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PHOTO QUIZ: Progressive trichomegaly, see page 35

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Editorial office 'The Netherlands Journal of Medicine'
Geeralien Derksen-Willemsen
Radboud University Nijmegen Medical Centre
Department of General Internal Medicine 463
PO Box 9101
6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59
Fax: +31 (0)24-354 17 34
E-mail: g.derksen@aig.umcn.nl
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EDITORIAL

A new editorial team for *the Netherlands Journal of Medicine*

M. Levi

Department of Medicine, Academic Medical Center, University of Amsterdam, the Netherlands, tel.: +31 (0)20-566 21 71, fax: +31 (0)20-691 96 58

The new year 2009 will mark the transfer of the editorial team of *the Netherlands Journal of Medicine* from Nijmegen to Amsterdam. After seven years, the Nijmegen group of Internal Medicine, under the supervision of Editors-in-Chief Jos van der Meer and subsequently Anton Stalenhoef,[1] will pass the responsibility to the Department of Medicine of the Academic Medical Center in Amsterdam.

First, many thanks and congratulations are in order for the Nijmegen editorial team, as they did a splendid job in managing the Journal for so many years. Maybe the utmost goal of journal editors is to succeed in leaving the Journal in a better shape than when they took over. It is more than fair to say that the Nijmegen team has achieved that goal. *The Netherlands Journal of Medicine* has steadily improved its quality over recent years and many interesting innovative additions were made to the Journal, such as the popular photo quiz. Importantly, the Journal is listed in the Index Medicus and appears in PubMed and other scientific databases soon after its publication, and is easily accessible through the web. All these improvements have resulted in an almost doubling of the impact factor under the Nijmegen reign. The Netherlands Society of Internal Medicine is very grateful to the Nijmegen editors, and in particular to Jos van der Meer, Anton Stalenhoef, Paul Smits, Joost Drenth, Jack Wetzels and Theo Thien, for their excellent work.

The Amsterdam group has a very difficult job on its hands if in a few years we are to leave a Journal that is in an even better shape than it is now. However, that is certainly our ambition and we are happy that we can build on the solid foundations that have been laid by our predecessors. The Department of Medicine at the Academic Medical Center has decided to form a large group of associate editors from each of the subdisciplines of medicine to cover the entire spectrum of our speciality. In addition, a group of junior associate editors, consisting of residents in training for internist and with ample research experience, has been formed to assist with peer review and editorial decision taking. The Journal will focus somewhat more on excellent review papers from national and international experts in their field and will continue to serve as a platform for the publication of interesting, original and clinically relevant case reports and case series,[2] including the popular photo quiz.

We are confident that *the Netherlands Journal of Medicine* will remain a high-quality monthly journal that is relevant for all specialists interested in internal medicine across the world and our ambition is to further improve the fame and reputation of the Journal in the coming years.[3] We hope and expect to receive the same support and positive spirit from the scientific-medical community in the Netherlands and abroad that our predecessors have met during their editorship and we foresee another series of fruitful years for *the Netherlands Journal of Medicine*.

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REVIEW

Adult issues in phenylketonuria

M.P.A. Hoeks[1], M. den Heijer[2], M.C.H. Janssen[1*]

Departments of [1]General Internal Medicine and [2]Endocrinology, Radboud University Nijmegen Medical Centre, the Netherlands, [*]corresponding address: tel.: +31 (0)24-366 62 60, e-mail: M.Janssen@aig.umcn.nl

ABSTRACT

Phenylketonuria (PKU) is a classical example of an inherited metabolic disease, in which mental retardation can be prevented successfully by using a diet. However, in adult PKU new problems occur, such as vitamin deficiencies, osteoporosis and the maternal PKU syndrome. The aim of this review article is to provide guidelines for the clinician to understand and manage PKU in adults.

KEYWORDS

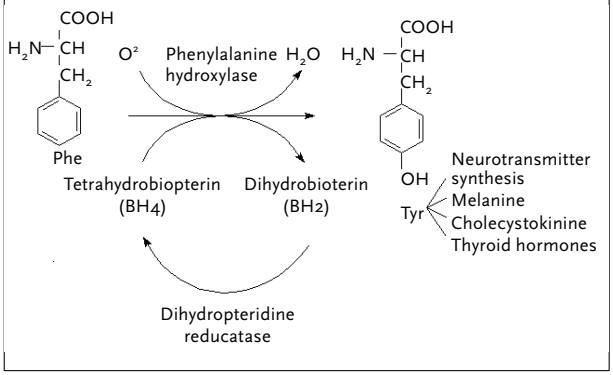
Adults, complications, phenylketonuria

INTRODUCTION

Phenylketonuria (PKU) is a hereditary metabolic disease, which is seen in approximately 1:18,000 newborns in the Netherlands.[1] In most cases it results from a deficiency of phenylalanine hydroxylase (PAH), an enzyme necessary for the conversion of phenylalanine (Phe) into tyrosine. Hyperphenylalaninaemia is the biochemical hallmark of PKU. Defects in the recycling of the cofactor required for PAH activity, tetrahydrobiopterin (BH4), can result in hyperphenylalaninaemia as well (*figure 1*). BH4 deficiency accounts for approximately 2% of the patients with elevated Phe levels.[2] Since 1974 all newborns in the Netherlands are tested for PKU within eight days after birth.[1]

The mode of inheritance of PKU is autosomal recessive. Nearly all cases are caused by mutations in the gene encoding PAH, which has been mapped to human chromosome 12q24.1.[3] Clinical and molecular studies have indicated that PAH deficiency is a highly heterogeneous disease.[4] To date, more than 400 different mutations have been characterised in the PAH gene in various ethnic groups. Genotype at the mutant PAH locus influences the biochemical phenotype in heterozygotes.[5]

Figure 1. *The metabolism of phenylalanine*



If untreated, PKU leads to the development of mental retardation and neurological disturbances.[6] Hyperactivity may occur as well as hypopigmentation and eczematous rash. The exact mechanism by which the elevated concentration of Phe causes mental retardation is unknown. Several hypotheses have been described. High Phe values can have a direct deleterious effect on the myelinisation *in cerebro*.[7] Another mechanism can be the high affinity of Phe for the transport across the blood brain barrier. This transporter is also used by e.g. tyrosine and tryptophan, which are precursors of dopamine and serotonin. Therefore, high Phe concentrations can lead to disturbances in dopaminergic and serotonergic neurotransmission.[8,9]

DIAGNOSIS

The diagnosis of PKU is based upon the finding of an elevated serum concentration of Phe (reference value 35 to 76 µmol/l). Classical PKU or just PKU is historically defined as a blood Phe level >1200 µmol/l, which indicates a complete deficiency of PAH. At that level phenylketones appear in the urine. In mild/atypical PKU levels of 600 to 1200 µmol/l are seen and hyperphenylalaninaemia (HPA) is classified

as blood Phe levels between 240 and 600 µmol/l.[10] In the Netherlands a successive BH4 loading test is performed to detect a possible BH4 deficiency.[11] The most useful laboratory method for newborn screening is tandem mass spectrometry.[12] Other methods include the Guthrie bacterial assay, fluorometric and chromatographic analysis.

PRINCIPLES OF TREATMENT

The principle of treatment in PAH deficiency is to reduce the blood Phe concentration sufficiently to prevent brain damage. The cornerstone of PKU treatment is dietary protein restriction, supplemented with all amino acids except Phe. It should be initiated as soon as possible after diagnosis.

