Netherlands
The Journal of Medicine
PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE

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Contents

Cover
Etching entitled 'Interior of an ichtyologist' by Annemarie Petri. For details about the artist, her work, and how to order see page 28.

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An annual subscription to The Netherlands Journal of Medicine (ISSN 0300-2977) consists of 11 issues. Issues within Europe are sent by standard mail and outside Europe by air delivery. Cancellations should be made, in writing, at least two months before the end of the year.

Subscription fee
The annual subscription fee within Europe is € 650,00, for the USA € 665,00 and for the rest of the world € 675,00. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Payment method
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EDITORIAL

Covenant between gastroenterology and internal medicine in the Netherlands: a major step forward

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ABSTRACT

Recently, the Netherlands Society of Gastroenterohepatology (Nederlands Genootschap Maag-Darm-Leverartsen; NGMDL) and the Netherlands Association of Internal Medicine (Nederlandse Internisten Vereniging; NIV) set up a covenant to optimise the collaboration between internists and gastroenterologists. Important points:

- **certification of endoscopic skills;**
- **training of residents of internal medicine with regard to pathology of the stomach, intestines and liver as well as to endoscopy, and the training in internal medicine of residents in gastroenterology;**
- **defining competence of gastroenterologists for night and weekend duties in internal medicine.**

On 15 March of this year, delegations of the Netherlands Society of Gastroenterohepatology (Nederlands Genootschap Maag-Darm-Leverartsen; NGMDL) and the Netherlands Association of Internal Medicine (Nederlandse Internisten Vereniging; NIV) convened to set up a covenant regarding the care for patients with gastrointestinal disorders. The basis of this covenant was to create optimal collaboration between the NGDML and the NIV. This was considered of great importance, in view of the rapidly approaching manpower problems.
After approval of the entire covenant by the councils for the speciality programmes of both societies ('Concilia'), their general assemblies also approved it in May 2002.

Because the covenant is considered a major step forward, I think it is important to publish the text here. It should be realised, however, that the present text is a translation of the Netherlands original and carefully formulated covenant. The translation may lead to slightly different interpretations, for which I cannot be held responsible. For legal purposes only the certified Dutch text should be used.

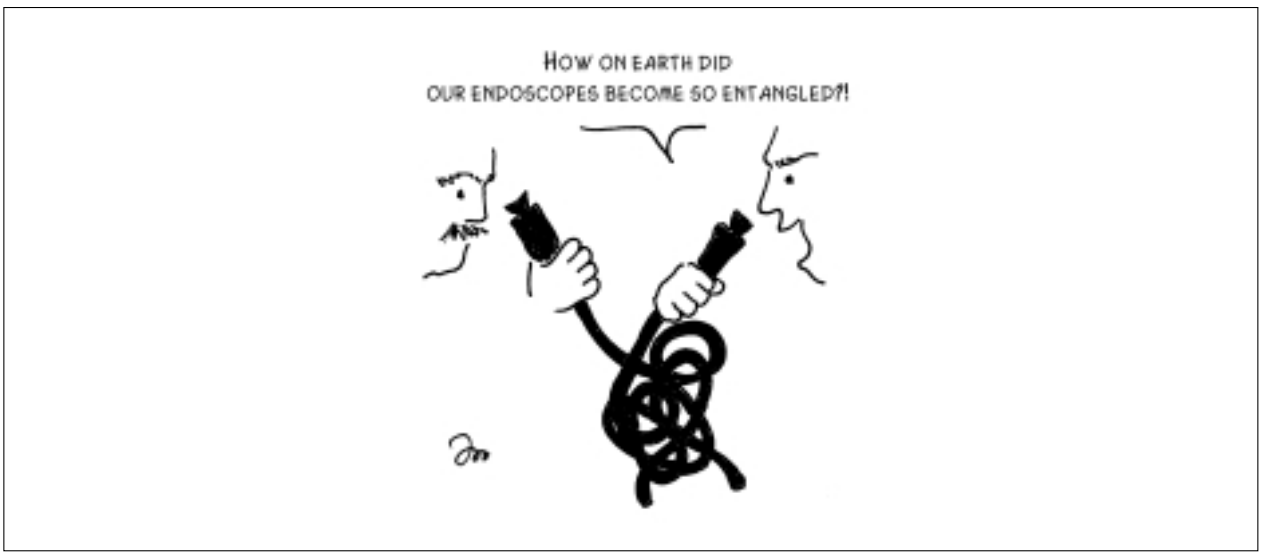
The following was agreed upon.

1. There is mutual recognition of each other's position and expertise, i.e., expert knowledge of the gastroenterologist in the field of diseases of the stomach, intestines and the liver, and the internist's generalistic approach. There is a preference for optimal collaboration within the context of a partnership.
2. When one or more gastroenterologists join a hospital (preferably within the context of a partnership of internists and gastroenterologists), quality assurance of both internal medicine and gastroenterology according to a well-founded manpower planning should be guiding. Preferably, this will lead to an extension of manpower for both specialities; in such extension of both specialities, quality is paramount.
3. The continuity and quality of facilities for endoscopies, diagnostic as well as therapeutic, should be seven days of the week (including nights, weekends and holidays) and is the responsibility of the partnership of internists and gastroenterologists. The partnership settles this through local and regional agreements, which are put in writing. The agreed schedule for endoscopy service will be inspected by the two societies during site visits.
4. Quality criteria for independent performance of endoscopic procedures will be formulated by the NGMDL and the NIV and these will be presented to the Netherlands

Society for Surgery. Based on these criteria, the following types of license can be provided:
- Partial certification, i.e., license for a limited array of endoscopies;
- Recertification, i.e., license for endoscopy if established requirements are met;
- Retrograde certification, i.e., license for endoscopy if established requirements are met by experienced endoscopists.

5. Within the context of the six-year training programme for internal medicine, two forms of training should be established:
   a. A four to six month training period (in hospital and/or in the outpatient clinic) to acquire specific knowledge of gastroenterohepatology. This period may take place within the first four years of the training programme for internists (common trunk). This period will not include endoscopy training.
   b. Those who have done the training period mentioned under 5a may take a further six to eight month gastroenterology course within the two final years of the internal medicine training; this will lead to a total of 12 months gastroenterology training during the six years of internal medicine residency. This programme contains a certified endoscopy training, which includes gastroscopy and sigmoidoscopy and if found suitable, colonoscopy. The basic assumption is that gaining experience in endoscopy cannot be detached from solid knowledge of the pathology of the stomach, intestines and liver. Other endoscopic procedures, such as ERCP, are not part of this package.
6. With regard to the competence of gastroenterologists for the night and weekend duties of internal medicine, the following criteria will be applied:
   - A licensed gastroenterologist with at least four years of training in internal medicine is allowed to perform night and weekend duties for internal medicine without a seconding internist.
   - A licensed gastroenterologist with three years of training in internal medicine is allowed to perform night and weekend duties for internal medicine with a seconding internist during the first three years. From the fourth year of license, it is allowed to perform night and weekend duties for internal medicine without a seconding internist.
   - A licensed gastroenterologist with two years of training in internal medicine is not allowed to perform night and weekend duties for internal medicine.
7. The duration and organisation of the speciality training programme for gastroenterology is decided by the gastroenterologists. The minimal duration of the internal medicine training will be two years, of which one year is devoted to general internal medicine. Preferentially, training periods dedicated to intensive care medicine and to oncology, and if possible to nephrology, are included in these two years.
8. If the criteria mentioned above are met, the NIV will not object to the nomenclature: gastroenterohepatologist.

This covenant with formulation of the criteria for the contents of the training periods will be further developed by the NGMDL and the NIV, together.
Meantime, it has become clear from the reactions of the membership of both the NGMDL and the NIV that this covenant meets with broad support. It does justice to the position of the internists, as well as that of the gastroenterologists, within the field of care for patients with disorders of the gastrointestinal tract.



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Gaucher disease: from fundamental research to effective therapeutic interventions

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ABSTRACT

Gaucher disease type 1 is the most common lysosomal storage disorder, with a prevalence of 1:50,000 in most countries. It is caused by an autosomally recessive inherited deficiency of the lysosomal enzyme glucocerebrosidase, leading to the accumulation of glucocerebroside in the macrophages. The lipid-laden macrophages are called Gaucher cells and can be found in the liver, spleen and bone marrow. Gaucher disease type 1 should be considered in any patient with an unexplained splenomegaly with or without bleeding diathesis, skeletal manifestations or hepatomegaly. The diagnosis is made by showing decreased glucocerebrosidase activity in peripheral blood leucocytes or by demonstrating previously defined DNA mutations. Detailed knowledge about the molecular defect has provided a rationale for therapeutic interventions and attempts have been made to correct the defect at gene level, protein level and by manipulation of metabolism. Clinical trials of gene therapy have been conducted but so far have not resulted in successful intervention. For moderate to severely affected patients, intravenous enzyme supplementation therapy is the treatment of choice, resulting in substantial clinical improvement in the majority of patients. For individuals with mild Gaucher disease, an oral substrate inhibitor can also be considered if intravenous treatment is a less attractive option.

INTRODUCTION

Gaucher disease is the most common of the lysosomal storage disorders, a subgroup of the inherited metabolic diseases. The disease is characterised by a deficiency of the lysosomal enzyme glucocerebrosidase (glucosylceramidase), which leads to the accumulation of glucocerebroside in the macrophages.[1,2] Based on the presence or absence of neurological symptoms, Gaucher disease can be divided into three phenotypes; type 1 (non-neuronopathic), type 2 (acute neuronopathic) and type 3 (subacute neuronopathic). Type 1 Gaucher disease is by far the most common form, accounting for 99% of the Gaucher cases. Since specialists in internal medicine will rarely be confronted with the neuronopathic forms, this review will focus on type 1 Gaucher disease.

In the past two decades, Gaucher disease has received much attention for being the first of the lysosomal storage disorders for which safe and effective enzyme therapy has been developed, thereby making Gaucher disease a prototype for other intracellular protein deficiency diseases. More recent developments include the discovery of specific disease markers, the use of a new therapy based on substrate reduction and clinical trials of gene therapy.

This review provides an overview of the current knowledge and describes the recent advances concerning Gaucher type 1 disease.

EPIDEMIOLOGY AND GENETICS

Type 1 Gaucher disease can be found in all ethnic groups, but is especially prevalent in the Ashkenazi Jewish population, occurring in about 1:400 to 865 people.[3-5] The prevalence in the general population has been estimated at about 1:50,000,[6] with a carrier frequency of 1:200.

