: NRTI, nucleoside analogue reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; II, integrase inhibitor; PI, protease inhibitor.

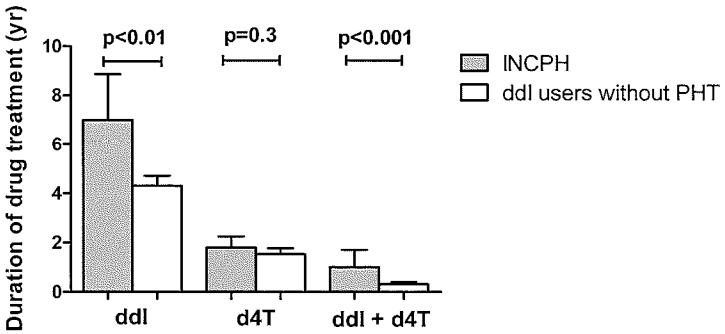

Figure 2 Mean differences in drug exposure to didanosine, stavudine and combination treatment with didanosine and stavudine (in years ± standard error of the mean) between HIV-positive INCPH patients (n=18) and HIV-positive patients exposed to didanosine without signs of portal hypertension (n=96).

patients diagnosed with INCPH were treated significantly longer with didanosine (86 vs. 51 months, p<0.01) and combination treatment with didanosine and stavudine (21 vs. 4 months, p<0.001) in comparison to control patients (figure 2). After adjustment for duration of didanosine treatment, similar results were obtained regarding the association between INCPH and patient characteristics at HIV diagnosis and at the start of didanosine (table 2). In multivariate analyses all three remained significantly associated with INCPH (p<0.01). Duration of stavudine treatment was similar in both groups (p=0.3).

DISCUSSION

This study highlights several important features of portal hypertension in HIV-infected patients. Firstly, we demonstrated that INCPH is a frequent cause of portal hypertension in Western HIV-infected patients living in areas with a low prevalence of chronic HCV and HBV infection. Secondly, our results underline the important role of didanosine in the pathophysiology of INCPH. Thirdly, risk factors for INCPH in HIV-infected patients exposed to didanosine are long-term treatment with this drug and short-term combination treatment with didanosine and stavudine.

Over the last decade, INCPH has been reported increasingly in HIV-infected patients (8-11, 14, 24). However, the clinical burden of this disorder remains a matter of debate. Based on data retrieved from national registries, the prevalence of HIV-associated INCPH is rare (15, 25). These data are subject to potential bias since INCPH can remain undiagnosed in the absence of complications of portal hypertension and be misclassified as cirrhosis because of similar radiological features (24). As a result, according to expert opinion, the prevalence of HIV-associated INCPH is higher than reported in these studies (8, 26). Up

to now, screening for portal hypertension in a large cohort of HIV-infected persons has not been performed. By screening a large cohort of HIV-infected patients (representative for the general Dutch and probably the Western HIV-population) we demonstrate that HIV-associated INCPH is a frequent cause of portal hypertension in a Western cohort of HIV-infected patients with a low prevalence of HCV and HBV infection. In this study, 21% of the HIV-infected patients with portal hypertension could be diagnosed with INCPH, whereas the presence of portal vein thrombosis and Budd-Chiari syndrome could not be demonstrated. The observation that INCPH has not been reported outside the setting of didanosine treatment is suggested to be a result of detection bias as clinicians might have pursued this diagnosis more readily in patients exposed to this drug. Our study results elucidate the controversy concerning the unique role of didanosine in the pathophysiology of INCPH since this disorder could only be diagnosed in patients exposed to didanosine. HIV-associated INCPH could not be diagnosed in any of the 915 HIV-infected patients that never had been exposed to didanosine. Therefore, it can be concluded that didanosine exposure is an essential factor in the development of INCPH.

The observation that only 4% of the HIV-infected patients exposed to didanosine develop INCPH indicates that this disorder is likely to be a multifactorial disease requiring additional elements. In order to identify these unknown covariates, we compared a cohort of well-defined INCPH patients to a group of HIV-infected patients exposed to didanosine but without clinical signs of portal hypertension. No patient characteristics at HIV diagnosis or the start of treatment with didanosine predictive for the development of INCPH could be identified. Since renal function, BMI and treatment dosage of didanosine at the start of didanosine were comparable between the 2 groups, a pathophysiological role for drug accumulation related to pharmacokinetic interactions is unlikely. Both groups were similar in follow-up after HIV diagnosis, suggesting that long-term HIV-infection has no crucial role in INCPH development. INCPH patients and patients treated with didanosine without signs of portal hypertension only differed in respect to HAART characteristics. Patients with INCPH were treated significantly longer with didanosine or combination treatment with didanosine and stavudine. These results validate the previously demonstrated association between INCPH and treatment with NRTIs with a high capacity of inhibiting host mitochondrial DNA polymerase, reinforcing the causal role of mitochondrial toxicity in the pathophysiology of this disorder (27). The previously suggested association between INCPH and long-term monotherapy with stavudine or nevirapine could not be confirmed (11, 25).

Currently, only a minority of HIV-infected patients receives didanosine treatment. At first sight, it can be suggested that our study results lack clinical importance. However, deriving from our study results, exposure to didanosine and not current treatment is a

risk factor for INCPH. A significant amount of HIV-infected persons currently seen on the outpatient clinic were treated with didanosine in the past (in The Netherlands 7.000 out of 18.000 HIV-infected patients) and are as such at risk for the development of INCPH. The actual prevalence of clinically diagnosed INCPH in the Dutch HIV-infected patients exposed to didanosine is 0.2% (25). By systematically screening a large cohort of HIV-infected persons we could identify a substantially larger INCPH prevalence of 4% in HIV-infected patients exposed to didanosine (23, 28). As a result, these study results suggest significant underdiagnosis of INCPH in HIV-infected persons and subsequently advocate for increased awareness of HIV-associated INCPH in HIV-infected persons treated with long-term exposure to didanosine and short-term combination therapy with stavudine and didanosine in order to avoid complications of portal hypertension such as variceal bleeding.

Since we screened a large group of HIV-infected patients for sonographical signs of portal hypertension, our study design does not allow us to assess the prevalence of INCPH without sonographical signs of portal hypertension or HIV-associated obliterative portal venopathy. The latter could only be evaluated by obtaining liver specimens. Taking into consideration the complications associated with liver biopsy, performing this procedure in a large asymptomatic cohort would be unethical. Another limitation of the study is the fact that in the case-control study, the cases were identified from 2 different cohorts whereas the controls were retrieved by screening a large cohort of HIV-infected patients. Nevertheless, both case groups were similar in age, gender and duration of exposure to didanosine. Finally, the overall prevalence of chronic hepatitis C infection in our cohort is significantly lower than that in Eastern Europe countries such as Italy. As a result our data regarding the prevalence of portal hypertension cannot be extrapolated to these countries.

We conclude that INCPH is a frequent cause of portal hypertension in HIV-infected patients in areas with a low prevalence of chronic hepatitis B/C infection and that didanosine is an essential factor in the development of HIV-associated INCPH. Risk factors for the development of this disorder are prolonged didanosine treatment or short-term combination treatment with didanosine and stavudine. Because of underdiagnosis of this disorder, screening for HIV-associated INCPH is warranted in HIV-infected patients with these risk factors.