One of the most controversial issues is whether to stop or continue dietary treatment throughout adulthood. Before 1970, clinicians believed that dietary treatment was only necessary until the end of childhood.[13,14] The rationale for this approach was that the brain is only vulnerable to the toxic effects of elevated Phe in the period of its maximal myelinisation throughout childhood.[15] Later on, concerns were raised about the safety of discontinuing the diet. Several studies suggested that elevated Phe concentrations in adults adversely affect cognitive function, neuropsychological and psychiatric outcome.[12] Apart from in France, this led to the recommendation of 'diet for life' in most treatment centres, although recommendations about blood Phe levels do vary. The actual recommendation of 'diet for life' is based on lack of sufficient evidence to support the relaxation of diet in adults. Channon *et al*. found that after discontinuing the diet, only subtle changes in cognitive performance occurred compared with adolescents who remained on a diet.[16]

According to the Dutch guidelines of management of adult PKU, the target blood Phe level is 120 to 600 µmol/l. In Germany the target level is 40 to 1200 µmol/l, in the UK 120 to 700 µmol/l and in the USA 120 to 900 µmol/l. In our centre we encourage those on diet to monitor regularly (one to three monthly) and take their nutritional supplements with the aim to keep the Phe level <600 µmol/l. There is a very real risk of vitamin B12 and other micronutrient deficiencies in those patients restricting their protein intake, but not taking vitamin supplements. Therefore, for those who choose to be off diet, we recommend that they eat a completely normal diet with full protein and vitamin intake. Side effects of dietary treatment such as vitamin deficiencies and osteoporosis, possibly due to long-standing dietary deficiency of protein, calcium, vitamin D, vitamin B12 or trace elements, can be prevented by this approach. The role of essential fatty acid supplementation in the diet of PKU patients remains to be explored.

The poor palatability of protein substitutes leads to a decrease in compliance with the diet, especially in adolescents. Furthermore time constraints, social pressure and dissatisfaction with restrictions challenge dietary compliance.[17] Consequently, the need for an alternative treatment is growing.

Many new approaches have been discovered in the last ten years. The most ultimate and definitive therapy for PKU patients would be a liver transplantation, because it would correct the molecular disorder. But it is too complicated and hazardous to be justifiable as an appropriate substitute for the diet.[18] However, the availability of the classic effective treatment militates against a therapy requiring immune suppressive therapy, with its attendant complications.

In 1999, it was discovered that some PKU patients can benefit from administration of the cofactor BH4. Many studies confirmed the decrease on the blood Phe levels.[19-21] In a large study, 38% of the PKU patients (not BH4 deficient) responded to BH4 administration with a decrease of ≥30% in Phe. The prevalence of responsiveness was unfortunately highest in patients with mild forms of PKU. Only 7 to 10% of the patients with classical PKU responded to BH4 therapy.[21] BH4 therapy may be used as an adjunct to dietary restriction. However, BH4 is not registered in the Netherlands and may have uncertain long-term availability; also there is minimal clinical experience and the potential costs may be high.

Another treatment that may be promising is the use of oral large neutral amino acids (LNAA). LNAA (e.g. phenylalanine, tyrosine, tryptophan, leucine, isoleucine) compete with each other at the blood brain barrier. High blood levels of LNAA can result in carrier saturation and competitive inhibition of Phe, resulting in a decrease in Phe.[22] LNAA cross the intestinal mucosa by carrier proteins similar to those of the blood brain barrier. The affinity of the LNAA at the blood brain barrier is much higher than in the intestinal mucosa, so high concentrations must be present in the intestines in order to influence brain Phe levels.

A double-blind, randomised, controlled trial with LNAA therapy showed a significant decrease in blood Phe levels with a mean of 39% from the baseline (from 932 µmol/l to 568 µmol/l).[22] An advantage compared with BH4 therapy is the lowering of Phe levels in all PKU patients, not only in milder forms. LNAA therapy is not available in the Netherlands at present.

Brain Phe is deducted from the blood Phe levels, but a linear relation may not be present. New techniques to noninvasively assess brain Phe would be most welcome.

At a more basic level, new modalities in PKU treatment are being studied, for example: somatic gene therapy and enzyme therapy.[23-27] However, these efforts have not led to an effective treatment so far. Although all of the new approaches may be useful in the future, the dietary treatment of PKU remains the most important clinical tool in PKU.

COMPLICATIONS IN ADULTHOOD

The adult complications in PKU consist of two components. On the one hand complications of high Phe levels can be seen, such as neurological and (neuro)psychological problems. On the other hand, complications occur as a cause of dietary protein restriction, for example vitamin deficiencies and osteoporosis.

NEUROLOGY/NEUROPSYCHOLOGY

Adults with PKU who were treated from early infancy have normal intelligence, but their neuropsychological test scores are somewhat lower than those of the general population, their parents, and unaffected siblings.[28] In many functional domains, such as motor speed, speech, language and some aspects of memory, treated PKU patients perform comparably with healthy controls. However, the domains of abstract reasoning, executive functioning, and attention have been identified as areas of weakness despite adhering to the diet.[29] Even continuously treated PKU patients show decreased performance at neuropsychological tests, which improves when Phe levels are lowered by a more strict PKU diet.[30]

Furthermore, adults with PKU who were continuously treated but relaxed the diet have displayed white matter abnormalities on structural magnetic resonance imaging,[31,32] which are considered to be indicative of a reduction in myelin.[33,34] These abnormalities disappear after reintroducing a strict diet. The hypothesis suggests that elevated Phe concentrations impact on myelin production and maintenance. Using a murine model, Dyer *et al*. have shown that Phe can inhibit the activity of HMG-CoA reductase, which leads oligodendrocytes to develop into non-myelinating oligodendrocytes. A strict diet on the other hand normalises the HMG-CoA reductase activity and produces myelinating oligodendrocytes.[34] However, this theory has not yet been confirmed in humans.

Neurological investigations in early-treated adults with PKU who discontinued the diet reveal a higher incidence of minor neurological signs including tremor, brisk deep tendon reflexes or clumsy motor co-ordination.[31,35,36] In rare cases severe neurological deterioration may occur after cessation of dietary treatment. The symptoms, including spastic paresis, late-onset epilepsy, ataxia and tremor, are very similar to those of untreated PKU patients.[35,37] Poor early control seems to be a risk factor in all reported cases. Neurological deterioration has not yet been reported in early-treated PKU patients.[38] However, it cannot be excluded that further neurological deterioration could emerge later in life.[36] In all patients with late-onset neurological deterioration it is mandatory to exclude a possible vitamin B12 deficiency.[38]

PSYCHOLOGICAL PROBLEMS

Severe behaviour and psychiatric problems are seen in profoundly retarded (untreated) adults with PKU in the third and fourth decade of life.[39] With introduction of a Phe-restricted diet, these symptoms are sometimes reversible.[40] It has been reported that early-treated adults with normal intelligence whose diet has been discontinued developed psychiatric and/or psychological problems, including depression, anxiety, social withdrawal, agoraphobia, low self-esteem, neurotic behaviour, and other phobias.[41-43] Other studies have found no difference between PKU patients and sibling controls on psychological outcome.