These figures could represent an underestimation, since a number of patients may well remain undiagnosed because of lack of symptoms or because physicians do not make the correct diagnosis. In the Netherlands, between 100 and 150 cases of type 1 Gaucher disease are known.[7]
Gaucher disease is transmitted in an autosomal recessive way. The 7.5 kb gene is located on chromosome 1q21 and encodes glucocerebrosidase.[8] More than 100 mutations have been described, of which the majority are point mutations.[9]
The most frequent mutations in the Ashkenazi Jews are the N370S and the 84GG mutations.[4,10] The N370S enzyme is present in normal amounts and shows considerable activity at low pH values but not at higher pH values.[11] Homozygotes for the N370S mutation often have a very mild form of the disease or are discovered as asymptomatic family members.[12] The heteroallelic presence of the N370S mutation is associated with non-neuronopathic disease only.[13,14] Alleles bearing the 84GG mutation are unable to direct synthesis of any protein at all ('null' mutation), and as such, this mutation has never been found in the homozygous state.[15] The combination of the N370S and the 84GG mutation results in relatively severe disease.[13]
The most prevalent mutations in Caucasian patients are the N370S and the L444P.[16] Homozygosity for the latter is associated with the neuronopathic forms of the disease.[16] Many of the Dutch patients have the N370S mutation in a heteroallelic form.[17]
There is a wide variability in clinical presentations of type 1 Gaucher disease and no strong correlations have been found between genotype and clinical expression.[18] A striking example of this is the description of a pair of identical twins, both carrying the N370S/N370S mutation, in which one subject has serious manifestations of the disease and the other has no symptoms at all.[19]

PATHOPHYSIOLOGY

The enzyme glucocerebrosidase catalyses the cleavage of glucose and ceramide from glucocerebroside, an intermediate in the degradation of complex glycosphingolipids, which are mainly present in cell membranes. Since macrophages degrade apoptotic and senescent blood cells and their precursors that are rich in this glycosphingolipid, it is not surprising that the lysosomes of these cells accumulate glucocerebroside when there is a deficiency in glucocerebrosidase activity. The lipid-laden macrophages are called Gaucher cells and are characterised by eccentric nuclei and a typical striated 'crumpled silk' cytoplasm (see *figure 1*).[20] Macrophages are especially found in the liver, spleen, bone marrow and, to a lesser extent, in the lung, and therefore these organs are predilection sites for excessive storage of undegraded glycolipid.

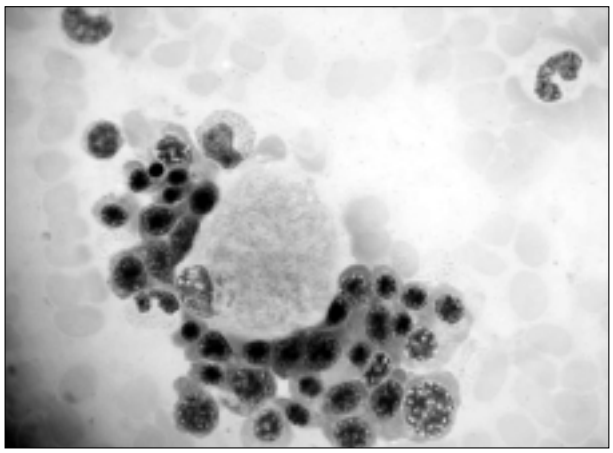

Figure 1
A microscopic view of a typical Gaucher cell, with a 'crumpled silk' cytoplasm and an eccentrically located nucleus, in the bone marrow aspirate of a patient with Gaucher disease

The glucocerebroside concentration in spleens can be increased 10- to 1000-fold, but high levels can also be detected in liver and bone marrow.[21] The increase in plasma concentration of glucocerebroside is far less spectacular, with an average of about twofold.[22,23] Other substances than glycolipid have also been found to be elevated in plasma and tissue of Gaucher patients. For example, tartrate resistant acid phosphatase 5B (TRAP), ferritin, angiotensin-converting enzyme (ACE), hexosaminidase and the lysosomal hydrolase chitotriosidase.[24] The last mentioned is by far the most elevated in symptomatic patients, with levels increased at least 100-fold and ranging to more than 4000 times the median normal value, while asymptomatic Gaucher patients show no or only slight increases.[25] Extensive studies have shown that chitotriosidase originates from the Gaucher cell and that plasma levels are closely associated with the total body burden of Gaucher cells. It is therefore a good marker to monitor disease progression and response to therapy.[24]
Since the presence of large numbers of storage cells in itself cannot explain all the phenomena observed in Gaucher patients, it has been suggested that the accumulated glucocerebroside activates macrophages, which induce inflammatory responses by releasing cytokines. Indeed, elevated levels of IL-1β, IL-6, IL-10 and M-CSF in sera from patients with Gaucher disease have been found,[26-29] as well as a trend towards elevated TNF-α mRNA.[30] In addition, glucocerebrosidase deficient mice showed a multisystem inflammatory reaction with inflammatory cell infiltration in several organs, lymphadenopathy, and elevated TNF-α and IL-1β expression. Evidence of B-cell proliferation was also found, as well as elevated serum IgG levels.[31] These findings also support the hypothesis that chronic stimulation of B cells occurs, which may be

the cause of the increased incidence of autoantibodies and the high frequency of gammopathies and multiple myeloma that have been found in patients with Gaucher disease.[32]

CLINICAL PRESENTATION

Type 1 Gaucher disease is a highly variable non-neuronopathic disease with a clinical picture that is dominated by a slowly to rapidly progressive hepatomegaly and splenomegaly, bone involvement, and a cytopenia. The mean age at diagnosis is 21 years,[13] but the age of onset can range from early childhood to the eighth decade. In general, early onset may be associated with a poor prognosis, but variability is the rule.

Haematology

Thrombocytopenia is the most common peripheral blood abnormality in patients with Gaucher disease, often leading to spontaneous bruising and bleeding.[33] Initially, this is the result of enhanced clearance of blood cells by the enlarged spleen. In a later stage of the disease or in patients who have undergone a splenectomy, replacement of the bone marrow by Gaucher cells adds to the development of cytopenia. Low levels of several clotting factors have also been found in patients with Gaucher disease, but the clinical expression of this derangement seems to be modest.[34] Anaemia and neutropenia are usually mild, but may result in pallor and palpitations or recurrent bacterial infection.[35]

Spleen

Splenomegaly is present in all but the very mildest cases of Gaucher disease and is often a presenting symptom.[36] In severely affected patients the spleen may be huge, sometimes weighing more than 10 kilogram, and interfering with normal food intake. Fibrotic areas and regions of extramedullary haematopoiesis sometimes present as nodules.[37,38] Splenic infarctions sporadically occur, presenting with local pain and tenderness, fever and abdominal guarding.[36]

Liver

The liver is increased in size in most patients, but gross enlargement, in which the liver may fill the entire abdomen, is typically found in splenectomised patients. The bulk of the liver may cause distress and episodes of pain occur. On physical examination the liver is usually hard and smooth. Between 30 and 50% of patients have elevated liver enzymes. However, hepatocytes appear not to be involved in the storage process and liver function is usually preserved.[38] Frank hepatic failure and cirrhosis with portal hypertension and ascites are uncommon but occur sporadically.[37,39]

Bones

The skeletal involvement probably leads to the most debilitating symptoms.[40] Bone disease in Gaucher is characterised by bone marrow infiltration of Gaucher cells as well as defective bone remodelling, leading to osteopenia, osteonecrosis and avascular infarction. Nearly all patients have signs of bone involvement, but the clinical presentation varies widely. Some patients experience chronic, ill-defined bone pain that can be debilitating and poorly correlated with radiographic findings. Pathological fractures, avascular necrosis of the femoral head, as well as instability of the spine with consequent vertebral compression and spinal cord involvement can result in severe mobility impairment.[40] Deformities of the distal femora can lead to the classical Erlenmeyer configuration. A number of patients experience one or more bone crises, which can occur spontaneously or follow a febrile syndrome and begin with a deep, dull, aching pain in the involved bone. These crises are usually very painful, requiring high doses of analgesics, and can last for weeks to months. Bacterial osteomyelitis should be excluded by appropriate cultures.

Lungs

Although relatively uncommon, pulmonary failure is one of the most serious consequences of Gaucher disease. It may result from infiltration of the lung by Gaucher cells or from left-to-right shunting, probably secondary to liver disease.[41] Massive visceromegalia or kyphoscoliosis following vertebral collapse can cause compression of the lung, which is probably a more frequent cause of respiratory disease.

DIAGNOSIS

Histological

The classical method for diagnosis of Gaucher disease was the detection of lipid-laden Gaucher cells in bone marrow, in a biopsy of the liver, or in a surgically removed spleen. However, the finding of Gaucher cells is not pathognomonic for Gaucher disease of as a diagnostic tool. So-called 'pseudo-Gaucher' cells can be found in several haematological diseases, including chronic granulocytic leukaemia,[42,43] lymphomas,[44,45] and multiple myeloma.[46] Since the development of the glucocerebrosidase assay and DNA mutation analysis, histological examinations are no longer necessary for diagnosing Gaucher disease.

Enzymatic

Glucocerebrosidase activity can be measured in peripheral blood leucocytes,[47,48] urine samples,[49] or cultured skin fibroblasts.[10] The typical adult Gaucher patient will have

enzyme activity that is 10 to 30% of normal values. The usefulness of this assay in the detection of heterozygotes is limited, since there is a considerable overlap of glucocerebrosidase activity between normal and heterozygous individuals.[50] The main advantage of this method is that it can establish the diagnosis regardless which disease mutations are present. A disadvantage is that glucocerebrosidase is relatively labile, and therefore, rapid transportation of refrigerated samples to a reference laboratory is required to obtain valid results.

DNA-mutation analysis

A major advantage of DNA-based diagnosis is that, since DNA is very stable, blood samples can be transported at ambient temperature without haste. The DNA can then be extracted from the leucocytes and stored for years. A second advantage is its potential to give some prognostic information, taking into consideration the limitations mentioned previously.

However, a major difficulty is that current technology permits routine examination only for previously defined mutations, but not for the entire sequence of the gene. As a consequence, it is important to realise that the presence of two apparently normal alleles does not rule out the diagnosis, and finding only one abnormal allele does not automatically mean that the patient is simply a carrier.[51]

THERAPY

Symptomatic treatment

Before enzyme supplementation therapy became available, treatment of Gaucher disease was only symptomatic. Splenectomy was the customary treatment in cases in which massive splenomegaly caused severe cytopenia or mechanical discomfort.[52,53] After removal of the spleen, a reversal of the cytopenia occurs almost invariably and the well-being of the patient usually improves considerably.[52,54] Splenectomy is now only indicated in the very severe cases in which life-threatening complications, such as bleeding, make rapid intervention necessary. For treatment of bone crisis, analgesics and bed rest are usually needed. Bacterial osteomyelitis may occur and requires extensive treatment with intravenous antibiotics. Orthopaedic procedures, such as hip and knee joint replacement or stabilisation of the spine, are often performed.

Bone marrow transplantation

Since macrophages are derived from haematopoietic stem cells, bone marrow transplantation is expected to cure Gaucher disease. Indeed, allogenic bone marrow transplantation in a number of patients with type 1 and 3 Gaucher disease showed good haematological and visceral responses.[55,56] However, bone marrow transplantation is a high-risk procedure with severe complications and this treatment is therefore not usually recommended for patients with type 1 disease.