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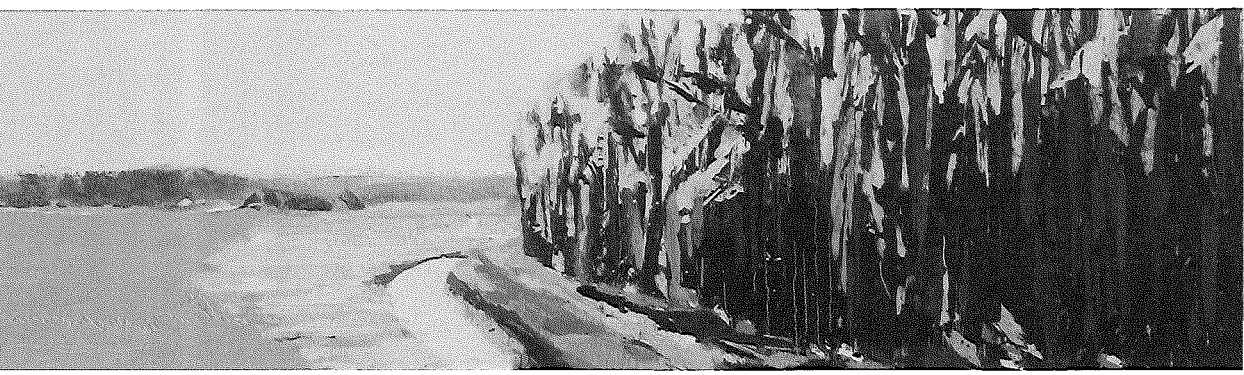

CHAPTER 8

SUMMARY AND FUTURE PERSPECTIVES

Portal hypertension is a clinical syndrome defined by a portal-caval venous pressure gradient exceeding 5 mm Hg (1). This increase of portal pressure eventually will lead to the development of a collateral circulation and splenomegaly. In the Western world, liver cirrhosis is the most frequent cause of portal hypertension (1). However, in a variety of disorders portal hypertension can develop in the absence of cirrhosis. This condition, referred to as noncirrhotic portal hypertension, is often classified based on the site of obstruction (prehepatic, intrahepatic and suprahepatic portal hypertension). Worldwide, the most common cause of noncirrhotic portal hypertension is schistosomiasis (2). In the Western world, chronic liver diseases such as non-alcoholic steatohepatitis, primary biliary cirrhosis, primary sclerosing cholangitis and congenital hepatic fibrosis together with extrahepatic portal vein thrombosis (PVT) and Budd-Chiari syndrome (BCS) are common causes of noncirrhotic portal hypertension (3-5). If all these conditions have been ruled out, the diagnosis of idiopathic noncirrhotic portal hypertension (INCPH) can be made. The international nomenclature about this condition has been ambiguous. In the Indian subcontinent this condition is known as noncirrhotic portal fibrosis, while in Japan and other Asian countries, it is referred to as idiopathic portal hypertension (6-8). In the Western world this condition has been variably termed hepatoportal sclerosis, idiopathic portal hypertension, incomplete septal cirrhosis and nodular regenerative hyperplasia (NRH) (9-12). Since all these entities share histopathological characteristics (obliterative vascular lesions) and clinical profile, INCPH can be viewed as a distinct single entity with various pathological aspects, rather than different clinicopathological entities. We therefore suggested that in future studies, the term INCPH will be used (regardless of patient origin) since it covers both the clinical and etiological aspects of the disorder. In contrast to the high prevalence of this disorder in India, INCPH is a rare disease in the Western world (13). As a result, little is known about its pathophysiology, clinical characteristics and outcome. Furthermore, extending Eastern study results to Western INCPH patients would be incorrect since different pathophysiological mechanisms have been postulated (6-7, 14). In the latter, thrombophilia leading to thrombosis of the small portal vein branches and medication exposure have been proposed as possible pathogenic mechanism whereas in Asian patients vascular damage due to enteric infections has been attributed an etiological role (14-15) **(CHAPTER II)**.

Histopathological data regarding Western patients with INCPH are scarce. Furthermore, it remains unclear which morphologic features, described in association with INCPH are distinctive for this disorder and which are related to changes in portal pressure. We established clear definitions of histological features reported in patients with INCPH. Afterwards, we evaluated these parameters systematically in liver biopsies of 70 INCPH patients and 23 biopsies of patients with recent (< 1 year) PVT as a control group for patients with noncirrhotic portal hypertension **(CHAPTER III)**. Hypoplastic portal tracts,

phlebosclerosis and nodular regeneration were more frequently observed in liver specimens of INCPH patients in comparison to specimens from patients with noncirrhotic PVT. As a result, they can be assumed to be INCPH specific. On the other hand, sinusoidal dilatation, paraportal shunting vessels and increased portal and parenchymal vessels may be interpreted as pressure-related morphologic signs of portal hypertension since they are equally present in patients with noncirrhotic portal hypertension and INCPH. Interestingly, despite similar occurrence of phlebosclerosis in patients with INCPH and HIV-associated INCPH, nodular regeneration was significantly more present in the latter. A possible explanation for these observed differences might be different pathophysiology between the two disorders. INCPH is a disorder with a heterogeneous spectrum of associated disorders (16), where the pathogenesis of HIV-INCIPH is considered to be more homogeneous with didanosine treatment being an essential element (**CHAPTER VI**). Since agreement on uniform nomenclature is an essential requirement for collaborative studies we established definitions of histological features reported in patients with INCPH. Despite the fact that these definitions appeared to be easily applicable and consensus between the scoring pathologists on the absence, presence and grade of the different morphological parameters was easily obtained during simultaneously scoring, the interobserver variability remains to be proven in additional studies. Except the presence of hypoplastic portal tracts, morphologic appearance of INCPH seems similar in Western patients and Eastern patients. Further study is warranted to explore the role of this histopathological element.

In order to assess the clinical manifestations, determinants of survival and outcome of patients with INCPH, we retrospectively studied all patients diagnosed between January 1992 and December 2010 with this disorder in 2 European tertiary referral centers (Erasmus MC University Hospital Rotterdam in The Netherlands and University Hospitals Leuven in Belgium) (**CHAPTER VI**). First, we confirmed some aspects of the disorder that have been reported previously in smaller cohort studies. We confirmed the observation that the majority of INCPH patients initially present with signs or complications of portal hypertension (mainly variceal bleeding and splenomegaly) in combination with normal liver function (14). However, as reported earlier, liver failure, hepatic encephalopathy and hepatopulmonary syndrome can occur and, if present, must be considered indications for liver transplantation (17). Our data also confirm that the differential diagnosis of INCPH and liver cirrhosis remains a challenge (14, 17). Before having histological specimens, 79% of our INCPH patients were radiologically and clinically misclassified as cirrhotics. Second, our study results also demonstrate important new insight in this complex disorder. The observation that INCPH patients at initial diagnosis were almost two decades older in comparison to the reported age in Indian patients supports the assumption that INCPH in the Western world can be regarded a distinct entity compared

to the disorder in Indian patients. Furthermore, we showed that both 10-year survival in Western INCPH patients is around 50%. Mortality is mainly related to INCPH associated disorders and medical conditions accompanying older age (neurological disorders, heart failure) and not to complications of portal hypertension or the development of liver failure. By comparing survival rates of the INCPH patients to survival rates in the general population of similar age and gender, we demonstrated a substantially higher mortality in the former. In uni- and multivariable analysis, ascites was an independent predictor of poor outcome. Finally, INCPH patients did not developed HCC during long-term follow-up. Large prospective multi-centre studies are needed to confirm these results.