Several studies showed reduced levels of noradrenalin and serotonin in PKU patients which has been linked to panic attacks,[44] and depression or agoraphobia,[45] respectively. High blood Phe levels lead to disturbances in dopaminergic and serotonergic neurotransmission through the same mechanism as described earlier.[44,46,47] Koch *et al*. showed a clinical improvement in depressed PKU patients, who were treated with LNAA.[48]

VITAMIN DEFICIENCIES

Vitamin B12 is only found in animal protein, the sources being meat, fish and poultry. Since the PKU diet is restricted in protein, vitamin B12 must be provided either combined with the amino acid supplement or from a separate supplement. Vitamin B12 deficiency can occur when PKU patients relax their diet in adolescence.[15] They tend to choose products that are low in animal protein and amino acid supplements are not taken properly.[49] Vitamin B6, folic acid and iron can also be deficient in a PKU diet, although it occurs less frequently than vitamin B12 deficiency. Methylmalonic acid (MMA) and homocysteine are both highly sensitive markers to assess vitamin B12 deficiency at tissue level.[50]

Vitamin B12 serves as a co-factor for two enzymes: L-methylmalonyl-coA mutase and methionine synthetase. Deficiency of vitamin B12 will lead to higher concentrations of homocysteine and MMA.[50] Vitamin B12 deficiency can cause a wide range of clinical symptoms including neuropathy, subacute combined degeneration of the cord, glossitis, anaemia, dementia and psychiatric states such as depression and psychoses.[15] The clinical manifestations of a vitamin B12 deficiency appear years after the start of an inadequate intake.[51] The large body storage, long half-life and the efficient re-absorption in the intestines are the main reasons.[52] However, low serum vitamin B12 levels correlate in only 25 to 50% with a vitamin B12 deficiency at tissue level.[53] Combined deficiencies of iron and vitamin B12 are frequently seen

in PKU patients, so haematological parameters such as haemoglobin and mean corpuscular volume are not good indicators for vitamin B12 deficiency.

OSTEOPOROSIS

Osteoporosis is an important cause of morbidity and mortality in later life. Diagnosis is usually made following a fracture but may be preceded by an asymptomatic period of osteopenia. One of the predisposing factors to fracture is thought to be failure in achieving an optimal peak bone mass in early adult life.[51] Modan-Moses *et al.* showed a decreased peak bone mass in PKU patients.[52] Many other studies have shown a decreased bone mineral density (BMD) in patients with PKU.[53-55] The exact mechanisms of osteopenia and osteoporosis in PKU and HPA patients remain unclear. Possible explanations include long-standing dietary deficiency in protein, calcium, vitamin D or trace elements, or a primary defect in bone turnover inherent to the disease itself.[52] PKU patients may be at risk for osteoporosis in later life. However, there is no evidence that a life-long dietary treatment can prevent or treat osteoporosis.

MATERNAL PHENYLKETONURIA

One situation where a phenylalanine-restricted diet is mandatory is for women with PKU and HPA who are pregnant or planning a pregnancy. Charles Dent was one of the first physicians to recognise the teratogenic effects of maternal Phe on the foetus in 1956.[56] These effects include mental retardation, facial dysmorphism, microcephaly, intrauterine growth retardation (IUGR), developmental delay and congenital heart disease (CHD).[56,57] In untreated pregnancies of women with PKU and Phe levels ≥1200 µmol/l, more than 90% of the offspring have microcephaly and mental retardation, 40% have IUGR and 12 to 15% have CHD. When the range of Phe is between 600 and 1200 µmol/l these teratogenic effects are less frequent, and when dietary treatment lowers the blood Phe level to 120 to 360 µmol/l, the offspring may be normal. This indicates a dose-response relation.[58]

During pregnancy, the placenta naturally selects for higher concentrations of amino acids, including Phe. There is an active transplacental Phe gradient during pregnancy. Phe levels are amplified (1.5 to 2.0 times) which leads to high Phe levels in the foetal circulation.[59] During the early stages of embryogenesis high Phe levels can have a deleterious effect on neural crest cell migration, thereby explaining facial dysmorphisms and cardiac anomalies. During fetogenesis Phe has deleterious effects on neuronal multiplication and myelinisation.[60] However, these hypotheses have only been tested in animal studies.

Not only the blood Phe levels are of importance. Too little maternal weight gain and low intake of either protein or calories can also interfere with foetal growth.[61]

The most important weapon for preventing embryopathy is preconceptional information, since a good metabolic state must be achieved before conception. However, it is still beneficial to the foetus to reduce levels within the first trimester in case of an unexpected pregnancy.

The Dutch guidelines recommend blood Phe levels of 120 to 240 µmol/l in pregnancy. It is advised to determine Phe levels once or twice a week. After three months, tyrosine should be supplemented. The critical period for the nervous system, cranium and heart is at five to eight weeks of pregnancy. Consequently, good metabolic control must be achieved before week 5, to prevent teratogenic effects on the developing foetus.[59] Recent data suggest that swings in blood Phe levels, even within the target ranges, have significant impact on the offspring neuropsychometric outcome.[62]

Nowadays, few teratogenic effects on newborns are seen, thanks to good metabolic control. Hanley and colleagues have shown that a small number of untreated or poorly treated PKU pregnancies result in normal offspring, while a few apparently well-treated pregnancies are not successful. The exact mechanisms remain unclear. However, teratogenesis is a multi-factorial phenomenon with protective and toxic mechanisms, so that poorly treated PKU mothers can have normal offspring, and apparently well-treated PKU pregnancies are not successful.

RECOMMENDATIONS

PKU patients should be encouraged to remain on a lifelong diet and take their nutritional supplements. Blood Phe levels should be monitored every three months. It is recommended to do a clinical review on patients every year. Not only dietary status and blood Phe level should be monitored, but all other items in *table 1* need to be considered. Full amino acid spectrum is recommended. A dedicated dietician should be part of the team caring for PKU patients. It is advised to assess bone marrow density (BMD) every two to five years. Treatment with calcium and vitamin D supplements should be given in every patient with PKU and a low BMD. Furthermore, women need to be informed about strict dietary control in (intended) pregnancy. All pregnant PKU patients should be under the control of a physician specialised in metabolic diseases, a dietician and a gynaecologist and should be offered a detailed ultrasound at 20 weeks of gestation. Pregnant PKU and HPA woman should be seen every three to four weeks and blood Phe levels must be monitored at least once a week.

Table 1. *Guidelines in the management of phenylalanine*

Laboratory values (once a year)	Additional tests
Phenylalanine (every 3 months)	Dual X-ray
Amino acid spectrum	Absorptiometry (every 2-5 years)
Vitamin B12	
Vitamin B6	
Folic acid	
Methylmalonic acid	
Iron status	
Ferritin	
Calcium	
Vitamin D	
Albumin	
Haemoglobin	
Mean corpuscular volume	
Creatinine	

CONCLUSION

PKU is a success story. Its ascertainment and treatment from early infancy has led to excellent outcomes. At the same time new problems occur with the PKU patients getting older. Knowledge of the adult issues in PKU will lead to a better understanding among clinicians and can overcome these difficulties, resulting in (almost) normal lives for PKU patients. Still many aspects of adult PKU remain unclear and need further elucidation in the near future.

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REVIEW

Eosinophilic oesophagitis: an enigmatic, emerging disease

R.A. Sprenger[1*], J.W. Arends[3], J.W. Poley[4], E.J. Kuipers[4,5], F. ter Borg[2]

Departments of [1]Internal Medicine, [2]Gastroenterology and [3]Pathology, Deventer Hospital, Deventer, the Netherlands, Departments of [4]Gastroenterology and Hepatology, and [5]Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, the Netherlands,
[*]corresponding author: tel.: +31 (0)570-64 66 66, email: r.a.sprenger@dz.nl

ABSTRACT

Eosinophilic oesophagitis is a disease that has emerged in recent years. It is often associated with dysphagia and oesophageal food impaction in adults. The disease is characterised by infiltration of eosinophilic granulocytes into the oesophageal mucosa. This infiltrate may be responsible for the subtle peristaltic abnormalities that can be found in these patients. Endoscopic findings are usually absent or nonspecific, although a discrete circular ring pattern of the mucosa may be noticed. Occasionally, overt endoscopic abnormalities (such as exudative changes and shearing of the mucosa) can be found. The presence of at least 15 intraepithelial eosinophilic granulocytes per high-power field in random biopsies from the whole length of the oesophagus is considered to be diagnostic. Gastro-oesophageal reflux needs to be excluded as it may lead to eosinophilic infiltration as well. Adequate diagnosis is relevant for treatment and the prevention of unnecessary further investigations. The disease responds well to the ingestion of fluticasone propionate and its long-term prognosis is generally good. But when fluticasone is discontinued recurrent symptoms are common, and some cases are severe, needing treatment with systemic corticosteroids.