Gene therapy

The idea that Gaucher disease will also benefit from gene therapy is based on the positive results of bone marrow transplantation, the lack of need for strict regulation of glucocerebrosidase secretion and the fact that Gaucher disease is a monogenic disorder. However, clinical trials of retroviral transfer of the normal glucocerebrosidase gene into CD34+ cells from patients with Gaucher disease showed gene-containing cells in peripheral blood only transiently and at very low levels.[57,58] Methods of more efficient gene transfer need to be developed to improve these results.

Enzyme supplementation therapy

Gaucher disease was the first of the lysosomal storage disorders that could be treated using enzyme supplementation therapy, which has been available in the Netherlands since 1991.[59] Clinical trials using modified enzyme from placental tissue (Ceredase, alglucerase,) and later enzyme produced by recombinant techniques (Cerezyme, imiglucerase, both manufactured by Genzyme Corp., Mass., USA) showed a dramatic clinical response to regular intravenous administration.[60-63] In general, patients report striking improvements in well-being, energy level and quality of life.[64,65] Improvement in cytopenia and decreases in splenic and hepatic size are apparent after 3 to 12 months of treatment. Splenic size decreases by approximately 20% and liver size by approximately 10% after six months of treatment.[60,62,63] Liver volume usually normalises while the spleen continues to show some enlargement, even after a long period of treatment. Bone marrow and mineral skeleton usually respond slower and a maximal response may take years to achieve. The most sensitive method for measuring bone marrow infiltration is quantitative chemical shift imaging (QCSI). QCSI determines the ratio between triglyceride and water content of the bone marrow, which is greatly reduced in type 1 Gaucher disease,[66,67] probably due to displacement of normal triglyceride-rich adipocytes by Gaucher cells.[68] There are no serious side effects associated with enzyme supplementation therapy. About 13% of patients develop IgG antibodies to enzyme replacement therapy with alglucerase, but anaphylactic reactions are very rare.[69] There is still controversy about the most effective dosing regimen, one that results in an optimal therapeutic effect, while decreasing the infusion rate and the cost of care (€ 100,000 to 300,000 per patient per year in the Netherlands). In the Netherlands an individualised dosage regimen is used, starting with a low dose which is adjusted according to the response to treatment.[63]

Substrate reduction

The orally administered compound OGT 918 (Zavesca™, Oxford Glycosciences, UK) is an inhibitor of glucosylceramide synthase, the enzyme which catalyses the first step in the synthesis of most glycosphingolipids. Studies in untreated patients showed improvements in all key clinical features and biochemical markers, although less impressive compared with enzyme supplementation therapy. The most common adverse effect was diarrhoea.[70,71] In practice, enzyme replacement therapy remains the first choice for patients with moderate to severe disease. For patients with a mild or minimal residual disease, the disadvantages of the side effects should be balanced against the advantages of oral administration. Further studies will be needed to identify those patients that will benefit most from OGT 918.

Gaucher disease provides a good example of how fundamental research can contribute to the development of effective therapeutic strategies. Current research in the pathogenesis of Gaucher disease is likely to clarify unsolved aspects, aiding in the better understanding and management of this disease, as well as in other lysosomal storage disorders.

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Lamivudine plasma levels in chronic hepatitis B patients

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ABSTRACT

Lamivudine has recently been registered for the treatment of chronic hepatitis B patients. The main therapeutic outcome in the studies on which the registration was based was a drop of HBV DNA below 10^7 genome equivalents/ml, the level of detection of the insensitive Abbott Genostics assay. However, as we have reported previously, with the use of sensitive PCR-based assays, individual differences in virological response to lamivudine can be detected.
As a first step in analysing the chain of events after oral intake of lamivudine we modified and validated a high-pressure liquid chromatography (HPLC) method to evaluate lamivudine plasma levels. Lamivudine levels in chronic hepatitis B patients who participated in a study on the efficacy of lamivudine were comparable to our reference curve, which was derived from eight chronic hepatitis B patients. From the reference curve, a mean area under the curve (AUC) of 4994 mcg/l.h (SD 1524), a mean t_{max} of 42 minutes (SD 11), and a mean C_{max} of 1.9 mg/l (SD 0.70) were calculated. Lamivudine exerts its action as the active triphosphate inside the hepatocyte after extensive handling. Therefore, additional steps in the pharmacokinetic process should be evaluated to explore the potential mechanisms that are responsible for the diversity in quantitative HBV DNA response to lamivudine.

INTRODUCTION

Lamivudine, the negative enantiomer of 2'-3' deoxy 3' thiacytidine, is a nucleoside analogue which has recently been registered for the treatment of chronically infected hepatitis B patients. In large phase III studies the favourable effect of this drug was shown on suppression of hepatitis B virus (HBV) DNA, a parameter expressing active viral replication, which is often followed by a decline in transaminases and improvement of liver histology.[1-3] The conclusions in these studies were based on the percentage of patients with a viral decline below the lower limit (approximately 10^7 genome equivalents/ml (geq/ml)) of the insensitive liquid hybridisation assays (Abbott Genostics, Abbott Laboratories, Abbott Park, IL). HBV DNA became undetectable in around 80% of patients measured with this test after six months of therapy.[1] However, if we look more carefully with more sensitive polymerase chain reaction (PCR)-based assays, individual differences in response to lamivudine become apparent.[4,5] Whereas some patients show a rapid decline in levels even below the threshold of the qualitative PCR assay (Roche Monitor, lower limit of detection 400 geq/ml), in others, the HBV continues to replicate actively even after six months of therapy. We previously reported on a cohort of long-term lamivudine-treated chronic hepatitis B patients in Rotterdam.[4] In the 19 patients in whom HBV DNA was still detectable by insensitive assays (Digene, liquid hybridisation assay, lower limit of detection 1.5 x 10^6 geq/ml) after six months of therapy, only three patients had a mutant virus that could explain this continuing active viral replication. Thus, ongoing active replication of the HBV must be based on some other phenomenon in the majority of patients.
Lamivudine is subject to several transport and activation steps from oral intake until incorporation into the pregenomic viral chain. Our hypothesis was that poor

uptake of lamivudine might be responsible for the suboptimal decline in HBV DNA in some patients.
In order to be able to address this issue, we modified and validated a high-pressure liquid chromatography (HPLC) method to measure lamivudine plasma levels in chronic hepatitis B patients. Moreover, we studied the availability of lamivudine in blood after a standard oral dose in chronic hepatitis B patients.

PATIENTS AND METHODS

Patients

In group A eight patients were evaluated for 24 hours after oral intake of a single dose of lamivudine 150 mg to obtain a lamivudine plasma reference curve. Patients fasted overnight and blood was withdrawn over a period of 24 hours at t=0, 15, 30, 45, 60 and 90 minutes and 2, 3, 4, 6, 8, 12, 18 and 24 hours after intake of lamivudine.
In group B nine patients, in whom the viral decline during lamivudine 150 mg therapy[6] was studied in detail, the lamivudine concentration in a serum sample taken six hours after start of lamivudine therapy was assessed. The pharmacokinetic reference curve was based on plasma samples. Therefore, the agreement between plasma and serum results was ascertained in 11 randomly selected patients on lamivudine who visited the outpatient clinic (group C).

High-pressure liquid chromatography of lamivudine in plasma and serum

Lamivudine in plasma and serum was assayed with an HPLC method slightly modified from Harker *et al.*[7] In short, the following procedure was used.
Sample extraction is performed using a solid-phase extraction method (Bond Elute Verify LRC; 10 cc/130 mg, Varian Inc., Harbor City, CA, USA), after activation of the column with subsequently 2 ml of methanol and 2 ml of acetic acid 1%. Next, a mixture of 1 ml of plasma and 1 ml of acetic acid 1% is applied to the column with a pressure of 5 mmHg for at least two minutes. The column is consecutively washed and dried with distilled water, methanol/acetic acid 10% (9:1) and distilled water again. Desorption is carried out four times with 0.5 ml of methanol/ammonia 25% (9:1) under a low vacuum. The four fractions are collected and evaporated to dryness with a gentle flow of nitrogen at 40°C and subsequently suspended in 300 µl of the mobile phase by vortex-mixing. Separation of the mixture is performed by HPLC, equipped with a BDS Hypersil C18 column (250 x 4.6 mm ID; 5 um), using a mixture of methanol (40 ml), acetonitrile (5 ml), glacial acetic acid (0.5 ml), and 0.1 M ammonium acetate in water (455 ml) as the mobile phase at a flow of 1 ml/min and at a temperature of 40°C. Quantification was based on UV detection at 270 nm, calibrated with a range of external standards in plasma, which were processed the same way.

Intra- and interassay variability

Eight calibration standards of lamivudine, with a concentration ranging from 0.1 mg/l to 7.5 mg/l, were analysed simultaneously six times (intra-assay variability), expressed as the average accuracy with percent of the deviation from the nominal concentration. The procedure was repeated on three separate days (interassay variability) expressed as a co-efficient of variation.

Correlation between lamivudine levels in plasma and serum

The concentrations of lamivudine in serum and plasma were compared by means of a linear plot, as well as a Bland and Altman plot.[8]

Modelling of pharmacokinetic data

From the 24-hour pharmacokinetic curves, the average area under the curve (AUC), the half-life of lamivudine (t1/2), t_{max}, and C_{max} were calculated. Lamivudine concentrations were fitted with the TOPFIT pharmacokinetic programme[9] using a one-, two- and three-compartment model using four weightings (1, $1/\sqrt{y}$, 1/y, and $1/y^2$). The Akaike criterion[10] was used to establish the best fit for our data.

Table 1
Patient characteristics

	GROUP A (N=8)	GROUP B (N=9)	GROUP C (N=9)
Age in years (median range)	37 (17-60)	28 (22-51)	29 (17-57)
Male/female	7/1	7/2	6/3
Cirrhosis	4	1	0 (n=8)
Additional medication	Patient 3: ferrofumarate Patient 5: furosemide, aldactone Patient 7: methotrexate	Patient 4: oral contraceptive	Patient 1: pantoprazole Patient 3: clinoril, cough medicine, doxazosine, losartan, atorvastatin, insulin Patient 4/9: paracetamol

RESULTS

Patient characteristics of group A, B and C are shown in *table 1*. In group A, 50% of the patients had an advanced stage of liver disease.

The lower limit of detection of the HPLC assay was determined at 0.005 mg/l and the lower limit of quantification at 0.1 mg/l. The higher limit of detection was arbitrarily determined at 7.5 mg/l. All calibration curves were linear between 0.1-7.5 mg/l with a variance between -15% to +10% in this range. A variety of drugs, which were co-administered frequently to these patients, did not interfere with the extraction and detection procedure.

The intra-assay variability showed an accuracy of 80 to 95%, which is comparable with data described in the literature (*table 2*).[7] The interassay variability was concentration dependent, 3 to 16.6% (*table 2*). Recovery of lamivudine in spiked plasma samples compared with non-processed standard solutions was 86% (± 6.7%).