In India, approximately 20% of patients presenting with portal hypertension have INCPH (8, 19). In contrast, in the Western world, it has been suggested that INCPH might be responsible for 3-5% of cases of portal hypertension (20). Regarding the prevalence in the general population, only one study is available. A histological review of 2500 autopsies demonstrated a 3% prevalence of INCPH histological features of which 5% had evidence of portal hypertension (10). However, since this study included liver specimens of autopsies, it is obviously biased to study the prevalence in the general population. Therefore, in order to assess to study the prevalence of INCPH in the general Western population we used the study results of the Rotterdam study (**CHAPTER V**). The Rotterdam Study is a large prospective population-based cohort study conducted among elderly inhabitants of Ommoord, a district of Rotterdam (21). Each participant completed an extensive interview and clinical examination that included, among others, anthropometric assessment and abdominal ultrasonography (22). Abdominal ultrasonography was performed in the first two cohorts of the Rotterdam Study (February 2009- February 2012). Various parameters were assessed according to the generally accepted sonographic guidelines (23). In 135 out of the 2.582 participants with a reliable spleen measurement was performed a spleen size of >12cm was present (5.2%). In 35 of these 135 participants an etiology could be retrieved. Clinical significant portal hypertension defined as portosystemic collaterals in combination with splenomegaly was only present in the patient with PVT and in 13 patients with liver cirrhosis. Patients with INCPH could not be diagnosed confirming that it is a rare disorder in the general Western population. In addition we identified determinants and upper limits of normal spleen size. Since the Rotterdam study is a population based study performed in the elderly, these prevalence data cannot be extrapolated to a younger population where INCPH might be more frequent.

Several reports have described the occurrence of INCPH in patients with human immunodeficiency virus (HIV) infection (24-30). Opinions regarding the prevalence of HIV-associated INCPH are discordant. According to some experts, the prevalence of INCPH in

HIV-infected patients is assumed to be substantial (31), whereas others agree that it is a rare disease (29-30). Furthermore, data on clinical outcome and survival in HIV-associated INCPH patients were lacking (26, 32-34). We demonstrated that HIV-associated INCPH is a rarely diagnosed disorder in the Netherlands (**CHAPTER VI**). By performing a systematic survey in all 25 designated HIV treatment centres we could identify 16 out of 18.085 HIV-infected patients with HIV-associated INCPH, signifying that 0.1‰ of the Dutch HIV population is diagnosed with clinically overt INCPH. In total, 7132 out of 18.085 Dutch HIV-infected patients have been exposed to didanosine. Taking into consideration that all INCPH cases were exposed to didanosine, an INCPH prevalence of 2‰ in HIV-infected patients exposed to didanosine can be calculated. However, these data can be an underestimation of its actual prevalence since awareness for the disorder is low and patients without clinical complications of portal hypertension might remain undiagnosed. In order to assess the risk factors of this disorder we performed a case-control study. All patients with HIV-associated INCPH were or had been exposed to didanosine. Prolonged didanosine exposure or short-term combination exposure with didanosine plus either stavudine or tenofovir are independent risk factors for the development of this disorder. These results are in line with previous studies indicating didanosine an indispensable role in the development of INCPH in HIV patients (28, 33-35). The observed association between INCPH and long-term exposure to antiretroviral drugs with the most potent ability to inhibit mitochondrial DNA polymerase-γ and the observed synergistic risk profile of concomitant exposure to didanosine and stavudine suggest mitochondrial toxicity in the development of HIV-associated INCPH. In comparison to INCPH patients outside the setting of HIV infection (CHAPTER IV), we demonstrated that mortality in HIV-associated INCPH is mainly related to HIV-associated disorders. Because of normal synthetic function, survival in patients with noncirrhotic portal hypertension has been considered to be better than survival in patients with cirrhosis. However, considering the rarity of HIV-associated INCPH, large international multicenter trials are required to achieve sufficient power to demonstrate significant differences in survival between cirrhotic and noncirrhotic HIV-infected patients with portal hypertension. Finally, our study results demonstrate that INCPH is not reversible after discontinuation of didanosine. Thrombocytopenia and clinical signs of portal hypertension sustained during follow-up (despite immediate interruption at INCPH diagnosis of didanosine and stavudine). Taking into account the histological features observed in liver specimens retrieved at INCPH diagnosis of these patients, this clinical observation is reasonable.

In chapter VI we identified didanosine exposure as an essential element for the development of INCPH. This observation is in line with previous reports. So far, INCPH has only been reported in HIV-infected patients exposed to didanosine treatment. Nevertheless, this unique role of didanosine in the pathophysiology of INCPH remains controversial

as the drug was widely used in HIV treatment in the past and reports on INCPH remain scarce. In addition, the observation that INCPH has not been reported outside the setting of this drug could be a result of detection bias. In order to assess this unique relationship we performed a systematical screening of a large population of HIV-patients (**CHAPTER VII**). Our study results elucidate the controversy concerning the unique role of didanosine in the pathophysiology of INCPH. INCPH could not be diagnosed in any of the 910 HIV-infected patients that never had been exposed to didanosine. Therefore, in line with previous observations, didanosine exposure can be regarded an essential factor in the development of INCPH. Furthermore, we demonstrated that HIV-associated INCPH is an underestimated disorder. The 4% prevalence of INCPH in HIV-infected patients exposed to didanosine is substantially larger than the 0.2% reported in Chapter VI. As a result, increased awareness for this disorder is warranted and patients treated with didanosine (in past or currently) should be screened for the presence of this disorder. The observation that only a minority of the HIV-infected patients exposed to didanosine develops INCPH indicates that this disorder is likely to be a multifactorial disease requiring additional elements. In order to identify these unknown covariates, we compared a cohort of well-defined INCPH patients to a group of HIV-infected patients exposed to didanosine but without clinical signs of portal hypertension. No patient characteristics at HIV diagnosis or the start of treatment with didanosine predictive for the development of INCPH could be identified. INCPH patients and patients treated with didanosine without signs of portal hypertension only differed in respect to HAART characteristics. Patients with INCPH were treated significantly longer with didanosine or combination treatment with didanosine and stavudine. The possible role of thrombophilia in HIV-associated warrants further investigation.

DIRECTIONS AND FUTURE RESEARCH

The chapters in this thesis have focused on different aspects of the histopathology, etiology, prevalence and clinical outcome of idiopathic noncirrhotic portal hypertension. Despite the fact that the studies reporting the clinical outcome are retrospective we believe that our findings lead to the further understanding of this disease in Western patients. In order to further study a rare disease like this, international cooperation is indispensable. In comparison to previous studies on BCS and PVT, studying INCPH in a collaboration network as the European Network for Vascular Disorders of the Liver (EN–Vie) would result in a unique patient cohort providing valuable clinical information and pathophysiological insight (36-37).