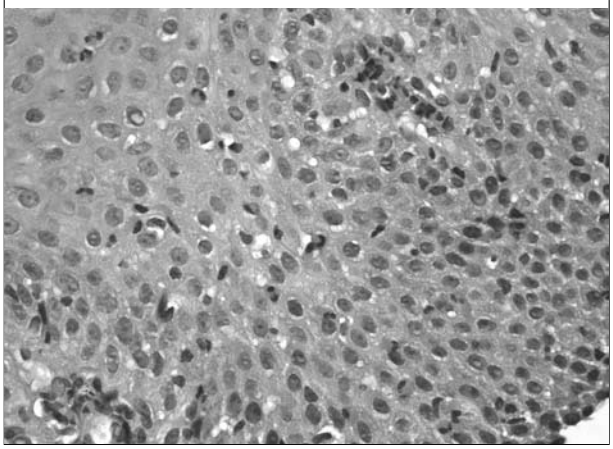
KEYWORDS

Eosinophilic oesophagitis, dysphagia, food impaction, intraepithelial eosinophilic granulocytes

CASE REPORT

A 50-year-old, previously healthy man presented to the emergency department because of complete obstruction of the oesophagus after eating meat. For several years he had been visiting the emergency department occasionally with the same problem. Repeated upper gastrointestinal (GI) endoscopies had never shown any abnormalities. Between these events the patient suffered from mild dysphagia that could be managed by drinking water. The patient had not changed his diet nor suffered weight loss. Treatment with high-dose omeprazole did not improve his symptoms. Recent extensive evaluation including 24-hour ambulatory pH monitoring, manometry and barium X-ray of the oesophagus had revealed no abnormalities. Emergency endoscopy showed obstruction of the oesophagus due to an impacted food bolus. After endoscopic removal no mucosal abnormalities or stenosis were visible. Multiple biopsies were taken from the mucosa in the proximal and distal oesophagus. Histological examination showed a marked intraepithelial infiltration consisting of >20 intraepithelial eosinophilic granulocytes (IEGs)/high-power field (HPF) (*figure 1*). The diagnosis of eosinophilic oesophagitis (EO)

Figure 1. *The oesophageal epithelium shows a prominent eosinophilic infiltrate (magnification inset: 400x)*



was made. Treatment with fluticasone propionate (FP) ('spray-and-swallow') 250 µg twice daily was instigated which led to complete resolution of symptoms. After nine months the patient stopped his medication of his own accord. Six weeks later he presented to our emergency department again with food bolus obstruction. Since then, continuous fluticasone treatment has been given and he has remained free of symptoms for more than a year of follow-up. He did not develop oropharyngeal or oesophageal candidiasis.

EPIDEMIOLOGY

EO was first described by Dobbins *et al*. in 1977.[1] Between approximately 1985 and 1995 it was considered to be a rare disease, primarily affecting children and adolescents. After 1995, an increasing number of affected adults have been reported.[2] The annual incidence of EO has been estimated to be around 1:100,000 children in Ohio, with an increasing prevalence reported in Switzerland over the last decade.[3] The prevalence was studied in a random sample of the Swedish population who underwent upper GI endoscopy for other reasons and about 1% of individuals met the histological criteria of EO.[4] It is estimated that the prevalence of EO is around 1:2500 in children and 1:4000 in adults.[5] The male-to-female ratio is approximately 3 to 1. More than 95% of reported cases are of the Caucasian race,[2,6] although it remains unclear whether this reflects true racial difference or is due to selection bias.

PATHOGENESIS

The cause of this disease is not known. In health the oesophagus has no eosinophils.[7] The recruitment of eosinophils is observed in a variety of inflammatory or infectious conditions and exposure to food and aeroallergens.[8,9]

The presence of intraepithelial eosinophilic granulocytes in the oesophageal mucosa – as anywhere in the digestive tract – may be a nonspecific phenomenon. It can be caused by any irritating agent, noticeably acid or non-acid refluxate, non-steroidal anti-inflammatory drugs or food stasis due to oesophageal motility disorders such as achalasia and systemic sclerosis.[7] Furthermore, eosinophilic infiltration of the oesophageal mucosa has also been found in asymptomatic subjects. Whether these findings represent 'early' EO and may progress to symptomatic EO or are incidental findings is not known.[10] We do know that during an allergic response, tissue injury, or infection eosinophils can be major effector cells and are able to release chemokines, lipid mediators, cytokines, and cytotoxic secretory products.[7]

Although eosinophils seem to play a major role in EO it is not the only critical contributor. A combination of environmental exposure, allergen sensitisation, eosinophils and other cells, molecules released and genetic predisposition, all interplay in EO pathogenesis.[11]

Most studies characterising the allergic phenotype have been performed in children.[6]

The high prevalence of atopic constitution among patients with EO and the good response of EO to elimination or elemental diets reinforce the link between the disease and the allergic aetiology.[11-14] The presence of food sensitisation and the response to elimination diets insinuate an immunoglobin (Ig)E-dependent mechanism. Although, as only a minority of EO patients present with food anaphylaxis, it indicates a distinct mechanism. As such, a local oesophageal population of allergen specific IgE producing B cells is possible.[11] Or there may be a mixed IgE- and non-IgE-mediated reaction. On the basis of allergy testing results EO is thought to be a polygenic allergic disorder.[15] Intriguingly patients with EO have sometimes reported seasonal variations in symptoms and oesophageal IEG levels,[16,17] suggesting it is not only a food but also an aeroallergen hypersensitivity.

Furthermore EO seems also to be associated with Th2-type immune responses and local or systemic Th2 cytokine overproduction.[11] American investigators showed that not only food but also environmental allergens induced a significantly higher production of specific Th2 cytokines (IL-5 and IL-13) by peripheral blood mononuclear cells (PBMCs) in patients with EO compared with healthy controls.[18]

Animal models have linked EO and allergic diseases and assess the sensitisation pathways that could occur in human EO.[11] Experimental models can be induced in mice by sensitisation or exposure, as well as by administration or overexpression of specific Th2 cytokines.[11,19,20] The experimental models demonstrate an intimate connection between the development of eosinophilic inflammation in the respiratory tract, skin and oesophagus, not only to external allergic triggers but also to intrinsic Th2 cytokines.[11,21,22]

A genetic predisposition for EO has been reported in children. An abnormal gene encoding for eotaxin-3, a key promoter of eosinophil attraction and inflammation, was found in nearly half of the children with EO.[23]

Of note, allergy and genetics do not explain all patients with EO. In adults the prevalence of atopy is much smaller and no genetic association has been established.[24]

Just like the aetiology, the development of dysphagia in EO remains a mystery. An important hypothesis on this field is the role of oesophageal dysmotility. Certain biologically active components, produced by IEGs, may induce motility disorders,[9] although there is no strong evidence and the

precise role in EO remains far from elucidated. Technological advances such as high-resolution manometry and combined manometry with impedance may provide new insight into the more subtle motility abnormalities.[25] It must also be noted that it remains uncertain whether the eosinophilic infiltration of the mucosa is the primary cause of dysmotility or the result of mucosal irritation by food stasis and impaction.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The single most common presenting symptom of EO is intermittent or persistent mild dysphagia occasionally leading to food impaction, which occurs in more than 90 and 60% of cases, respectively.[2,6] These symptoms do not lead to weight loss.[26] Endoscopic abnormalities may be absent or inconspicuous. But when present, typical findings are a fine ring pattern, vertical furrows, white spots or mucosal fragility.[2,8,27-30] Recently a prospective study showed that the prevalence of EO in patients with solid food dysphagia and a normal-appearing oesophagus is approximately 10%.[3] And only one third of the patients with EO had any of the typical findings. It was also observed that when one of the typical findings was present, EO was only histologically present in 38% of the cases.[3] So the endoscopic findings are nonspecific and have been reported in other oesophageal diseases, such as gastro-oesophageal reflux disease (GERD), achalasia and other motility disorders.[6] Especially GERD is an important differential diagnosis and should be excluded after a thorough work-up to justify a diagnosis of true EO.[13,31,32] Occasionally, impressive endoscopic findings have been described consisting of white exudates, a small calibre oesophagus and a Schatzki's ring.[2,6,30] It is unclear whether these patients, usually suffering from severe symptoms, represent a distinct subgroup of EO.