The relation between the concentration of lamivudine in plasma and serum was linear as observed by a line with a slope of 0.997 and an intercept at (0.0). The Bland and Altman plot showed a mean of the difference between the serum and plasma level of 0.02 mg/l (SD ± 0.0411).

For group A, a mean AUC of 4994 µcg/l.h (SD 1524), a mean t_{max} of 42 minutes (SD 11), and a mean C_{max} of 1.9 mg/l (SD 0.70) were calculated (*figure 1*).

If we compare the six-hour serum concentration of lamivudine in group B (median 0.35 mg/l; range 0.28-0.52) with the same time point in group A (median 0.32 mg/l; range 0.15-0.48), these concentrations are within the same range.

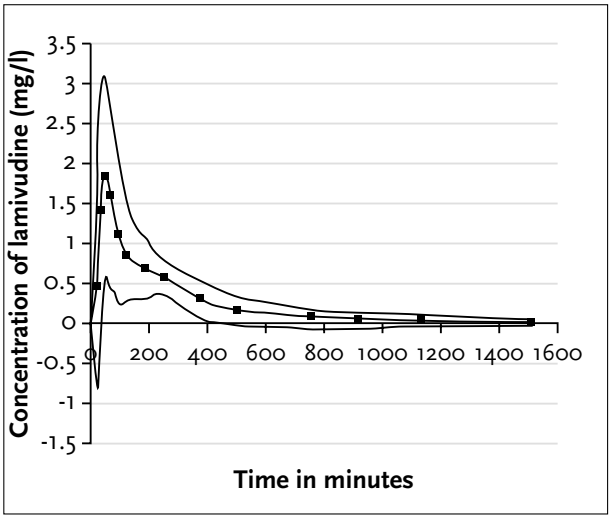

Figure 1
Pharmacokinetic reference curve (± 2 x SD) based on eight chronic hepatitis B patients treated with lamivudine 150 mg once a day (group A)

DISCUSSION

If the inhibitory effect of lamivudine on HBV replication is studied with a sensitive PCR-based assay with a dynamic range between 400-10^9 geq/ml, a wide variation in response between individual patients is observed. In a previous study, we showed that this could only in part be explained by the emergence of a mutation in the catalytic site of the polymerase gene of the HBV.[4] In this study we made a first step in further exploration of host-dependent mechanisms which might explain the variability of response to lamivudine.

Table 2
Intra- and interassay variability

	INTRA-ASSAY VARIABILITY			INTERASSAY VARIABILITY		
THEORETICAL VALUE (MG/L)	MEAN (N=6)	STANDARD DEVIATION	% COEFFICIENT OF VARIATION	MEAN (N=6)	STANDARD DEVIATION	% COEFFICIENT OF VARIATION
0.1	0.096	0.009	9.4	0.094	0.016	16.6
0.21	0.189	0.009	4.8	0.19	0.018	10.0
0.56	0.495	0.022	4.4	0.487	0.026	5.4
1.04	0.875	0.013	1.5	0.882	0.043	4.9
1.53	1.293	0.018	1.4	1.296	0.05	3.9
2.18	1.819	0.11	6.0	1.884	0.102	5.4
3.24	2.583	0.037	1.4	2.69	0.13	5.0
7.5	6.177	0.18	2.9	6.22	0.18	3.0

The pharmacokinetic process of any drug, including lamivudine, is characterised by a sequence of events: absorption, distribution, metabolism and elimination. Lamivudine is highly soluble, dissolves rapidly once in the stomach and is absorbed in the small intestine by passive diffusion. Food reduces the rate of absorption but not the extent: t_{max} is prolonged and c_{max} is reduced, but the AUC is not altered.[11,12] The absolute bioavailability is reported to be around 80%, with a mean volume of distribution of 1.3 l/kg, indicating considerable distribution into deeper tissues.[13]
In chronic hepatitis B patients, lamivudine acts in the liver, the target organ for viral replication. Lamivudine probably enters hepatocytes through active uptake by pyrimidine nucleoside transporters.[14,15] In the cytoplasm of the hepatocyte, lamivudine is phosphorylated to the mono-, di- and triphosphate by deoxycytidine kinase, cytidine monophosphate kinase and pyrimidine nucleoside diphosphate kinase, respectively. The diphosphate is present in highest concentrations inside the hepatocyte and the conversion of the diphosphate to the triphosphate is the rate-limiting step.[16] This extensive bioactivation makes the drug prone to individual differences between patients. Less than 10% of lamivudine is metabolised by the liver, only 5 to 10% of lamivudine is metabolised to a trans-sulphoxide metabolite and excreted in urine, while around 70% of the drug is excreted unchanged in urine.[17,18]
In this study, we modified and validated the HPLC assay for detection of lamivudine in plasma. Only few data on pharmacokinetics of lamivudine in compensated chronic hepatitis B patients have been published.[18] Our pharmacokinetic parameters are comparable to the published data.
Measurement of levels of lamivudine in daily practice may be useful for two purposes. If the level is within the normal range, this ascertains that patients have been compliant with therapy on the one hand and that on the other hand absorption, the first pharmacokinetic step, is adequate. As can be observed from our data, levels of lamivudine in plasma six hours after intake of lamivudine (group B) are in the same range as in patients in group A at six hours. These data, however, should be interpreted with caution, since group characteristics may vary. Recent studies have stressed the potential influence of co-administered drugs on lamivudine kinetics. This is either caused by the increase of phosphorylation of lamivudine (e.g. hydroxyurea, methotrexate)[19] or because of reduction of the excretion ratio of lamivudine in urine (e.g. trimethoprim).[20]
Our kinetic data show that the lamivudine concentrations in group B are well above the *in vitro* IC_{50} even five hours after the maximum concentration in plasma has been reached.[16] Plasma levels have been measured after intake of the first dose of lamivudine but these levels may change during long-term therapy. Previous data do not indicate that lamivudine accumulates during long-term application, but these data ascertain sufficient levels of lamivudine above the *in vitro* IC_{50} throughout the 24-hour period.[16] In contrast, in seven out of the eight patients in group A of our study, levels of lamivudine 24 hours after intake are undetectable. This may necessitate re-opening the discussion on twice daily dosing in patients with hepatitis B virus infection. The key question here will be how these plasma levels relate to the levels of the phosphorylated lamivudine inside the human hepatocytes. Half-life of lamivudine triphosphate in human lymphocytes infected with the human immunodeficiency virus (HIV) has been calculated to be substantially longer (10.5-15.5 hours) than lamivudine serum half-life.[21] Conflicting data on the half-life of lamivudine triphosphate in hepatocytes have been published: 3.6 to 8 hours in primary duck hepatocytes[22] versus 17 to 19 hours in HepG2 cell lines.[16] Therefore, research into human hepatocytes is needed both to address the dosing issue, as well as to better understand the differences between individual patients.
Absorption of lamivudine is a passive process and may therefore be the least important reason for variation in response to lamivudine between patients. In contrast, uptake of lamivudine in hepatocytes is an energy-driven active process, after which lamivudine is phosphorylated inside hepatocytes. Phosphorylation is mediated by host enzymes and the efficacy of the process from parent drug to active triphosphate and persistence of the active triphosphate in the hepatocyte may vary between individual patients due to genetic polymorphism. Therefore, to be able to explain differences in viral decline between patients infected with the same virus, patient-to-patient differences in conversion from lamivudine to phosphorylated lamivudine should be explored further.

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The yield of UGIE: a study of a ten-year period in the 'Zaanstreek'

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ABSTRACT

Background: Study of the occurrence of abnormalities in the oesophagus, stomach and duodenum, and of changes in specific diagnoses.

Methods: All consecutive upper gastrointestinal endoscopies (UGIEs) carried out in a period of ten years were included.

Results: In ten years 14,927 diagnostic UGIEs were performed (7335 men (49%) and 7592 women (51%)). These procedures were done at the request of the general practitioner in 4995 (33%) cases. A steady yearly increase in the number of open-access UGIEs was noted. Each year a mean of 796 abnormalities was seen in the oesophagus, a mean of 437 in the stomach, and of 162 in the duodenum. The presence of hiatal hernia and reflux oesophagitis significantly increased in the ten years ($p<0.001$). The numbers of Barrett's oesophagus and oesophageal cancer remained constant. Gastritis showed a gradual increase ($p<0.001$), while the number of gastric ulcers found per year decreased significantly in the ten-year period ($p<0.001$). Gastric malignancy remained constant. Presence of duodenal ulcers and bulbitis significantly decreased ($p=0.01$ and $p=0.06$, respectively).

Conclusions: The number of UGIEs carried out at the request of the general practitioner has significantly increased. Peptic ulcer disease shows a significant decrease, while reflux disease increases.

INTRODUCTION

Since its introduction, upper gastrointestinal endoscopy (UGIE) has been widely used, resulting in an increasing UGIE workload. The advantage of the direct visual inspection of the oesophageal, gastric and duodenal mucosa is obvious. Biopsy specimens can be taken for histological or microbiological examination. For this reason UGIE is considered the investigation of choice in cases of dyspepsia, or in the presence of reflux or alarm symptoms, and is mandatory for a precise diagnosis in cases of these upper abdominal symptoms.[1]
As a direct result of the use of gastroscopy, many data on the occurrence and prevalence of diseases in the upper part of the digestive tract have been collected.
There are many reports in the literature on the yield of UGIE. However, no data are present on the yearly yield of these UGIEs. Hence, there is no information on actual changes in specific UGIE morbidity patterns in patients sent for UGIE.
A cross-sectional study, including all consecutive UGIE reports from a ten-year period, was undertaken to study the yearly occurrence of abnormalities in the oesophagus, stomach and duodenum, and changes in the presence of specific diagnoses.

MATERIAL AND METHODS

All consecutive diagnostic UGIEs carried out in a period of ten years (January 1992 to December 2001) in De Heel Zaans Medical Centre, a community hospital in the 'Zaanstreek', were included. The UGIEs were performed at the request of internists, gastroenterologists, and

sometimes paediatricians, cardiologists or surgeons. In addition, there is an open-access facility for general practitioners.

Two experienced endoscopists performed all the procedures. After obtaining informed consent, UGIE was performed with Olympus endoscopes (Paes Nederland BV, Zoetermeer). In 1992, fibreoptic endoscopes were used. From 1993 onwards, the EVIS 100 video-endoscopes were introduced. Since the beginning of 2000, this system has been gradually replaced by the EXERA 160 system of Olympus. UGIE was carried out without sedation or local anaesthesia.

The results were noted in a written standardised report. If clinically indicated, biopsy specimens were taken to confirm the macroscopic diagnosis.

Hiatal hernia was defined as a distance of more than 2 cm between the diaphragm and the Z line. Defective or insufficient lower oesophageal sphincter closure was defined as a widely open sphincter during introduction as well as retrieval of the endoscope. Oesophagitis was scored according to the well-known Savary-Miller system. Endoscopic gastritis was judged to be present if nodularity was seen in the antrum or if erosions or intramucosal bleeding were present.[2] In addition the presence of red stripes, especially in the gastric antrum, was considered an endoscopic sign of gastritis. Barrett's oesophagus was judged to be present if the typical macroscopic appearance of cylindrical epithelium was present in the tubular oesophagus. Bulbitis was defined as the presence of erosions in the duodenal bulb.