Previous studies have demonstrated a high prevalence of thrombophilia (mainly protein S and C deficiency) in INCPH patients (30-50%) (14, 32). However, these studies are

hampered by small study size and potential selection bias. Furthermore, in patients with HIV-associated INCPH, low activity of protein S has been reported. It has been hypothesized that this low activity is a result of functional inactivation of circulating protein S by antiprotein S IgG (32). The high levels of inhibitory circulating antiprotein S in HIV-infected patients might be explained by a persisting abnormality of B-cell activation. In patients with BCS, it has been demonstrated that hypofibrinolysis may also be involved in the etiology of the disorder in addition to thrombophilia (38). In INCPH patients, hemostatic research has been limited to thrombophilia, the fibrinolytic system has not been studied so far.

Finally, previous studies have shown clear differences in the distribution of etiologic factors between patients with BCS and noncirrhotic PVT. Comparing both patient groups, a myeloproliferative disorder was more common in BCS patients compared to patients with PVT. This observation provides evidence for the hypothesis that different factors may promote thrombosis in different vascular systems (site-specificity of thrombosis). Whether there are also differences in etiologic factors underlying INCPH versus BCS and PVT has not been investigated. Large prospective international multicentre studies are needed to study these different pathophysiological aspects, taking into account the rarity of the disorder.

Regarding the treatment of INCPH patients, current guidelines are limited to the treatment of portal hypertension and its complications. Etiological treatment of INCPH is currently not available. Based on the high prevalence of thrombophilia in INCPH patients, several authors incriminated thrombosis of small intrahepatic portal veins as an important etiological factor in the development of this disorder (14, 27). Furthermore, patients with INCPH are at risk for the development of portal vein thrombosis (14, 33, 39-40). In patients with HIV-associated INCPH, the incidence appears to be higher (33, 40-41). As a result, anticoagulation therapy has been proposed by several authors in order to prevent disease progression and to maintain portal vein patency (14, 33, 42).

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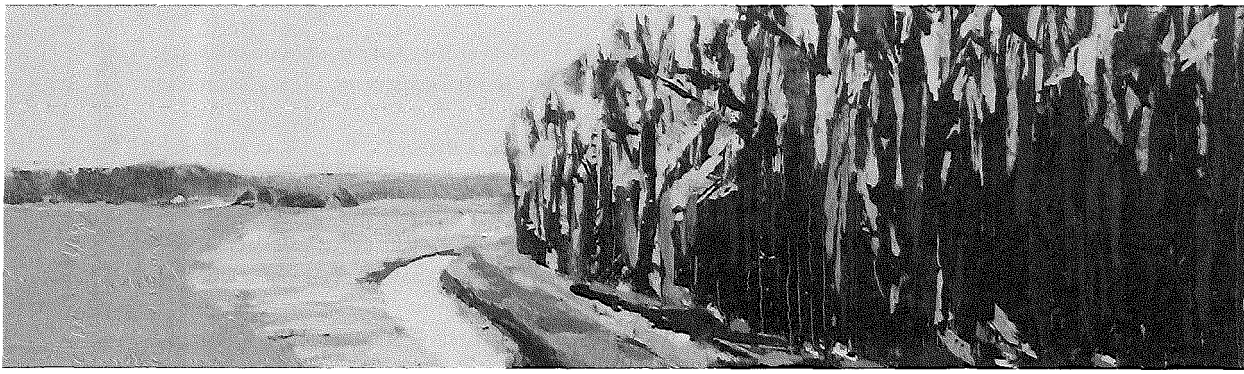

NEDERLANDSE SAMENVATTING

Portale hypertensie is het gevolg van een veneuze obstructie van de hepatische bloedflow. Deze obstructie kan leiden tot een milt vergroting en het ontstaan van collateralen waaronder spataders in het maagdarm systeem. Afhankelijk van het niveau van de veneuze obstructie spreekt men van prehepatisch, intrahepatisch of posthepatische portale hypertensie. Levercirrose is de meest voorkomende oorzaak van portale hypertensie in de westerse wereld. Portale hypertensie kan echter ook ontstaan in de afwezigheid van levercirrose. Er wordt dan gesproken van niet-cirrotische portale hypertensie. De meest voorkomende oorzaak van niet-cirrotische portale hypertensie wereldwijd is chronische schistosomiasis. Andere frequente oorzaken van niet-cirrotische portale hypertensie in de westerse wereld zijn: niet-alcoholische leververvetting, primaire biliaire cirrose, primaire scleroserende cholangitis, congenitale hepatische fibrose, extra hepatische vena porta trombose en het Budd-Chiari syndroom (2-4). Indien al deze oorzaken uitgesloten zijn kan de diagnose van een idiopatische niet-cirrotische portale hypertensie (INCPH) gesteld worden.
In tegenstelling tot de hoge prevalentie van dit ziektebeeld in India is INCPH een zeldzame ziekte in de westerse wereld (8). Het spreekt dan ook voor zich dat er weinig bekend is over de ontstaansmechanismen, de klinische karakteristieken en prognose van het ziektebeeld.
Het doel van het proefschrift is een beter inzicht te krijgen in deze aspecten van dit zeldzame ziektebeeld. In de eerste hoofdstukken van dit proefschrift worden de morfologische aspecten, prognose, overleving en het voorkomen in de algemene oudere populatie van INCPH beschreven. In de laatste hoofdstukken zal dieper ingegaan worden op INCPH bij HIV-geïnfecteerde patiënten.

In **Hoofdstuk II** wordt een overzicht gegeven van verschillende aspecten van dit ziektebeeld (verschillende definities, voorkomen, klinische presentatie, morfologische bevindingen, behandeling en overleving).

In **Hoofdstuk III** worden de microscopisch bevindingen in leverweefsel van INCPH patiënten vergeleken met leverweefsel van patiënten met niet-cirrotische portale hypertensie in het kader van een vena portae thrombose. Hypoplastische portale velden, phlebosclerose en nodulaire regeneratie werden meer waargenomen bij patiënten

met INCPH en kunnen dan ook beschouwd worden als INCPH specifiek. Bij patiënten met HIV-geassocieerde INCPH kwam meer nodulaire regeneratie voor dan bij INCPH patiënten zonder HIV.

In **Hoofdstuk IV** worden de klinische presentatie, determinanten van overleving en klinische uitkomst van patiënten met INCPH bestudeerd. De meerderheid van INCPH patiënten presenteert zich met complicaties van portale hypertensie (voornamelijk gastro-intestinale bloedingen en splenomegalie) en een normale leverfunctie. INCPH patiënten ontwikkelen tijdens het verloop van de ziekte zelden leverfalen, hepatische encefalopathie of een hepatopulmonair syndroom, maar indien deze complicaties optreden moet levertransplantatie overwogen worden. Verder toont deze studie ook aan dat de 10-jaarsoverleving bij INCPH patiënten rond de 50% ligt. Het overlijden van deze patiënten is meestal een gevolg van de INCPH-geassocieerde aandoeningen (o.a. HIV-infectie en maligniteiten) en aandoeningen die voorkomen bij hogere leeftijd (neurologische aandoeningen en hartfalen). Overlijden ten gevolge van leverfalen of slokdarmbloedingen is uiterst zeldzaam.