Obviously, the frequently observed obstruction of a normal-appearing oesophagus by well-chewed food can only be due to an underlying motility disorder. Although standard oesophageal motility studies have failed to demonstrate abnormalities in a large proportion of patients with EO, 24-hour manometry is able to demonstrate oesophageal dysmotility in patients with EO.[33] Because manometric alterations can be intermittent or remain undetected by the usual measurement techniques, the true incidence of dysmotility in EO may be underestimated.[34]

The diagnosis of EO is made when suggestive symptoms and, if present, endoscopic findings are supported by biopsy specimens demonstrating an abnormal accumulation of IEGs. Although the exact number of IEGs/HPF required for diagnosis remains a matter of debate, most experts believe that the presence of more than 15 IEGs/HPF in the oesophageal squamous epithelium with concurrent symptoms establishes the diagnosis of EO.[6,32]

In 2007 medical experts made consensus recommendations for the diagnosis of EO.[6] They stated that intraepithelial eosinophils should be counted in the most intensely inflamed HPF of the biopsy (x400) to generate a peak count. They concluded that a peak count of 15 IEGs/HPF is an absolute minimum number to make the diagnosis of EO. If all HPFs are counted, the mean eosinophil number may be less than 15 because of focal inflammation in the biopsy specimens, but at least one HPF must contain at least 15 IEGs.[6]

The distribution of histological abnormalities may be patchy and therefore the amount of IEGs may vary throughout the length of the oesophagus.[35] Therefore, it is recommended to take multiple biopsies from the entire length of the oesophagus. Furthermore, the presence of IEGs in the distal oesophagus only may be suggestive of reflux disease rather than EO,[1,8] suggesting that it would be prudent to collect biopsies at this level in a separate container.

As previously stated, the differential diagnosis primarily includes GERD, which should be excluded properly. Most authors suggest that a trial with a high-dose proton-pump inhibitor, without effect on symptoms of the oesophagus, is required to exclude GERD. It is unknown whether non-acid GERD (demonstrable with the use of impedance monitoring[26]) plays a role in EO.[36] At present no recommendations can be given with regards to the value of impedance monitoring in the work-up of these patients.

As achalasia is part of the differential diagnosis of EO, oesophageal manometry is mandatory, although strong supporting literature is lacking and consensus recommendations currently see no diagnostic value in patients with EO.[6] Eosinophilic infiltration of the oesophagus in the setting of a generalised eosinophilic gastroenteritis has been reported and some have advocated taking jejunal biopsies to rule out this condition.[31] Eosinophilic gastroenteritis, however, has quite a different clinical manifestation and contrary to EO includes abdominal pain, nausea, vomiting, and diarrhoea.[37] For this reason, we have not adopted this policy in our patients.

Finally, hypereosinophilic syndrome must be ruled out, especially, when there are extra-gastrointestinal manifestations and splenomegaly, cutaneous, respiratory, neurological or cardiac findings are present. Missing this diagnosis can have important implications, as cardiac and neurological involvement can be life-threatening.[38]

TREATMENT AND PROGNOSIS

The mainstay of treatment consists of topical steroid ingestion.

The literature concerning EO is dominated by paediatric studies. Two randomised controlled trials concerning the use of FP in children were carried out.[39,40]

American investigators assigned 36 children randomly to oral FP or placebo for three months. Histological remission was observed significantly more frequently in the fluticasone group (50 *vs* 9%). Clinically, vomiting improved significantly in FP responders, but other clinical symptoms did not reach a significant improvement with FP treatment.[39] Another American research group from Indianapolis treated 80 children with either FP or systemic corticosteroids and demonstrated a greater degree of histological improvement but no significant difference in clinical remission rates.[40] In adult patients only case series have been published. The investigators treated 21 patients with 220 µg FP, swallowed twice a day for six weeks: all patients experienced symptomatic relief.[41] Remedios *et al.* confirmed the symptomatic effectiveness of FP in 19 adult patients with concurrent significant histological improvement.[28]

Although topical corticosteroids have been proven to be effective, all studies, including a recently published prospective study,[42] show a high relapse rate after stopping treatment (as in our case). This highlights the need for maintenance treatment. As long-term treatment is needed in EO, systemic corticosteroid use is precluded by its long-term side effects including cataract, growth retardation in children, osteoporosis and adrenal suppression. Using oral FP, these systemic effects can be minimised, thus enhancing compliance.[28] Acute and severe exacerbations of EO, however, can still be treated with systemic corticosteroids.[22]

It must be noted that before high-dose inhaled FP can be fully implemented as maintenance treatment for EO it is important for future trials to further investigate the use of possible systemic side effects. And continuing evaluations of dose and duration of therapy are needed. Proposed mechanisms through which corticosteroids influence EO are inhibition of pro-inflammatory mediators, down-regulation of chemotactic factors and induction of apoptosis.[9]

Other proposed treatments are exclusion diets, elementary diets, gastric acid suppression, and stabilisers of eosinophilic trafficking and activation.

The selective elimination of foods with demonstrable allergic effects in the patient under consideration has been reported as an effective strategy in individual patients. In children, the use of an elemental diet has also been shown to be effective.[14,43] However, the long-term implementation of such therapy is hampered by nutritional deprivation, psychological problems, unnecessary food aversion and loss of quality of life.

As mentioned earlier, a trial of high-dose proton pump inhibitor therapy is justified to exclude acid GERD as part of the differential diagnosis of EO, but as gastric acid plays no part in the pathogenesis of true EO, long-term treatment with proton-pomp inhibitors is not effective.[6]

Treatments that target eosinophilic trafficking and activation have been assessed in pilot studies. A case series using the leukotriene inhibitor montelukast showed symptomatic but no histological improvement and unfortunately a quick relapse after therapy cessation.[44] Mepolizumab, a humanised monoclonal antibody directed against IL-5, was infused intravenously once a month in one patient with EO unresponsive to topical and oral steroid therapy. Mepolizumab produced symptomatic, endoscopic, and histological improvement.[45] Following this initial success, ongoing placebo-controlled trials have been set up to further analyse the use of mepolizumab in EO.

Therapies focussing at controlling a suggested allergy component of EO, using antihistamines and cromolyn – a mast cell stabiliser and also an inhibitor of eosinophil mediator release and T-cell function – showed limited success in case reports.[13,46]

Recently there have been promising results in case reports concerning the use of purine analogues – azathioprine (AZA) and 6-mercaptopurine – showing clinical and histological response. Two of the three studied patients experienced relapses after ceasing AZA therapy.[47]

Finally, EO, although chronic, does not appear to limit life expectancy and no associated oesophageal malignancies have ever been reported.[6,48]

CONCLUSION

EO is a mucosal inflammatory disorder, most likely leading to oesophageal dysmotility with an unclear pathogenesis which is increasingly recognised in children but also in adults. The diagnosis should be considered in patients with unexplained dysphagia, especially when there are no or only subtle endoscopic abnormalities of the oesophagus. Histological biopsies of normal appearing mucosa throughout the length of the oesophagus are pivotal in establishing the diagnosis. Absence of endoscopic signs of GERD and a negative PPI trial of the oesophagus are prerequisites for making a definite diagnosis of EO. The use of FP aerosol is the most successful long-term treatment, with minimal side effects. With such treatment the long-term prognosis seems good.