Twice a year, all the UGIE results of the preceding six months were stored in a computerised database system. Statistical analysis was performed with chi-square test for contingency tables. A value below 0.05 was considered statistically significant.

RESULTS

In a period of ten years 14,927 diagnostic UGIEs were performed in 7335 men (49%) and 7592 women (51%). The mean number of gastroscopies a year was 1492 (range 1384-1631). Because of direct endoscopic follow-up, 1126 procedures were carried out due to previously diagnosed abnormalities. This was mostly because of upper gastrointestinal bleeding or follow-up because of gastric ulcer or cancer. The results of these UGIEs were, obviously, excluded from the present analysis. However, these follow-up UGIEs were included in the analysis of the applicants for gastroscopy.

UGIE was requested by the general practitioner in 4995 (33%) cases, by internists and gastroenterologists in 9786 (66.1%) cases, and in 146 (0.9%) cases the UGIE was performed at the request of paediatricians, surgeons or cardiologists. In the ten-year period a steady increase in the number of UGIEs carried out at the request of general practitioners was noted, while the number requested by specialists showed a parallel decrease (*figure 1*).

In 32% of the UGIEs (range 26-37%) carried out at the request of specialists no macroscopic abnormalities were detected; this was also the case in 32% (range 30-34%) of open-access gastroscopies (p=ns). The number of UGIEs revealing no macroscopic abnormalities showed very little yearly fluctuation in the ten-year period (*figure 2*), regardless of whether the procedure was done at the request of the general practitioner or a specialist.

Per year a mean of 796 macroscopic abnormalities (range 651-874) was seen in the oesophagus, a mean of 437 (range 377-513) in the stomach, and a mean of 162 (range 134-218) macroscopic abnormalities was diagnosed in the duodenum. The total number of endoscopic diagnoses exceeds the total number of patients as in some patients multiple diagnoses were made. The total number of yearly macroscopic diagnoses showed little fluctuation. Few UGIEs were inconclusive (the patient changed his mind about consent, or the patient forcefully retrieved the endoscope before the procedure was completed).

Hiatal hernia and insufficiency of the lower oesophageal sphincter closure occurred in a mean of 33.7% of cases (range 29.5-38.2%); oesophagitis was seen in 15.8% of gastroscopies (range 11.5-17.8%). Barrett's oesophagus

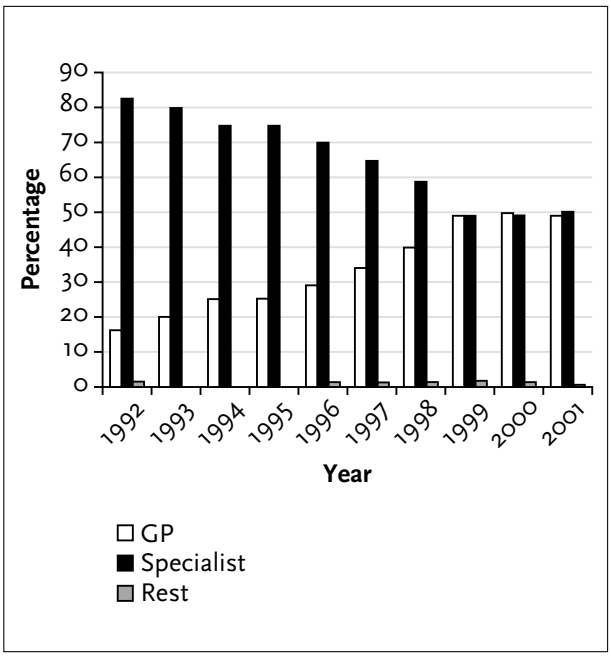

Figure 1

Number of endoscopies performed a year at the request of general practitioners and specialists

Rest indicates endoscopies performed at the request of surgeons, paediatricians or cardiologists.

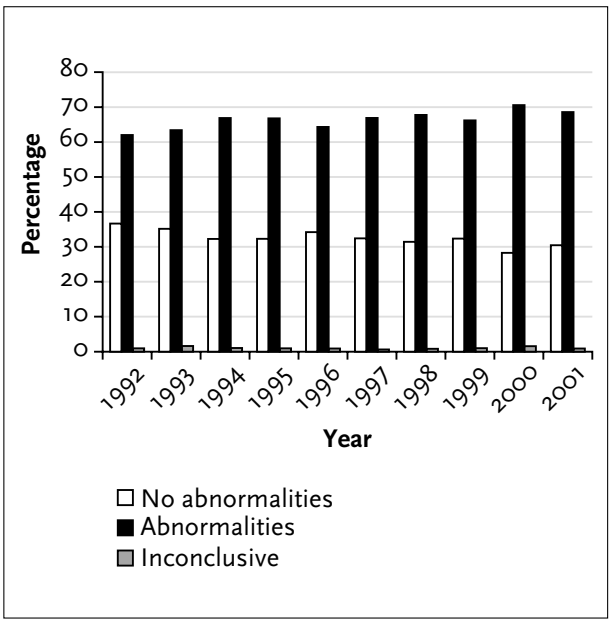

Figure 2
The annual number of endoscopies with macroscopic abnormalities and no abnormal findings

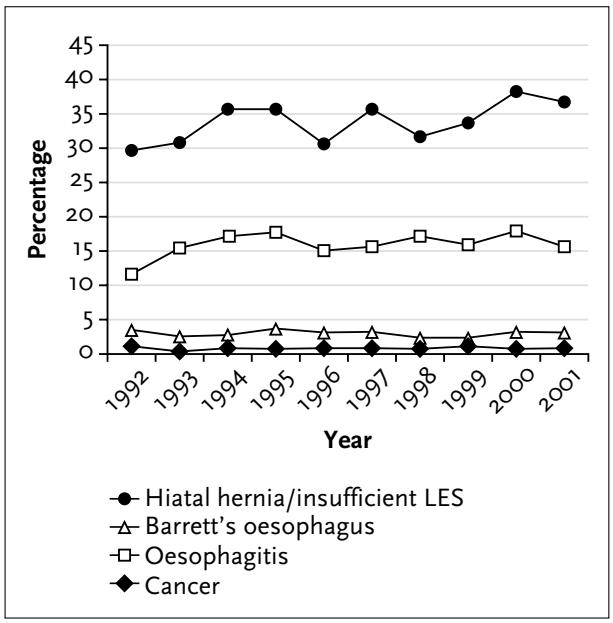

Figure 3
Relevant endoscopic findings seen in the oesophagus in the consecutive years

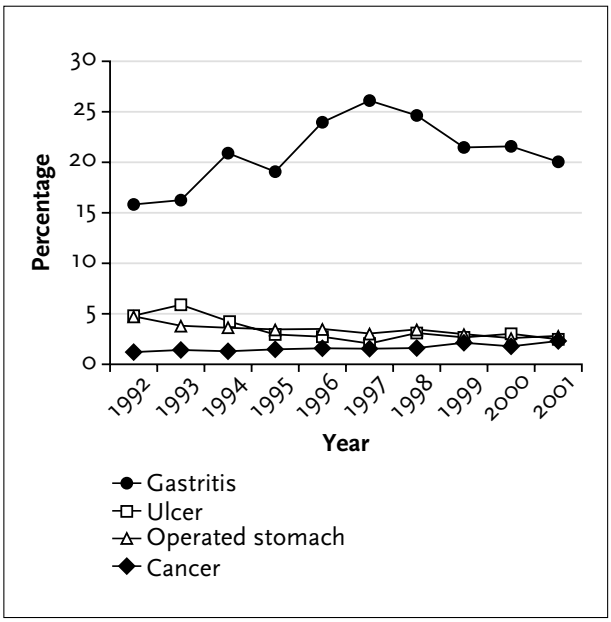

Figure 4
Relevant endoscopic diagnoses seen in the stomach in the consecutive years

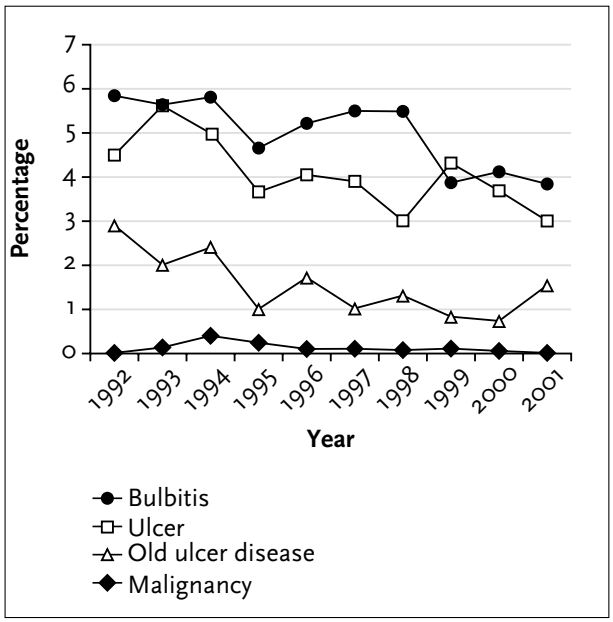

Figure 5
Relevant endoscopic diagnoses seen in the duodenum in the consecutive years

was present in 3% of the patients (range 2.6-3.7%), and cancer of the oesophagus was diagnosed in 0.7% (range 0.2-1.0%) (*figure 3*). The presence of hiatal hernia and insufficient lower oesophageal sphincter closure showed a statistically significant increase in ten years ($p<0.001$), while the number of patients with reflux oesophagitis showed a less impressive, but still significant increase ($p<0.001$). The number of patients with Barrett's oesophagus and oesophageal cancer remained very constant each year. Relevant diagnoses in the stomach were endoscopic gastritis 20.9% (range 15.9-26.1%), ulcer 2.3% (range 1.8-5.8%), operated stomach (Billroth resection or gastroenterostomy) 3.3% (range 2.8-4.6%), and malignancy located in the stomach 1.5% (range 1.1-2.3%) (*figure 4*).

Gastritis showed a gradual increase (p<0.001), while the number of gastric ulcers significantly decreased yearly in the ten-year period (p<0.001). Gastric malignancy remained constant.
In the duodenum ulceration was present in 4% (range 3-5.6%), bulbitis in 5% (range 3.8-5.8%), scarred bulbus in 1.5% (range 0.7-2.9%), and malignancy in 0.09% of the patients (range 0-0.4%) (*figure 5*). The numbers of duodenal ulcers and bulbitis significantly decreased (p=0.01 and p=0.06, respectively).
Table 1 shows the diagnostic yield of UGIEs carried out at the request of general practitioners or specialists. Overall the specialist UGIEs more often yielded a Barrett's oesophagus (mean percentage 3.5% versus 2% of the general practitioners' UGIEs) and ulcer disease (7.5% versus 3.8%). The number of cancers, reflux oesophagitis, bulbitis, hiatal hernia and insufficient lower oesophageal sphincter closure, and gastritis showed no difference.