In **Hoofdstuk V** werd het voorkomen van portale hypertensie (cirrotisch en niet-cirrotisch) bestudeerd in een oudere populatie. Bij 2582 deelnemers van de Rotterdam studie (een prospectief bevolkingsonderzoek uitgevoerd bij de oudere inwoners van Ommoord) kon een betrouwbare miltmeting uitgevoerd worden. Bij 135 van deze deelnemers kon een vergrote milt (>12cm) aangetoond worden. Bij 35 van deze 135 kon hiervoor een oorzaak aangetoond worden. Bij geen van deze deelnemers kon INCPH gediagnosticeerd worden. Bij 1 deelnemer kon een vena porta thrombose aangetoond worden. Bij 13 deelnemers kan levercirrose aangetoond worden. Deze studie bevestigd dat het een zeldzame aandoening is in de algemene oudere westerse populatie.

In **Hoofdstuk VI** werd aan de hand van een systematische vragenlijst bij alle HIV behandelingscentra in Nederland aangetoond dat HIV-geassocieerd INCPH een zeldzaam gediagnosticeerd ziektebeeld is in Nederland. Vervolgens werden de risicofactoren voor het ziektebeeld bestudeerd aan de hand van een patiënt-controle onderzoek. Langdurige behandeling met didanosine of kortdurende combinatie therapie met didanosine en stavudine of tenofovir bleken onafhankelijke risicofactoren voor het ontstaan van het ziektebeeld. In vergelijking met patiënten en INCPH buiten de context van HIV infectie toonden wij aan dat mortaliteit in HIV geassocieerde INCPH voornamelijk gerelateerd is aan de HIV geassocieerde aandoeningen (o.a. maligniteiten en systeem ziekten) en niet aan leverfalen.

In **Hoofdstuk VII** wordt de rol van behandeling met didanosine in het ontstaan van INCPH bestudeerd aan de hand van een systemische screening van een groot cohort HIV-geïnfecteerde patienten (met en zonder didanosine blootstelling). INCPH kon niet gediagnosticeerd worden in de 910 HIV-geïnfecteerde patiënten die nooit behandeld werden met didanosine. Als gevolg kan besloten worden dat didanosine blootstelling een essentiële factor is in het ontstaan van INCPH. Daarenboven werd aangetoond dat het ziektebeeld te weinig gediagnosticeerd wordt en dat bijgevolg een verhoogde waakzaamheid voor dit ziektebeeld noodzakelijk is bij artsen die verantwoordelijk zijn voor de behandeling van HIV-geïnfecteerde patiënten. Waarom slechts een minderheid van de patiënten die blootgesteld werden aan didanosine INCPH ontwikkelden blijft onduidelijk. Er konden geen patiënten karakteristiek op het moment van HIV diagnose of bij de start van didanosine voorspellend voor de ontwikkeling van het ziektebeeld aantoont worden.

Tenslotte wordt in **hoofdstuk VIII** een beschouwing gegeven van alle bevindingen uit dit proefschrift in het licht van mogelijk toekomstig onderzoek.

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Beste Nadia, ook jou wil ik bedanken voor de enorme steun die je mij de voorbije jaren gegeven hebt. Ons verhaal begon pas echt 10 jaar geleden toen we gedurende 3 jaren samen het appartement in "de Consciencestraat" gedeeld hebben. Mijn god, wat was dat een geweldige tijd. We zaten op dat moment in een vergelijkbare fase van ons leven waardoor we elkaar ontzettend goed aanvoelden. Het gevolg is een fantastische vriendschap. Ik kan je dan ook oprecht zeggen dat je voor mij de zus bent die ik nooit heb gehad. Ik waardeer jouw vriendschap enorm en ook al zien we elkaar veel te weinig, de momenten dat we elkaar zien zijn geweldig. Ik beloof plechtig dat ik na het beëindigen

van mijn promotiewerk meer tijd zal vrij maken voor jou en je geweldige familie. Ook jou wens ik nog heel veel succes met je carrière, zowel familiaal als professioneel.

Beste Alain, Christophe, Marika, Sofie, Inge, Piet, Gert, Pieter, Isabel, Bart, Koen, Bert, Jan, Björn, Ben, Roel, An-Sofie, Claudia, Wim, David, Erwin, Mieke, Fi, Erica, Ann, Philippe, Kris, Jan, Frederik, Lotte, Hedwig, Marco, Inge en Bruno bedankt voor jullie steun en vriendschap. Op jullie kon ik steeds terugvallen voor een luisterend oor of juist een avondje doorzakken met een goed glas wijn om eens totaal niet serieus te zijn en te kunnen ontspannen. Jullie stonden altijd klaar om mij door de pieken en de dalen te halen die verbonden zijn aan promoveren. Ik weet dat ik jullie de voorbije 5 jaren schromelijk heb verwaarloosd. Ik meen oprecht dat ik de komende jaren ga proberen dit goed te maken en de tijd aan jullie ga spenderen die jullie verdienen. Enkele vrienden wil ik nog specifiek bedanken.

Beste Alain, 10 jaar geleden begonnen we samen op de afdeling Hematologie in het Universitair Ziekenhuis van Antwerpen. Jij als staflid, ik als derde-jaars AIOS. Van jou heb ik heel veel geleerd. Naast de liefde voor het wetenschappelijk onderzoek leerde jij mij bovenal relativeren (what doesn`t kill you only makes you stronger). Big brother, je bent een dierbare vriend wiens mening mij aan het hart ligt. Ook jij ligt mede aan de basis van dit proefschrift. Alain ik wens je nog heel veel succes in de toekomst.

Beste Isabel, wij zaten bijna dagelijks 2 uur (in het beste geval, maar meestal veel langer) samen op de trein. Jouw aanwezigheid maakte deze tijd dragelijk. Hoe vaak hebben we niet gevloekt bij de zoveelste uitval van de internationale trein door sneeuw, bladeren op het spoor, ongeval met persoon of meestal zonder verklaring of communicatie. Gelukkig was jij er om tegen te ventileren. Sinds kort woon je achter mijn hoek waardoor we, ondanks het feit dat we niet meer samen op de trein zitten, nog vele leuke momenten kunnen beleven.

Beste Inge, zonder jou had op de cover van dit boekje alleen tekst gestaan. Hartelijk bedankt voor het assisteren in lay-out en illustratie voor mijn promotieboekje. Wat een geweldig idee om samen de treinrit van Rossendaal naar Rotterdam af te leggen en als 2 maniakale Japanners aan de lopende band fotos te nemen van het landschap om te gebruiken voor je fantastische creatie.

Beste Wim en David, ook jullie wil ik specifiek bedanken. De jaren die we samen hadden zijn mij enorm dierbaar en hebben mij deels gemaakt tot wie ik nu ben. Wim, jouw positivisme en gedrevenheid werken insiprerend. Ik ben blij dat ook jij gekozen hebt om gastro-enteroloog te worden. Jij wordt ongetwijfeld grote concurrentie.