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ORIGINAL ARTICLE

Comparison of different methods to investigate postprandial lipaemia

A.J.H.H.M. van Oostrom[1,2], A. Alipour[3], T.P. Sijmonsma[2], C. Verseyden[2], G.M. Dallinga-Thie[4], H.W.M. Plokker[1], M. Castro Cabezas[3*]

[1]Department of Cardiology, St. Antonius Hospital, Nieuwegein, the Netherlands, [2]Department of Vascular Medicine, University Medical Center Utrecht, the Netherlands, [3]Department of Internal Medicine, St. Franciscus Gasthuis, Rotterdam, the Netherlands, [4]Laboratory of Vascular Medicine, Erasmus Medical Center, Rotterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)10-461 72 67, fax: +31 (0)10-461 26 92, e-mail: m.castrocabezas@sfg.nl

ABSTRACT

Postprandial hyperlipidaemia has been associated with coronary artery disease (CAD). We investigated which of the generally used methods to test postprandial lipaemia differentiated best between patients with premature CAD (50±4 years, n=20) and healthy controls. Furthermore, the effects of rosuvastatin 40 mg/day on postprandial parameters were assessed. Standardised oral fat-loading tests (OFLT) and ambulant self-measurements of daylong capillary triglycerides (TGc) were performed. Total responses of individual lipoproteins, plasma TG (TGp) and remnant-like particle cholesterol (RLP-C) were estimated as area under the curve (AUC). Most AUCs were highest in untreated patients and reached control levels after rosuvastatin. From all AUCs, RLP-C-AUC was best associated to TGp-AUC in untreated patients and controls (adjusted R^2=0.84, β=0.92, p<0.001). From all parameters of postprandial lipaemia, TGc-AUC differentiated best between untreated patients and controls (adjusted R^2=0.48, β=0.70, p<0.001) and between patients on and off-treatment (adjusted R^2=0.34, β=-0.60, p<0.001). Our findings indicate that the real-life TG load, instead of metabolic ward testing, is the best parameter of postprandial lipaemia to identify patients with premature coronary sclerosis and to evaluate postprandial effects of statin treatment.

KEYWORDS

Capillary triglycerides, lipoproteins, postprandial hyperlipidaemia, remnant-like particle cholesterol, rosuvastatin

INTRODUCTION

Fasting hypertriglyceridaemia is an independent risk factor for coronary artery disease (CAD).[1] It has been suggested that fasting plasma triglyceride (TG) concentrations are the best predictor of postprandial lipaemia.[2,3] Postprandial hyperlipidaemia is frequently present in patients with premature CAD and could therefore constitute a concealed risk factor.[4] Furthermore, exaggerated postprandial lipaemia has been observed even in fasting normolipidemic subjects.[5,6]

The usual tool to investigate postprandial lipaemia is measurement of plasma TG (TGp) and lipoprotein fraction separation during a standardised oral fat-loading test (OFLT) under metabolic ward conditions.[7,8] Recently, remnant-like particle cholesterol (RLP-C) quantification has been described to estimate the cholesterol and TG levels in atherogenic remnant lipoprotein particles.[9] Furthermore, the total TG load to which subjects are exposed during the day can be estimated by means of ambulant self-determined daylong capillary triglyceridaemia (TGc). This technique has been shown to correlate with postprandial lipaemia in the metabolic ward.[10-12]

We investigated which of the above-described methods to investigate postprandial lipaemia provides the best differentiation between patients with premature CAD before and after treatment with rosuvastatin and between those patients and matched controls.

SUBJECTS AND METHODS

Participants

The study protocol was approved by the Independent Ethics Committee of the Institutional Review Board of the

University Medical Center Utrecht and the St. Antonius Hospital Nieuwegein. Male patients aged 40 to 55 years with angiographically established CAD without any atherosclerotic event in the six months prescreening were recruited from both centres. The selection of patients was carried out by screening patients' files at random and selecting the subjects fulfilling the criteria. Exclusion criteria were diabetes mellitus, renal and/or liver disease, apolipoprotein E2/E2 genotype, body mass index >30 kg/m^2, smoking and alcohol intake >3 units/day. Fasting plasma lipids after washout of lipid-lowering medication for four weeks, fulfilled a cholesterol >5 mM and plasma TG >1.7 mM. Age- and waist-matched healthy males with fasting plasma cholesterol <6.5 mM and plasma TG <2.3 mM were recruited by advertisement. Exclusion criteria were a positive family history for premature CAD, the use of drugs known to affect lipid metabolism and the exclusion criteria used in the patients.

Study design

On each hospital visit, the participants were fasting overnight for >12 hours and did not drink alcohol on the day before. On the morning of the first visit, anthropometric measurements were performed, blood samples were drawn and the subjects received instructions for daylong TGc measurements. The second visit comprised the first OFLT and was followed by rosuvastatin 20 mg/day treatment for one month, only in the patients. Hereafter, patients visited the outpatient clinic for pill counting and control of safety parameters. Subsequently, the patients started on rosuvastatin 40 mg/day for one month, followed by a second OFLT under the same conditions as the first test. Patients self-measured daylong TGc at baseline (four weeks off treatment) and after four weeks of 40 mg/day rosuvastatin. Controls performed TGc self-measurements for one period only.

Oral fat-loading test

After inserting a venous cannula for blood sampling, subjects rested for 30 minutes before administration of the fat load. Cream was ingested within five minutes at a dose of 50 g fat and 3.75 g glucose per m^2 body surface.[13] During each test, the participants remained supine and were allowed to drink mineral water only. At regular time intervals up to ten hours postprandially blood samples were obtained in sodium EDTA (2 mg/ml) and kept on ice and centrifuged immediately for 15' at 800 g at 4°C, finally plasma was stored at -80°C.

TGc self-measurements and dietary intake

By a process of dry chemistry and colorimetry, TGc was self-measured with a TG-specific point-of-care testing device (Accutrend GCT; Roche Diagnostics, Mannheim, Germany) as described.[10-12] The measurement range for TGc is 0.80 to 6.86 mM, in the case of TGc outside this range, we used the lower or upper limit, respectively, for calculations. TGc were self-measured on three different days (preferably Monday, Wednesday and Friday; not in weekends) at six time points: fasting, pre- and exactly three hours post lunch and dinner, and at bedtime. The results were recorded in a diary, evaluated with the subjects afterwards and compared with automatically recorded data in the memory of the device. Subjects were requested to refrain from heavy physical activity; normal daily activities such as riding a bike to work were allowed. When one or more measurements were missing for a day, the data for that particular day were not used. The mean daylong TGc profile was used for statistical analysis. Results were compared with recently described cut-off levels for high (>42.5 mmol.h/l) and abnormal (between 29.5 and 42.5 mmol.h/l) daylong TGc in males.[14]

Subjects were asked to consume their usual diet, intake was unrestricted concerning the frequency and composition of the meals and was recorded in the TGc diary. Quantities of intake were estimated according to instructions provided by a dietician and by using a table with standardised portion sizes.[15] Foods consumed were converted into nutrients by using the Dutch Nutrient Database and compared with the general Dutch diet.[16]