DISCUSSION

In the literature no data have been published on the yearly yield of UGIEs, except for data on the yield of a total number of gastroscopies in a certain time. This is the first study, at least in the Netherlands, in which the yearly yield of UGIE is studied.
In the 1980, the number of UGIEs showed a dramatic escalation. This was especially true after the introduction of open-access facilities for general practitioners.[3] The annual demand in the 1990s was calculated at 12 per 1000 of the population.[4] Since the Zaanstreek has a population of approximately 132,000, this figure, which was calculated in the United Kingdom in 1989, turned out to be astonishingly accurate. The annual number of diagnostic UGIEs ranges from 1384 to 1631, and thus is in accordance with this figure. In disagreement with an earlier report,[5] the total number of UGIEs did not rise significantly. In addition there was no difference between men and women, both sexes equally often underwent an UGIE. Despite the general assumption that UGIE workload is increasing, this is not supported by the data from the present study. There is almost no waiting time for gastroscopy in the Zaanstreek. The average time between the decision to do an UGIE and the actual gastroscopy varies from one to ten days. The waiting time will only be longer in case of holidays. Hence, it can be assumed that all patients in the region who have a clinical reason for undergoing UGIE are actually sent to the UGIE department of De Heel Zaans Medical Centre. This certainly indicates that there is a good balance between supply and demand of gastroscopies in this region.
In accordance with other reports, the number of UGIEs performed at the request of the general practitioner significantly increased.[6] The explanation is not only the presence of an open-access facility, but also the more prominent place of gastroscopy in the work-up of dyspepsia

Table 1
Relevant macroscopic abnormalities seen in endoscopies done at the request of general practitioners and specialists

	1992 %	1993 %	1994 %	1995 %	1996 %	1997 %	1998 %	1999 %	2000 %	2001 %	MEAN %
Barrett's oesophagus											
GP	2.0	0.7	2.3	3.6	3.6	2.5	1.8	1.2	2.5	3.0	2.0
Specialist	4.0	3.2	3.1	3.7	3.0	3.8	2.5	3.6	4.4	3.6	3.8
Ulcer											
GP	12.8	14.0	11.5	6.9	6.9	4.5	5.6	4.9	4.6	4.8	3.8
Specialist	8.2	10.3	8.5	6.2	6.6	6.4	6.2	8.8	8.6	5.6	7.5
Cancer											
GP	2.4	1.6	2.3	1.7	1.9	1.7	3.0	3.7	2.1	2.7	2.3
Specialist	2.5	1.8	2.4	2.3	2.3	2.7	1.7	2.2	2.3	3.4	2.4
Reflux oesophagitis											
GP	9.5	13.8	11.5	16.5	20.2	20.0	17.0	18.7	22.8	19.2	16.9
Specialist	12.0	15.7	19.5	18.3	12.7	13.0	17.6	12.7	12.2	11.4	14.5
Bulbitis											
GP	6.6	5.6	6.9	7.8	5.0	4.2	4.7	4.1	5.0	3.8	5.4
Specialist	6.1	5.5	3.4	3.5	5.3	6.2	6.0	3.6	3.1	3.8	4.7
Hiatus hernia/insufficient LES											
GP	31.8	35.7	38.5	42.5	36.1	38.0	36.2	41.2	42.1	40.4	38.3
Specialist	29.0	29.4	34.6	32.8	28.0	33.8	28.2	30.4	33.7	32.7	31.3
Gastritis											
GP	12.0	16.7	21.0	20.4	25.6	27.7	26.7	22.3	22.7	20.4	21.6
Specialist	16.8	16.0	20.8	37.4	23.1	25.3	23.4	20.5	20.1	19.4	22.3

and reflux disease that was introduced in general practice in the Netherlands in 1996. The number of gastroscopies carried out at the request of internists and gastroenterologists showed a parallel decrease in these ten years. This clearly indicates that the majority of patients with upper abdominal symptoms were no longer referred to the outpatient clinic. The general practitioner deals with the treatment of dyspepsia and reflux in the majority of cases.

The number of UGIEs revealing no macroscopic abnormalities is in accordance with the literature.[7,8]

No abnormal macroscopic findings were detected in approximately 33% of cases. No significant difference was present between open-access UGIEs and procedures done at the request of specialists. UGIEs at the request of specialists more frequently yielded ulcers and Barrett's oesophagus. The explanation could be that ulcers are responsible not only for dyspepsia but more importantly for bleeding (manifest or occult) and anaemia, which are reasons for referral. Patients with Barrett's oesophagus belong to the oldest age cohorts and often have comorbidity, which is a reason for specialist consultation. As the diagnostic yield is almost identical, these results provide very strong evidence in favour of unrestricted open-access policy.[9]

The presence of peptic ulcer disease showed a gradual and significant decrease in the ten-year period. The obvious explanation for this observation is the instalment of anti-*Helicobacter pylori* therapy, which is generally used in cases of ulcer disease since 1993. Also many patients with documented ulcer disease in the past underwent anti-*H. pylori* therapy without confirmation of the diagnosis by UGIE. Another explanation for the decreasing numbers of ulcers is the decreasing acquisition of *H. pylori*.

The number of cases of cancer diagnosed on a yearly basis remained very constant. Each year the same number of cancers, oesophageal as well as gastric, are diagnosed. There is no obvious explanation for this observation, but it is clearly in contradiction with data in the literature, reporting a definite increase in oesophageal cancer and a decrease in stomach cancer.

The results of UGIE with respect to gastritis and malignancy are in accordance with the literature.[7] The explanation for the rise in endoscopic gastritis is obviously the introduction of the video-UGIE. The macroscopic detection of gastritis has improved significantly. The visualisation of the gastric mucosa is much better with the video systems, as more details can be seen. The total number of UGIEs revealing peptic ulceration, carcinoma, oesophagitis, and bulbitis are in keeping with another study in the Netherlands.[10]

The results of this study clearly indicate changes in morbidity patterns. Possibly this has implications for future planning in UGIE, especially since the number of general internists performing gastroscopies is expected to decrease, while there are currently not enough gastroenterologists. These data can be used in planning healthcare expenditures. The increasing numbers of patients with reflux disease (reflux oesophagitis as well as hiatal hernia, or defective lower oesophageal sphincter) implicate a rise in the use of acid suppressive therapy. The decreasing numbers of peptic ulcer, obviously often related to *H. pylori*, point to a decrease in the use of anti-*H. pylori* therapy in the near future. The relation to diagnostic yield of UGIE and ethnicity of the patients must be the goal of future studies since important differences in morbidity patterns can be present if people of different ethnic origins are compared.[11]

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CASE REPORT

Vitamin K deficiency and bleeding after long-term use of cholestyramine

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ABSTRACT

Although it has been long known that in theory the use of cholestyramine can cause coagulopathy due to reduced absorption of vitamin K, only a few cases have been reported. In those cases the coagulopathy occurred within a few weeks to months after the start of therapy. We report a patient with severe pruritus due to intrahepatic cholestasis, who was on cholestyramine therapy for over 25 years before haemorrhage occurred. This case demonstrates that one should be aware of the possibility of depletion of fat-soluble vitamins during the long-term use of cholestyramine.

INTRODUCTION

Pruritus is a distressing manifestation of intrahepatic cholestasis. Severe pruritus, causing unrest and sleep deprivation, needs to be treated. The exact pathogenesis of pruritus in patients with cholestasis remains unknown. One of the possible explanations is bile acid deposition on nerve endings in the skin.[1,2] It is possible that besides bile salts also endogenous opioids play a role in pruritus.[3,4]

The pruritus of intrahepatic cholestasis can be relieved by cholestyramine, an anion exchange resin that binds bile acids. This effective binding leads to the lowering of the free bile salt concentration in the jejunum and reduced enterohepatic circulation of bile salts. As a response, the liver will increase bile salt synthesis. If the compensatory capacity of the liver is reached during cholestyramine therapy, the bile salt concentration will fall, leading to steatorrhoea and malabsorption of fat-soluble vitamins.[5] Until now, four cases of coagulopathy due to cholestyramine therapy have been reported (*table 1*). Recently we saw a patient with a coagulopathy after the use of cholestyramine for more than 25 years.

Table 1
Published cases of hypoprothrombinaemia after treatment with cholestyramine

AUTHORS	TIME BETWEEN START CHOLESTYRAMINE THERAPY AND BLEEDING	BLEEDING TREATED WITH
Gross, Brotman[5]	Three weeks	10 mg vitamin K i.m.
Acuña, Ceron[6]	Two weeks	40 mg vitamin K i.m.
Visintine, Michaels *et al.*[7]	Four months	Parenteral vitamin K
Shojania, Grewar[8]	Eight months	Blood transfusion and 5 mg vitamin K intravenously

CASE REPORT

Sixteen months after birth (October 1974), our patient suffered from his first period of jaundice; the level of bile acids in his blood was 219 mmol/l (normal <10 mmol/l). Cholestyramine therapy was initiated to relieve his pruritus. In the following years recurrent periods of jaundice occurred.

The patient was first diagnosed as having benign recurrent intrahepatic cholestasis (BRIC). Later on, he appeared to have multiple episodes of hepatic inflammation, which is uncommon in BRIC. A liver biopsy showed bile duct paucity, consistent with the diagnosis of Byler's disease (progressive familial intrahepatic cholestasis, PFIC). Patients with Byler's disease type 1 and 2 typically show a low serum gamma-glutamyl transpeptidase (GGT), which is discordant with the severe cholestasis.[9] This was also observed in our patient.

The patient was first admitted in 1990, because of severe pruritus. Ursodeoxycholic acid was tried instead of cholestyramine, but the pruritus worsened and cholestyramine was started again. During the following years he took doses of cholestyramine varying from 8 to 24 g daily. In addition, ursodeoxycholic acid and rifampicin were used intermittently and several other drugs were tried to relieve the pruritus, namely simvastatin, naltrexone and clomipramine.

On 24 August 2000 the patient was admitted to hospital. His ankle had become swollen a week before admission, without any known trauma. He had also noted that a few days after the ankle, his thumb had started to swell too. His general practitioner found microscopic blood in the urine and sent him to hospital.