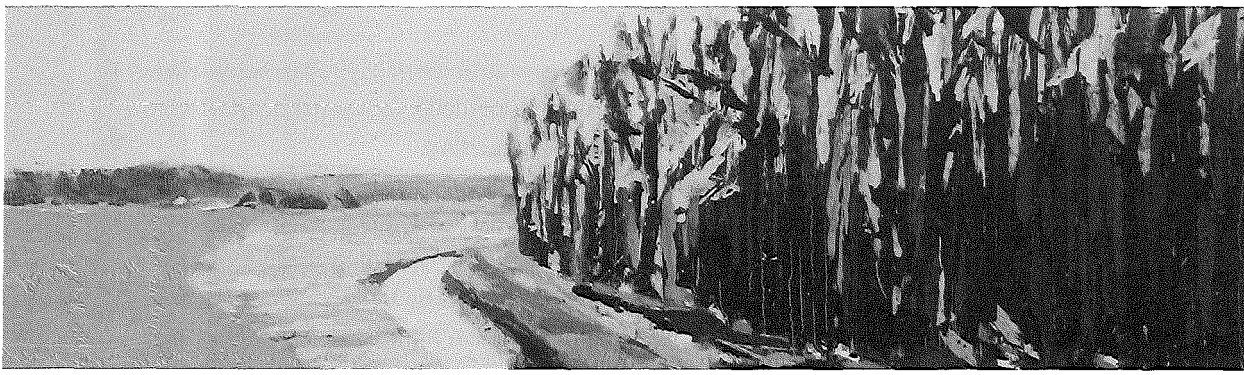
En, last but not least, mijn ouders. Lieve mama en papa, via deze weg wil ik jullie bedanken voor de geweldige opvoeding die jullie mij gegeven hebben. Jullie hebben altijd in mij geloofd en waren er steeds op de momenten dat ik het nodig had. Jullie liefde,

warmte en steun is onevenaarbaar. Zonder de kansen die jullie mij geboden hebben zou dit alles niet tot stand zijn gekomen. Jullie zijn verantwoordelijk voor wie ik nu geworden ben, op alle gebied. Bedankt voor mijn geweldige opvoeding.

Uiteraard ben je niets zonder een lieve familie. Jullie zijn de continue factor in mijn leven.

Beste tante Jess, tante Muis, Sophie, Elisah, Bernd, Thierry, Nancy, tante Maggy en natuurlijk Charlotte mijn petekindje, ook jullie wil ik hartelijk bedanken voor de voorbije jaren. Jullie hebben allemaal ongelooflijk bijgedragen aan dit boekje. Mocht ik er niet voldoende zijn geweest dan zien jullie hier een van de belangrijkste redenen daarvoor. Beste nonkel Eddy helaas kon je hier niet bij ons aanwezig zijn. Onze gedachten zijn bij jou.

Het laatste plekje heb ik bewaard voor jou Tom. Jij bent zonder twijfel de persoon die de voorbije 5 jaren het meeste heeft geleden onder mijn promotie. Jij hebt alle pieken en dalen van het promotietraject meegemaakt. Lieve Tom, hoe kan ik je bedanken. Eerst en vooral, bedankt voor je continue steun en begrip. Ik weet niet hoe vaak je naar mijn geklaag hebt moeten luisteren of mijn frustratie (en daaruit voort vloeiende onredelijkheid) hebt moeten tolereren, maar dat het vaak was weet ik zeker. Bedankt dat je telkens je tijd nam om hiernaar te luisteren en mee naar oplossingen te zoeken. Jouw nuchtere (eigen aan een industriële ingenieur weet ik nu) kijk bracht vaak orde in mijn chaotische gedachtegang (na de initiële irritatie). Ik apprecieer enorm de ruimte die je me hebt gegeven om dit project af te ronden. Men zegt dat promoveren een ware test is voor een relatie; we hebben het overleefd!



CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 11 juli 1976 te Wilrijk. Na het behalen van zijn eindexamen aan het Sint-Jan Berchmanscollege te Antwerpen, startte hij de studie geneeskunde aan de Universiteit van Antwerpen. In 1997 verrichtte hij een wetenschappelijke stage op de afdeling hematologie aan het Universitair Academisch Centrum Groningen. Tijdens een stage in London (King's College Hospital) en dankzij het enthousiasme van Peter Michielsen en Sven Francque werd zijn interesse voor de hepatologie gewekt. In oktober 2001 startte hij zijn opleiding als Maag-, Darm- en Leverarts aan de universitair van Antwerpen (opleider prof.dr. P. Pelckmans). Tijdens zijn opleiding voerde hij een multicentrische studie uit ter evaluatie van de laboratorium variatie in de parameters van de MELD score. Tevens onderzocht hij de waarde van de thrombine generatie test bij patiënten met levercirrose. In 2006 ontving hij de Paul Capell prijs (Belgian study of Thrombosis and haemostasis) voor dit werk. Op de wintermeeting van de BASL (2007) ontving hij de clinical research grant.

Na zijn specialisatie in 2008 startte hij als staflid op de maag- darm- en leverziekten in het Erasmus Medisch Centrum te Rotterdam alwaar hij zijn promotie startte onder begeleiding van prof.dr. H.L.A. Janssen. Verder heeft hij meegewerkt aan een grote epidemiologische studie (The Rotterdam Study) naar het voorkomen van leverziekten in de algemene populatie. Andere interesse gebieden zijn de abdominale echografie en infectieziekten.

Vanaf mei 2013 zal hij werkzaam zijn als gastro-enteroloog in het Academisch Ziekenhuis Nikolaas alwaar hij de hepatologie zal uitbreiden als onderdeel binnen de gastrogroep.

Hij is een culinair liefhebber en volgt een professionele scholing tot kok aan het Provinciaal Centrum voor Volwassenenonderwijs te Antwerpen.

Hij is woonachtig met zijn partner te Antwerpen.

PhD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student: J.N.L. Schouten
Erasmus MC Department: Gastroenterology and Hepatology
Research School: Erasmus MC

PhD period: 2008-2013
Promotor(s): Prof. Dr. H.L.A. Janssen
Supervisor:

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills		
- Biomedical English Writing and Communication		
- Research Integrity		
- Laboratory animal science		
Research skills		
Biostatistics for Clinicians (Rotterdam, NIHES)	2012	30
In-depth courses (e.g. Research school, Medical Training)		
Academic Hospital Groningen: scientific research July-September 1997 (Laboratory of Hematology).	1997	360
King's College Hospital London: Liver Transplantation.	2000	300
Department of Gastroenterology and Hepatology Erasmus MC.	2008	
Oral presentations		
Hyperferritinemie, een diagnostische aanpak. Huisartsen bijscholing. Antwerp, February, 2007.	2007	12
Comparison between Endogenous Thrombin Potential (ETP) and INR to assess liver function in patients in liver cirrhosis. Belgian week of gastroenterology. Ostend, February, 2007.	2007	12
Comparison between INR and PT of the ability to assess liver function in patients with liver cirrhosis. Belgian week of gastroenterology. Ostend, February, 2007.	2007	12
Influence of different laboratory methods on MELD calculation in the Belgian liver transplant centres. Annual Scientific Meeting of the Belgian Transplantation Society. Brussels, March 2007.	2007	12
Hyperferritinemie, een diagnostische probleem. Bijscholing biochemische opleiding. Antwerp, May 2007.	2007	6
A rare cause of fulminant liver failure. BASL Wintermeeting. Brussels, December 2007.	2007	12
Differences in prioritization for liver transplantation due to interlaboratory variability in MELD score parameters; need for improvement of the model? Eurotransplant meeting 2009. Leiden, October 2009.	2009	6
Doppler in Levertransplantatie, Gynaecologische & Abdominale Echocursus. Leuven, October 2009.	2009	12