Analytic determinations

Plasma HDL cholesterol obtained after precipitation with phosphotungstate/$MgCl_2$, and cholesterol and TG in plasma and in the isolated lipoprotein fractions were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits respectively (Roche Diagnostics, Germany). LDL cholesterol was calculated by the Friedewald formula. Plasma apolipoprotein B (apoB) was measured by nephelometry using monoclonal antibodies (Behring Diagnostics, Germany). Plasma RLP-C isolation was based on removal of apoA-I-containing particles (HDL) and most apoB100-containing particles (LDL, nascent very-low density lipoprotein (VLDL)) by immunoseparation (Japan Immunoresearch Laboratories, Takasaki, Japan), to leave apoB48 remnants of intestinal origin and apoB100-apoE remnants of hepatic origin in the unbound fraction.[17] Cholesterol was analysed by an automated enzymatic assay (RLP-C) using a Cobas Mira S auto-analyser (ABX Diagnostics, Montpellier, France). Lipoproteins were subfractionated by discontinuous density gradient and consecutive ultracentrifugation as described.[18] The resulting lipoprotein fractions correspond with chylomicron, VLDL1 and VLDL2. Plasma insulin was measured by ELISA (Mercodia, Uppsala, Sweden). Plasma glucose, creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and creatinine kinase were measured by dry chemistry colorimetry (Vitros 250; Johnson & Johnson, Clinical Diagnostics, Rochester, NY, USA). ApoE

genotype was determined as described.[19] For estimation of insulin sensitivity the HOMA index (homeostasis model assessment = glucose x insulin/22.5) was calculated.

Statistics

Data are expressed as mean±SD in the text and tables and as mean±SEM in the figures. Daylong TGc was calculated as mean integrated area under the 14-hours TGc curve (TGc-AUC) and as incremental integrated area (dTGc-AUC, calculated by subtracting the baseline TGc value from following measurements) by the trapezoidal rule using GraphPad Prism version 3.0. During the ten-hour OFLTs, total and incremental AUCs were calculated as well. Differences between patients and controls were tested by Student's t-test. Effects of treatment and postprandial effects when compared with T=0h, were tested using repeated measures analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons. Bivariate correlations were calculated using Spearman's correlation coefficients. Binary logistic regression analysis was performed to study which parameters differentiated best between patients and controls and between patients on and off-treatment. Linear regression analysis was performed to study predictors of postprandial lipaemia. TG, insulin and HOMA values were log transformed before analysis due to non-parametric distribution. For statistical analysis SPSS version 10.0 was used. P values <0.05 (two-tailed) were considered statistically significant.

RESULTS

Baseline characteristics

From 26 healthy subjects who responded, five were excluded (obesity: n=2, excessive use of alcohol: n=1, current smoking: n=1 and apoE2/E2 genotype: n=1). From the remaining subjects, 20 were matched for age and waist circumference with 20 CAD patients who met the inclusion criteria (*table 1*). At the start of the study, CAD patients reported a total dietary intake and relative contribution of different nutrients comparable with that of controls on a regular Dutch diet (data not shown). At the first OFLT, the untreated patients showed a less favourable fasting lipid profile and (based on HOMA) were more insulin resistant when compared with controls (*tables 1* and *2*). Baseline characteristics and fasting plasma lipids in the patients have been published elsewhere.[20] During treatment, no significant changes were observed, neither in the parameters depicted in *table 1*, nor in the self-reported dietary intake nor in physical activity (data not shown). None of the subjects started smoking. In addition, the co-medication of the patients was unchanged along the study and included aspirin (n=19), β-blockers (n=13) and angiotensin-converting enzyme (ACE) inhibitors (n=11). Rosuvastatin 40 mg/day was well tolerated and significantly improved all studied fasting lipid parameters (*table 2*).

Table 1. *Baseline characteristics of the study subjects (mean (SD))*

	Controls (n=20)	CAD patients (n=20)
Age (years)	50 (5)	50 (4)
Body mass index (kg/m²)	25.3 (2.1)	26.4 (1.4)
Waist (m)	0.92 (0.10)	0.96 (0.05)
Systolic blood pressure (mmHg)	126 (13)	129 (12)
Diastolic blood pressure (mmHg)	84 (6)	87 (8)
Glucose (mM)	5.2 (0.7)	5.2 (0.5)
Insulin (mU/l)	3.36 (3.36)	8.70 (7.19)**
HOMA index	0.77 (0.74)	2.04 (1.72)**

HOMA = homeostasis model assessment. **p<0.005.

Table 2. *Fasting plasma lipids and safety parameters of the study subjects (mean (SD))*

	Controls (n=20)	CAD patients (n=20)	
		Baseline	Rosuvastatin 40 mg/d
Plasma triglycerides (mM)	1.69 (0.59)	2.21 (0.87)*	1.52 (0.61)#
Capillary triglyceriwdes (mM)	1.29 (0.52)	3.08 (1.27)**	1.78 (0.66)##
Cholesterol (mM)	5.2 (0.9)	6.3 (1.0)**	3.6 (0.6)**##
LDL cholesterol (mM)	3.2 (0.8)	4.4 (1.0)**	1.8 (0.5)**##
RLP cholesterol (mM)	0.36 (0.11)	0.55 (0.22)**	0.33(0.11)##
HDL cholesterol (mM)	1.21 (0.25)	0.93 (0.23)**	1.10 (0.32)#
Non-HDL cholesterol (mM)	4.0 (0.9)	5.4 (0.9)**	2.5 (0.6)**##
Cholesterol/HDL cholesterol	4.4 (1.2)	7.1 (1.7)**	3.5 (0.9)**##
Apolipoprotein B (g/l)	0.97 (0.21)	1.26 (0.19)**	0.70 (0.14)**##
Creatinine (μM)	88 (7)	91 (12)	91 (12)
ASAT (U/l)	Nd	32 (10)	35 (12)
ALAT (U//)	29 (11)	34 (23)	40 (18)*
Creatinine kinase (U/l)	145 (87)	130 (91)	159 (117)

Nd = not determined; RLP = remnant-like particle. *p<0.05, **p<0.005 *vs* matched controls, #p<0.05; ##p<0.005 *vs* baseline.

Postprandial plasma TG and RLP-C

Fasting TGp, TGp-AUC and TGp-dAUC were higher in the untreated patients when compared with controls (*tables 2* and *3*, and *figure 1*). After treatment, TGp and TGp-AUC were reduced to control levels (-31%, p<0.05 and -23%, p<0.005, respectively), whereas dTGp-AUC was unaffected. In all conditions, postprandial plasma RLP-C increased towards a maximum at T=5 hours, followed by a gradual decrease towards baseline values, except for the untreated patients, where late postprandial RLP-C remained elevated (*figure 1*). Fasting plasma RLP-C and RLP-C-AUC were higher in the untreated patients when compared with controls and significantly reduced to control values by rosuvastatin (*tables 2* and *3*, and *figure 1*).

Postprandial lipoproteins

Significant postprandial changes of TG and cholesterol were not seen in IDL, LDL and HDL fractions (data not shown). In general, fasting cholesterol and cholesterol AUCs in the chylomicron, VLDL1 and VLDL2 fractions were significantly higher in the patients and reached control levels after treatment (*figure 2: left panels, table 3*). Fasting plasma TG and TG-AUC in those fractions were also higher in the patients, the reductions by treatment were small and did not reach control levels (*figure 2: right panels, table 3*).