At the time of admittance the patient was taking cholestyramine 8 g and rifampicin 300 mg, both twice daily. Physical examination showed a moderately ill man. His ankle was red, swollen, shiny and painful. The bleeding was classified as periarticular. His length was measured at 1.76 m, weight 69 kg, temperature 38.2°C, pulse 96 beats/minute and the BP was 130/90 mmHg. Laboratory test showed a prothrombin time (PT) >90 sec (control 12.6 sec), activated partial thromboplastin time (APTT) >120 sec (control 33 sec), thrombin time (TT) 13.6 sec (control 16.5 sec), fibrinogen 8.8 g/l, factor II 5%, factor V 99%, factor VIII 198%, factor IX 4%, factor X 7%, factor XI 174%, factor XII 145%, D-dimer <0.5 and an antithrombin III of 200%. His kidney function was normal. Further laboratory values showed moderately elevated liver enzyme values, except for the GGT which is typical for Byler's disease: total bilirubin was 39 µmol/l, conjugated bilirubin 31 µmol/l, alkaline phosphatase (ALP) 169 U/l, GGT 23 U/l, aspartate aminotransferase (ASAT) 140 U/l, alanine aminotransferase (ALAT) 216 U/l and lactate dehydrogenase (LDH) 899 U/l (haemolytic, later 547 U/l). Urine was positive for blood, with over 30 erythrocytes per viewing and 1-5 leucocytes per viewing. The values of the coagulation factors are typical for a coagulopathy due to a deficiency of vitamin K, with isolated low values for factors II, IX and X. Unfortunately factor VII was not measured. The values returned to normal within 12 hours after giving 10 mg of oral vitamin K. Because of the apparent malabsorption of fat-soluble vitamins, the patient was discharged with the additional medication of vitamins A, E and K. The patient has had no complaints since, except for pruritus, which is still difficult to control with medication.

DISCUSSION

In all four known case reports concerning coagulopathy due to the use of cholestyramine, bleeding occurred soon after the start of therapy, varying from two weeks to eight months.[5-8] In the present case, the bleeding only occurred after using cholestyramine for more than 25 years. This shows that caution should be taken when treating a patient with long-term cholestyramine, even if no sign of coagulopathy is present at the beginning of therapy. There is no known aggravating circumstance for this coagulopathy to suddenly develop in this patient and the patient had no history of bleedings. PT, APTT and TT values from previous years showed normal results.

The malabsorption of the different fat-soluble vitamins during treatment with cholestyramine has been the subject of many studies. Although some show no influence of cholestyramine on the absorption of fat-soluble vitamins,[10-12] others have found malabsorption.[5,9,13-17] It can therefore be concluded that malabsorption of fat-soluble vitamins when using cholestyramine is an existing phenomenon.

Besides cholestyramine, the patient was also taking rifampicin. The influence of rifampicin on vitamin K concentration is a controversial subject,[18,19] but rifampicin might have contributed to the development of the coagulopathy. However, the patient had been on the combined treatment of cholestyramine and rifampicin for two years. It remains unclear if it is necessary to add fat-soluble vitamins to the therapy with cholestyramine. The prevalence of these deficiencies is not known and there are no cost-benefit studies available.

Due to the short half-life and low body storage of vitamin K, it is believed not to be useful to monitor haemostasis on a three to six month basis. Vitamin K deficiency-related coagulopathy can probably develop within a few days. The best option seems to keep in mind that malabsorption can occur and to warn patients that bleeding may happen. In case of vitamin K deficiency, spontaneous cutaneous purpura, epistaxis and gastrointestinal, genitourinary or gingival bleeding often occur. Other types of bleeding can

also develop.[20] The patients should alert their doctor immediately if they notice bleeding without trauma. Then, fat-soluble vitamins should be added to the therapy. Since no severe haemorrhage has been reported thus far, such a wait-and-see attitude seems justified.

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CASE REPORT

Anthranoid self-medication causing rapid development of melanosis coli

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ABSTRACT

It is widely known that long-term use of anthranoid-containing laxatives is the cause of melanosis coli. We describe a case of melanosis coli, which occurred in a 39-year-old liver transplant patient who took an over-the-counter product containing aloe, rheum and frangula. The typical brownish pigmentation of the colonic mucosa developed in a period of ten months. The anthranoid medication was stopped and follow-up colonoscopy one year later showed normal looking mucosa once more. However, in contrast to previous examinations, a sessile polypoid lesion was found in the transverse colon. Histology showed tubulovillous adenoma with extensive low-grade dysplasia. Since there have been preliminary reports suggesting a possible role of anthranoid-containing laxatives in the development of colorectal adenomas and cancer, their use should be discouraged.

INTRODUCTION

Anthranoid-containing laxatives frequently cause melanosis coli, a condition which may be associated with an increased risk for colorectal cancer.[1,2] These products are widely used and self-administered for constipation. Few studies have actually documented how long it takes before the colonic lesions develop.[3] We detected melanosis coli in a patient ten months after he started to use an anthranoid-containing laxative.

CASE REPORT

A 39-year-old patient underwent liver transplantation in 1999 for end-stage primary sclerosing cholangitis. Since 1977 he was also known to suffer from ulcerative colitis which had been clinically quiescent during recent years. In January 2000 surveillance colonoscopy revealed no significant abnormalities. In particular, no abnormal mucosal discoloration was noted (*figure 1*) and colonic histology was normal. In contrast, in January 2001 a further colonoscopy showed a marked brownish pigmentation of the mucosa of the entire colon, compatible with melanosis coli (*figure 2*). Macrophages loaded with pigment were found in all biopsies taken from different areas of the colon (*figure 3*). At the time of the last examination he was being treated with mesalazine, tacrolimus, etidronate, vitamin D, psyllium and occasionally with polyethylene glycol (the last two because of constipation). Further medical history revealed that for the last ten months he had been taking two tablets of another product called 'Rheum Frangula' daily. This product consists of aloe, rheum and frangula, all three anthranoid-containing laxatives, which are known to be the cause of melanosis coli. We advised our patient to stop taking this product. A year later colonoscopy showed normal looking mucosa; biopsies showed no evidence of melanosis. However, in contrast to previous examinations, a large sessile polypoid lesion was found in the transverse colon. Histological examination showed tubulovillous adenoma with extensive low-grade dysplasia. The patient was listed to undergo total colectomy in the near future.

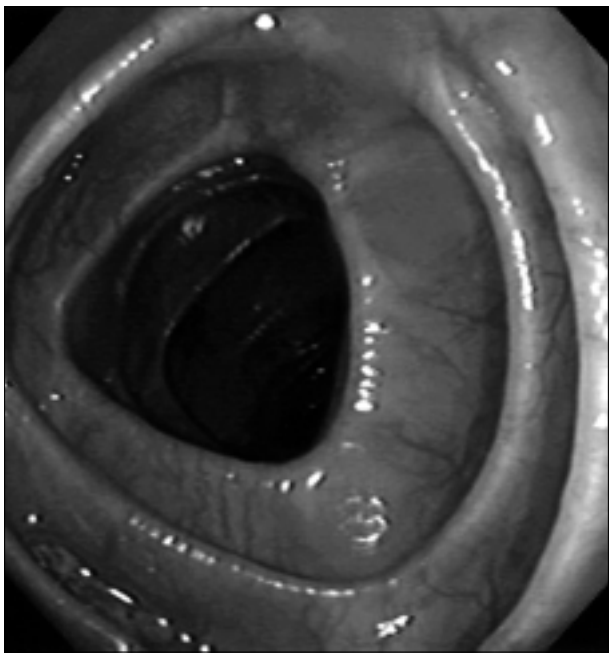

Figure 1
Colonoscopy in January 2000 showing a normal appearing mucosa

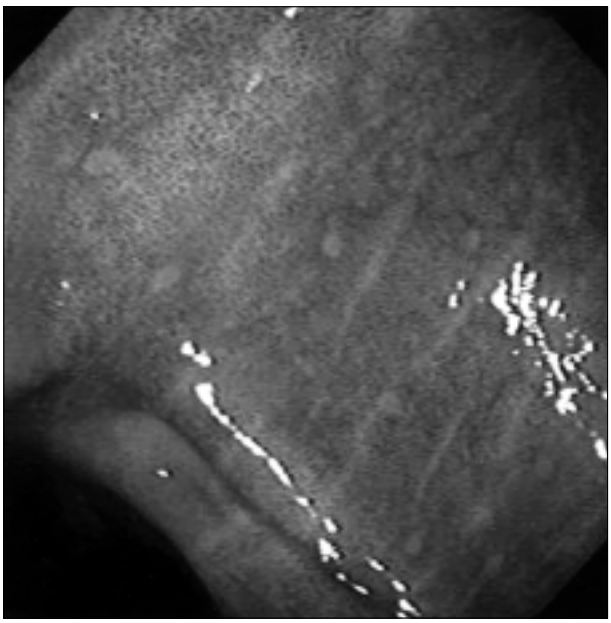

Figure 2
Colonoscopy in January 2001 showing diffuse brownish pigmentation of the colonic mucosa compatible with pseudomelanosis coli

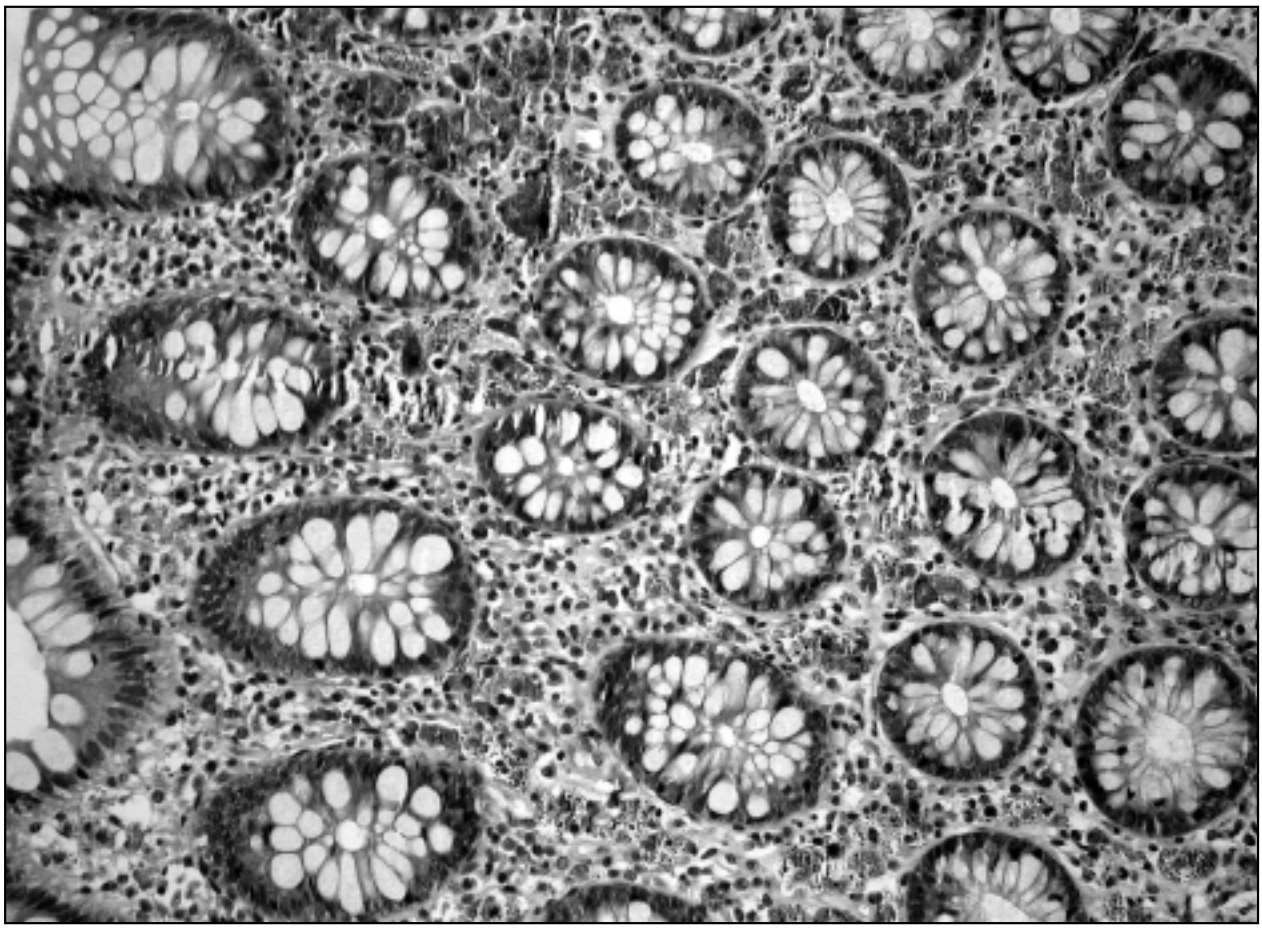

Figure 3
Colonic biopsy showing macrophages loaded with pigment (haematoxylin and eosin staining, 40x)

DISCUSSION

It is well known that anthranoid-containing laxatives frequently cause melanosis coli, a disease entity which can be identified by a brownish pigmentation of the colonic mucosa. Long-term use of anthranoids is generally believed to be necessary to cause melanosis coli. However, as long as 50 years ago it was documented that this condition can develop within periods varying from only 3 to 13 months.[3] A clearly established picture of melanosis coli was found in our patient after ten months.