Managing HBV: 3 years clinical experience with entecavir in Erasmus Rotterdam. BASL Wintermeeting, Brussels, 2009	2009	12
JNL Schouten, B Hansen, R De Knegt, P Taimr, A Hofman, H Janssen. Sonographic determination of normal spleen length in an elderly Caucasian population: results of a population based study. 22nd Euroson, Copenhagen, August 2010	2010	8
Ultrasound in liver disease. Leuven, October 2010.	2010	10
Drug Induced Liver Injury en rol van monitoring van de levertesten. Diner Pensant Rotterdam. June 2011.	2011	10
Ultrasound in bile duct disorders. Leuven, October 2011.	2011	10
E Koehler, JNL Schouten, B Hansen, B Stricker, H Janssen. Gamma glutamyl transferase and aminotransferase levels are independently associated with all-cause mortality in elderly: results of a population-based study. 62nd AASLD, San Francisco, November 2011.	2011	20
JNL Schouten, B Hansen, B Stricker, H Janssen. Low liver-related mortality in heavy drinkers: results of a long term follow-up study. 62nd Annual Meeting of the AASLD, San Francisco, USA, November 2011.	2011	20
Hepatitis Masterclass, Diagnostiek: Fibroscan en Echografie. Utrecht, November 2011.	2011	10
Belgian week of gastroenterology, Coagulation disorders in cirrhosis. Ostend, February, 2012.	2012	20
Dermatologendagen Hepatotoxiciteit van systemische medicatie in dermatologie, Arnhem, March 2012.	2012	6
Pathologic Liver course: INCIPH, idiopathic non cirrhotic portal hypertension. Amsterdam, March 2012.	2012	6
Non cirrhotic portal hypertension, Dutch Liver week. Rotterdam June 2012.	2012	6
EASL, Monothematic conference vascular liver disorders. Tallin, August 2012.	2012	20
Hepatitis Masterclass, Diagnostiek: Fibroscan en Echografie. Utrecht, September 2012.	2012	4
NASH symposium. Zeist, October 2012.	2012	15
Poster presentations		
A Gadisseur, JNL Schouten, S Francque, I Vangenechten, F Vertessen, M van der Planken, P Michielsen. The automated Endogenous Thrombin Potential (ETP) test to reflect coagulation changes in patients with cirrhosis of the liver. XXIst Congress of the International Society on Thrombosis and Haemostasis, Geneva, July 2007.	2007	12
JNL Schouten, S Francque, H van Vlierberghe, I Colle, F Nevens, J Delwaide, M Adler, P Starkel, D Ysebaert, A Gadisseur, P Michielsen. Influence of different laboratory methods on MELD calculation in the Belgian liver transplant centres. 13th ESOT, Prague October 2007.	2007	12
JNL Schouten, S Francque, H van Vlierberghe, I Colle, F Nevens, J Delwaide, M Adler, P Starkel, D Ysebaert, A Gadisseur, P Michielsen. Influence of different laboratory methods on MELD calculation in the Belgian liver transplant centres. 58th AASLD, Boston, November 2007 (president's choice).	2007	8
JNL Schouten, A Gadisseur, S Francque, P Michielsen, Z Berneman. Comparison between Endogenous Thrombin Potential (ETP) and INR to assess liver function in patients in liver cirrhosis. 58th AASLD, Boston, November 2007.	2007	8

JNL Schouten, E Koehler, B Hansen, A Hofman, B Stricker, H Janssen. Prevalence of non-alcoholic fatty liver disease (NAFLD) and tobacco consumption (cigarettes, cigars and pipes): results of a population-based study (Rotterdam study). 61st AASLD, Boston, November 2010.	2010	10
JNL Schouten, F Nevens, M Van Den Born, H Janssen. Outcome of Western patients with idiopathic non-cirrhotic portal hypertension (INCPH) is not determined by complications of portal hypertension: results of a large observational study. 61st AASLD, Boston, November 2010.	2010	30
E Koehler, JNL Schouten, B Hansen, B Stricker, H Janssen. Decreased prevalence of hepatic steatosis in the elderly: a population-based study. 61st AASLD, Boston, November 2010.	2010	15
JNL Schouten, E Koehler, BE Hansen, BH Stricker, HLA Janssen. Tobacco consumption is no independent risk factor of hepatic steatosis: results of a population-based study. 61st Annual Meeting of the AASLD, Boston, USA. November 2010.	2010	8
E Koehler, JNL Schouten, B Hansen, B Stricker, H Janssen. Higher physical activity level is associated with decreased prevalence and severity of non-alcoholic fatty liver disease in elderly. 62nd AASLD, San Francisco, November 2011	2010	6
E Koehler, JNL Schouten, B Hansen, B Stricker, H Janssen. Nonalcoholic fatty liver disease is not associated with cognitive impairment in elderly: results of a population-based study, 62nd AASLD, San Francisco, November 2011.	2011	4
E Koehler, JNL Schouten, B Hansen, B Stricker, H Janssen. Gamma glutamyl transferase and aminotransferase levels are independently associated with all-cause mortality in elderly: results of a population-based study. 62st AASLD, San Francisco, November 2011.	2011	4
JNL Schouten, E Koehler, BE Hansen, A Hofman, BH Stricker, HLA Janssen. Normal ranges of serum alanine aminotransferase levels in an elderly Caucasian population. 62nd Annual Meeting of the AASLD, abstract 1630, San Francisco, USA, 2011.	2011	15
E Koehler, C de Keyser, JNL Schouten, B Hansen, HLA Janssen, B Sticker. Association between statin use and non-alcoholic Fatty Liver disease in a population-based study. 63rd annual meeting of the AASLD 2012, Boston.	2011	5
JNL Schouten, M van der Ende, P Taimr, RJ de Knegt, T Koëter, H Rossing, B Hansen, HLA Janssen. HIV-associated Idiopathic Noncirrotical Portal hypertension. Risk factors and association with didanosine, 63rd annual meeting of the AASLD 2012, Boston.	2011	20
E Koehler, JNL Schouten, BE Hansen, B Stricker, L Castera, HLA Janssen. Prevalence and risk factors of severe fibrosis in the elderly: Transient Elastography in the Rotterdam study. 63rd annual meeting of the AASLD 2012, Boston.	2012	10
E Plompen, JNL Schouten, NJ van Blijderveen, BE Hansen, FW Leebeek, BH Stricker, HLA Janssen. Genetic risk factors and history of venous thromboembolism in relation to liver fibrosis in the elderly. 63rd annual meeting of the AASLD 2012, Boston.	2012	15
H Chi, BE Hansen, JNL Schouten, P Taimr, HLA Janssen, RJ de Knegt. Complication and rate in relation to the number of biopsy passes in percutaneous liver biopsy. 63rd annual meeting of the AASLD 2012, Boston.	2012	2
EM Koehler, JNL Schouten, BE Hansen, BH Stricker, HLA Janssen. Predictors of Nonalcoholic Fatty liver disease in non-diabetic lean elderly individuals. 63rd annual meeting of the AASLD 2012, Boston.	2012	6