Anthranoid-containing herbal laxatives damage epithelial cells, leading to changes in absorption, secretion and motility.[4] They can induce cell loss, shortening of mucosal crypts and increased cell proliferation.[2] It remains controversial whether melanosis coli is associated with an increased risk for colorectal cancer, as reported by Siegers *et al.*[1] Other studies, however, have either failed to confirm this[5] or found an increased risk for colorectal adenomas but not for cancer.[6] Our patient clearly was at risk for developing colonic neoplasm considering his long-standing ulcerative colitis in association with primary sclerosing cholangitis and the use of immunosuppressive medication after liver transplantation.[7,8] Therefore, the role of the short-term use of the laxative in the development of this patient's adenoma is highly speculative. From a practical point of view, it may be wise and prudent to discourage the use of anthranoid-containing laxatives, also considering the availability of safe alternatives.

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Netherlands
The Journal of Medicine

CASE REPORT

Enteropathy-associated T-cell lymphoma presenting with eosinophilia

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ABSTRACT

Hypereosinophilia can be related to various diseases; when it occurs without an obvious cause it is called idiopathic hypereosinophilic syndrome (IHES). We describe a patient with increasing eosinophilia, which in spite of extensive diagnostic procedures initially remained unexplained. However, during follow-up it became apparent that this patient had a lethal enteropathy-associated T lymphoma (EATL) causing the hypereosinophilia.

INTRODUCTION

Hypereosinophilic syndromes (HES) may result either from a myeloid neoplasm or from reactive eosinophilia.[1] Reactive eosinophilia is the result of the action of cytokines and chemokines produced by benign or neoplastic T-helper cells or other cells. Infections, allergies, skin disease, connective tissue diseases and malignancies can all lead to cytokine production by T cells. If no cause can be found, the HES is provisionally designated as idiopathic HES (IHES).[2,3]
Primary intestinal T-cell lymphoma is a rare disease and is often related to coeliac disease. Hence, the new WHO classification designates primary intestinal T-cell lymphomas as enteropathy-associated T lymphoma (EATL).[4] A relationship with eosinophilia, however, is seldom reported, in contrast to other types of T-cell non-Hodgkins lymphoma (T-NHL).[5-8]

We describe a patient with persisting and increasing eosinophilia, which in spite of extensive diagnostic procedures initially remained unexplained. However, during follow-up it became apparent that this patient had an EATL.

CASE REPORT

A 68-year-old male without a relevant medical history presented with a two-week history of a mild cough, slight dyspnoea and pain in his left side. Physical examination at presentation did not reveal any specific signs.
Laboratory analyses showed a white blood cell count of 48.8 x 10^9/l with 85% mature eosinophils. The blood film showed no myeloblasts and a normal morphology of eosinophils. Low values of the plasma total protein (54 g/l) and albumin (26 g/l) (reference range 55-65 g/l and 36-46 g/l, respectively) were noted. Other laboratory values were normal, including the IgE level.
Total protein excretion in the faeces varied between 1.2 and 1.5 g/24 hours, consistent with protein-losing enteropathy. An extensive work-up for allergic, connective tissue, malignant and infectious diseases was negative.
The bone marrow aspirate was hypercellular with 65% mature eosinophils, without evidence of acute leukaemia or chronic myeloproliferative disease. Cytogenetic analysis of the bone marrow showed clonal loss of the Y chromosome. Flow-cytometric analysis of peripheral blood revealed no abnormalities. On duodenoscopy, flattened mucosal folds were seen. The histology showed subtotal villous atrophy, with denudation focally increased intraepithelial lymphocytes (IEL) and cytoplasmic vacuolation indicative of malabsorption. The lamina propria contained an

increased chronic infiltrate with abundant eosinophils. The biopsy was signed out as 'villous atrophy not typical for coeliac disease'. The CT abdomen showed no abnormalities. A provisional diagnosis of IHES was made with probable involvement of the small intestine responsible for a protein-losing enteropathy. Four months later the absolute eosinophil count rose from 48.8 x 10^9/l to 159.0 x 10^9/l in four weeks, with lowering of the serum albumin (24 to 17 g/l). Therapy was started with 60 mg of prednisolone a day, initially leading to a decrease in the eosinophil count to 138 x 109/l, followed by an increase two weeks later. Treatment with hydroxyurea (3 g a day) was initiated because of therapy-resistant IHES. Three days after starting hydroxyurea, the patient was admitted to hospital because of an acute abdomen and an exploratory laparatomy was performed. A mass was found in the distal part of the jejunum and proximal ileum with perforation. Postoperatively the patient became septic and died three weeks after the initial operation because of total respiratory insufficiency due to acquired respiratory distress syndrome (ARDS). At that time the eosinophil count again rose to 265 x 10^9/l, despite continuing hydroxyurea therapy. Consent was not given for a post-mortem examination. Histopathological examination of the resected specimen revealed a bulky transmural tumour with ulceration of the mucosa and localisation in mesenteric lymph nodes. The tumour predominantly consisted of eosinophils intermingled with sheets of monomorphic medium-sized blasts. The adjacent mucosa showed subtle architectural changes without villous atrophy but with a focal increase of IEL.

Detailed immunophenotypic analysis of the, morphologically normal, intraepithelial and subepithelial lymphocytes revealed a substantial population of T cells with an abnormal phenotype: CD2+, CD3+, CD4+, CD7+ and CD5-, CD8- and CD30-. The reversed CD4/CD8 ratio, as compared with IEL of the normal intestine and in uncomplicated coeliac disease, and loss of CD5 were indicative of a neoplastic T-cell proliferation. The phenotype of the tumour mass was CD7+, CD43+, CD45R0+, TIA1+ and focally CD30+, CD2-, CD3-, CD4-, CD5-, CD8-, CD56-, ALK-, CD68-, MPO-, CD79a- and Granzyme-B-.
Additional DNA clonality studies using a PCR heteroduplex analysis of the *TCRG* genes revealed clonal VγI-Jγ1.1/2.1 and VγI-Jγ1.3/2.3 PCR products in the studied colon tissue biopsy,[9] thereby supporting the diagnosis T-NHL.[9,10] Based on the combined histopathological, immunophenotypic and molecular genetic findings we hypothesise that this patient had an EATL associated with a progressive HES.

DISCUSSION

This patient presented with eosinophilia initially interpreted as IHES with involvement of the small intestine, which has been described earlier.[11,12] Conventional cytogenetic analysis of bone marrow cells revealed the clonal loss of the Y chromosome. The frequency of cells with Y loss increases with age and is significantly greater in cases with myelodysplastic syndrome (MDS), myeloproliferative disorder (MPD), B-cell disease and especially acute myelogenous leukaemia than in controls.[13]
However, the follow-up showed the eosinophilia to be a paraneoplastic sign and the leading symptom of a T-NHL classified as an EATL. Although in principal all T-NHL can give rise to eosinophilia this is mostly associated with unspecified peripheral T-NHL.[4] Only a few cases associated with EATL are reported.[5-8] Our case illustrates that in the differential diagnosis of a HES it is insufficient to evaluate T-NHL as a general entity: all clinicopathological entities as described in the WHO classification should be evaluated rigorously.[4]
EATL is difficult to diagnose in an early stage as this disease usually presents with massive abdominal tumour load and perforation. However, in our case, in retrospect the duodenoscopy and biopsy findings were typical for ulcerative jejunitis and provided a clue to the nature of the underlying disease.[7] Because of the known aggressive course and therapy resistance, this most probably would not have changed the clinical outcome. As our case demonstrates, in clinical practice general imaging studies may not be sufficient to rule out the possibility of T-NHL in patients with massive eosinophilia.

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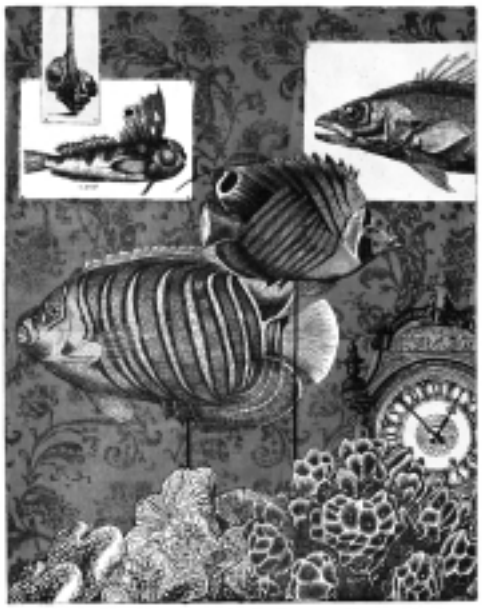
ABOUT THE COVER

'Interior of an ichthyologist'

Annemarie Petri



This month's cover, entitled 'Interior of an ichthyologist', shows an etching made by Annemarie Petri. Annemarie (1965) works and lives in The Hague, where she attended the Free Academy from 1983 to 1989. Different works of art interest her, such as mixed media, graphics and etching. This etching is a multiple colour print. While designing such etches she uses the collage technique, which is made up from fragments of old engraving, book illustrations and wallpaper.

The subject she choose, 'interiors of scientists of nature', expresses the binomial attitude that adults have regarding imagination. On the one hand, the desire of a playing child to enter fully into a world of imagination and on the other hand, the consciousness that this world of fantasy is not real.

A limited edition of original prints (size 36 x 46 cm) of this month's cover is available at a price of € 285. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.