EP Plompen, JNL Schouten, BE Hansen, BH Stricker, FW Leebeek. Von Willebrand factor levels are independently associated with liver stiffness: results of a population based study. 63rd annual meeting of the AASLD 2012, Boston	2012	15
International conferences, seminars and workshops		
Hepatology days of Beaujon hospital. January 2007.	2007	10
Kidney ultrasound. VENEB, May 2007.	2007	10
7th international meeting on therapy in liver disease. Barcelona, September 2007.	2007	30
8th European Transplant Fellow Workshop. Oslo, October 2007.	2007	35
AASLD postgraduate course. Boston, November 2007.	2007	20
Hepatology days of Beaujon hospital. January 2008.	2008	10
EASL Clinical School, Acute liver failure and liver transplantation. London, July 2008.	2008	36
AASLD postgraduate course. San Francisco, November 2008.	2008	20
EASL Clinical School, metabolic liver disease. Turin, June 2009.	2009	36
8th international meeting on therapy in liver disease. Barcelona, September 2009.	2009	30
Molecular Medicine Postgraduate School, SNPs and Human Diseases. November 2009.	2009	36
AASLD postgraduate course. Boston, November 2010.	2010	10
EASL postgraduate course. Berlin, March 2011.	2011	15
AASLD postgraduate course. San Francisco, November 2011.	2011	15
TIPS symposium. Rotterdam, January 2012.	2012	4
Barcelona Clinic Liver Cancer, Advancement of therapy in Hepatocellular Carcinoma. Barcelona, March 2012.	2012	20
Pathology: 19th Amsterdam Summer Course. March, 2012.	2012	20
EASL postgraduate course. Barcelona, April 2012.	2012	20
Biostatistics for Clinicians. Rotterdam, NIHES, April 2012.	2012	35
Mini Symposium Protease inhibitors for the treatment of Hep C: The future has just begun. May 2012.	2012	4
HCC symposium, State of the Art and future strategies. May, 2012.	2012	4
Dutch Liver Week, Abdominal Echo graphics. Rotterdam, June 2012.	2012	16
Monothematic conference vascular liver disorders. Tallin, March 2012.	2012	25
Didactic skills		
- Teach the teacher (training activities) 2x year	2008-2012	25
Other		
-		
2. Teaching activities		
Course Abdominal ultrasound, for midwifes, Pathology of the gallbladder and the bile ducts.	2011	10
Dutch Covigilance Centre Lareb: cirrhosis and it's complications. 's Hertogenbosch.	2011	10
Bachelor; Minor Education: Gastroenterology and Hepatology.	2011-2013	30
Bachelor; Clinical transplantation medicine.	2011-2012	30
Bachelor: Disorders in food, metabolism and hormone regulation.	2011-2012	60
EASL Barcelona: Early morning session, workshop, Non cirrhotic portal hypertension. Barcelona.	2012	15

Larebdagen: Cirrhosis and it's complications. 's Hertogenbosch.	2012	10
Diner Pensant: Drug induced liver injury en rol van monitoring van de levertesten.	2012	10
EASL Monothematic Conference Vascular Diseases of the Liver. Estonia, Tallin.	2012	30
Hepatitis Masterclass, Fibroscan en echografie.	2012-2013	15

Further: in addition all other oral presentations

Lecturing

-

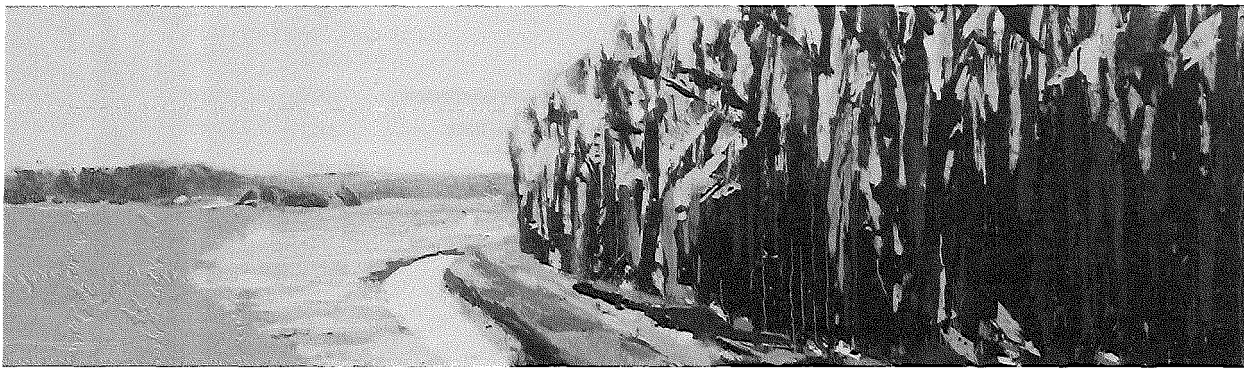
Tutoring

Lisanne Plompen/ PhD	150
Edith Koehler/ PhD	200
Maarten Rossing/keuzeonderzoek	50
Thijmen Koeter/keuzeonderzoek	50

Other

- *Memberships*

EASL	European Association for the study of the Liver
AASLD	American Association for the study of the Liver
NVGE	Nederlandse Vereniging voor Gastro-enterologie
NVH	Nederlandse Vereniging voor Hepatologie (Dutch Association for Hepatology)



ABBREVIATIONS

ABC: abacavir
ALP: alkaline phosphatise
ALT: alanine aminotransferase
AP: alkaline phosphatise
ASH: alcoholic liver disease
AST: aspartate aminotransferase
ATA: atazanavir
ATA: atazanavir
BMI: Body Mass Index
D4T: stavudine
DDI: didanosine
DNA: deoxyribonucleic acid
EBV: esophageal variceal bleeding
ENOS: Endothelial nitric oxide synthetase
fAPV: fosamprenavir
FTC: emtricitabine
GGT: gamma glutamyl transferase
HAART: highly active antiretroviral therapy
HAS: Hepatitis activity score
Hb: haemoglobin
HCC: hepatocellular carcinoma
HIV: Human immunodeficiency virus
IDV: indinavir
II: integrase inhibitor
INCPH: Idiopathic non-cirrhotic portal hypertension
INOS: Inducible nitric oxide synthetase
INR: International normalized ratio
IQR: interquartile range; ULN: upper limit of normal.
IVD: intravenous drug use
kPa: kilopascal
LAM: lamivudine
LPV/r: lopinavir/ritonavir

LS: liver steatosis
LTD: liver test disturbances
MSM: men who have sex with men
NAFLD: non-alcoholic fatty liver disease
NASH: non-alcoholic steatohepatitis
ND: not done
NNRTI: non-nucleoside reverse transcriptase inhibitor
NR: nodular regeneration
NRH: Nodular regenerative hyperplasia
NRTIs: nucleoside/nucleotide reverse transcriptase inhibitors
NVP: nevirapine
OLT: orthotopic liver transplantation
PCD: protein C deficiency
PHT: portal hypertension
PI: protease inhibitor
PNT: Partial nodular transformation
PSD: protein S deficiency
PSS: portosytemic shunting.
PVT: portal vein thrombosis
SPSS: Statistical Package for Social Sciences for Windows
TE: transient elastography
TEN: tenofovir
TIPS: transjugular intrahepatic portosystemic shunting
WBC: white blood cell count.
ZVD: zidovudine