Daylong capillary triglyceridaemia

TGc-AUC was elevated twice in the untreated patients, while dTGc-AUC was not different from controls (*figure 1*). Four controls and nine untreated patients were identified with abnormal TGc-AUC; the other patients had high TGc-AUC, the other controls normal TGc-AUC. After treatment, average TGc-AUC was significantly reduced to normal limits (<29.5 mmol.h/l), although TGc-AUC and fasting TGc remained higher when compared with controls. All treated patients showed improvement of TGc-AUC, resulting in normal, abnormal and high TGc-AUC in 12, 6 and 2 patients respectively.

Regression analyses

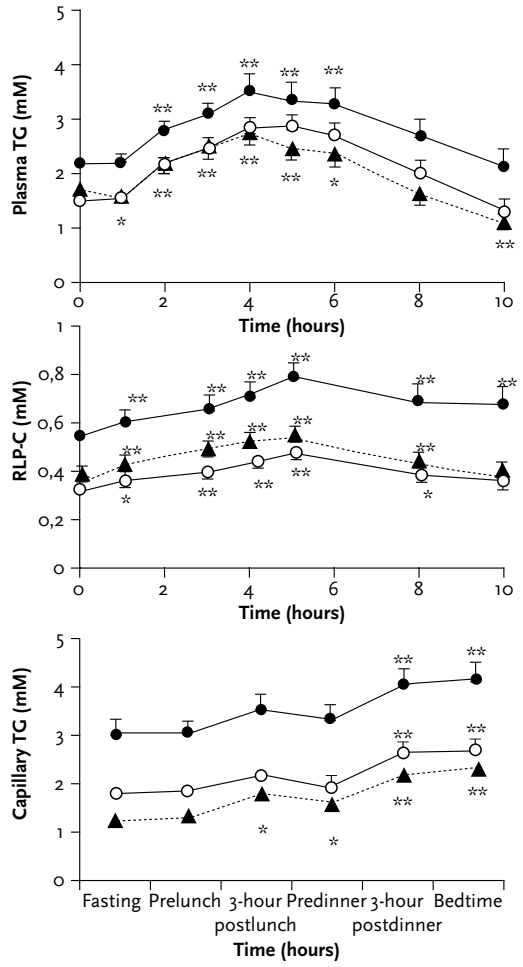
Table 4 shows that the different parameters of postprandial lipaemia (expressed as AUCs) were strongly associated. From all AUCs of *table 3*, RLP-C-AUC was the best determinant of TGp-AUC (adjusted R^2=0.84, β=0.92, p<0.001) and of TGc-AUC (adjusted R^2=0.67, β=0.82, p<0.001). When fasting lipid parameters from *tables 2*

Table 3. *Postprandial plasma lipids of the study subjects (mean (SD))*

	Controls (n=20)	CAD patients (n=20)	
		Baseline	Rosuvastatin 40 mg/d
TGp-AUC (mmol x h/l)	20.2 (8.4)	28.5 (9.2)**	22.0 (7.1)##
TGp-dAUC (mmol x h/l)	3.35 (3.83)	6.43 (4.21)*	6.83 (3.66)*
TGc-AUC (mmol x h/l)	23.6 (8.0)	47.5 (15.5)**	29.2 (8.6)*##
TGc-dAUC (mmol x h/l)	6.01 (3.62)	4.83 (6.79)	4.25 (5.16)
RLP-C-AUC (mmol x h/l)	4.69 (1.63)	6.87 (2.54)**	4.04 (1.17)##
RLP-C-dAUC (mmol x h/l)	1.06 (0.78)	1.37 (0.91)	0.75 (0.91)#
Fasting chylo-chol (mM)	0.019 (0.016)	0.027 (0.025)	0.013 (0.017)#
Chylo-chol-AUC (mmol x h/l)	0.53 (0.34)	1.16 (0.92)*	0.59 (0.26)##
Chylo-chol-dAUC (mmol x h/l)	0.34 (0.28)	0.89 (0.78)*	0.45 (0.23)#
Fasting VLDL1-chol (mM)	0.14 (0.12)	0.37 (0.21)**	0.18 (0.12)##
VLDL1-chol-AUC (mmol x h/l)	1.91 (1.32)	4.32 (1.85)**	2.54 (1.11)##
VLDL1-chol-dAUC (mmol x h/l)	0.47 (0.49)	0.61 (0.73)	0.78 (0.75)
Fasting VLDL2-chol (mM)	0.15 (0.09)	0.32 (0.16)**	0.18 (0.08)##
VLDL2-chol-AUC (mmol x h/l)	1.53 (0.91)	2.85 (1.50)**	1.64 (0.61)##
VLDL2-chol-dAUC (mmol x h/l)	0.07 (0.41)	-0.39 (0.70)	-0.11 (0.48)
Fasting chylo-TG (mM)	0.02 (0.03)	0.12 (0.17)	0.04 (0.08)
Chylo-TG-AUC (mmol x h/l)	1.95 (1.27)	4.96 (3.64)**	3.16 (1.60)*#
Chylo-TG-dAUC (mmol x h/l)	1.71 (1.08)	3.73 (3.19)*	2.77 (1.49)*
Fasting VLDL1-TG (mM)	0.36 (0.31)	0.91 (0.62)**	0.54 (0.34)*
VLDL1-TG-AUC (mmol x h/l)	4.82 (2.94)	11.15 (6.04)**	8.98 (3.84)**
VLDL1-TG-dAUC (mmol x h/l)	1.17 (1.04)	2.08 (2.45)	3.55 (2.57)**#
Fasting VLDL2-TG (mM)	0.15 (0.10)	0.36 (0.35)*	0.25 (0.14)*
VLDL2-TG-AUC (mmol x h/l)	1.72 (1.05)	3.46 (3.13)*	2.52 (1.23)*##
VLDL2-TG-dAUC (mmol x h/l)	0.274 (0.445)	-0.186 (1.603)	0.003 (0.819)

RLP = remnant-like particle; Chylo = chylomicron; chol = cholesterol; AUC = area under the curve; dAUC = incremental area under the curve, TG = triglycerides. *p<0.05; **p<0.005 *vs* age- and waist-matched controls; #p<0.05; ##p<0.005 *vs* untreated patients.

Figure 1. *Mean±SEM postprandial plasma triglycerides (TGp, upper panel) and plasma remnant-like particle cholesterol (RLP-C, middle panel) after a standardised oral fat load and self-measured diurnal capillary triglycerides (TGc, lower panel) in 20 CAD patients off lipid-lowering medication (closed bullets) and after rosuvastatin 40 mg/day (open bullets) in comparison with matched controls (n=20, dotted line)*



***p<0.05 ; **p<0.005 *vs* T=0 hours.**

and *3* were also included, TGp-AUC was best predicted by RLP-C-AUC and fasting TGp (adjusted R^2=0.86, β=0.32, p<0.001), whereas the best model to predict TGc-AUC included RLP-C-AUC and fasting TGc (adjusted R^2=0.86, β=0.32, p<0.001). When fasting TGp and TGc were excluded from this analysis (since these parameters are part of TGp-AUC and TGc-AUC), RLP-C-AUC remained the single best determinant. Logistic regression analysis with the study group (controls and untreated patients) as dependent variable and the significantly different AUCs from *table 3* as independent variables resulted in TGc-AUC as the best discriminator of patients and controls (adjusted R^2=0.48, β=0.70, p<0.001); when all significantly different parameters from *tables 2* and *3* were included, the best discriminators were fasting TGc and TGp and the total cholesterol/HDL cholesterol ratio (adjusted R^2=0.65, β=0.36, p=0.04). Similarly, from all significantly different AUCs from *table 3*, TGc-AUC was the only discriminator of treated and untreated patients (adjusted R^2=0.34, β=-0.60, p<0.001). When all significant different parameters of *tables 2* and *3* were included, non-HDL-C was the only determinant (adjusted R^2=0.77, β=-0.88, p<0.001).

DISCUSSION

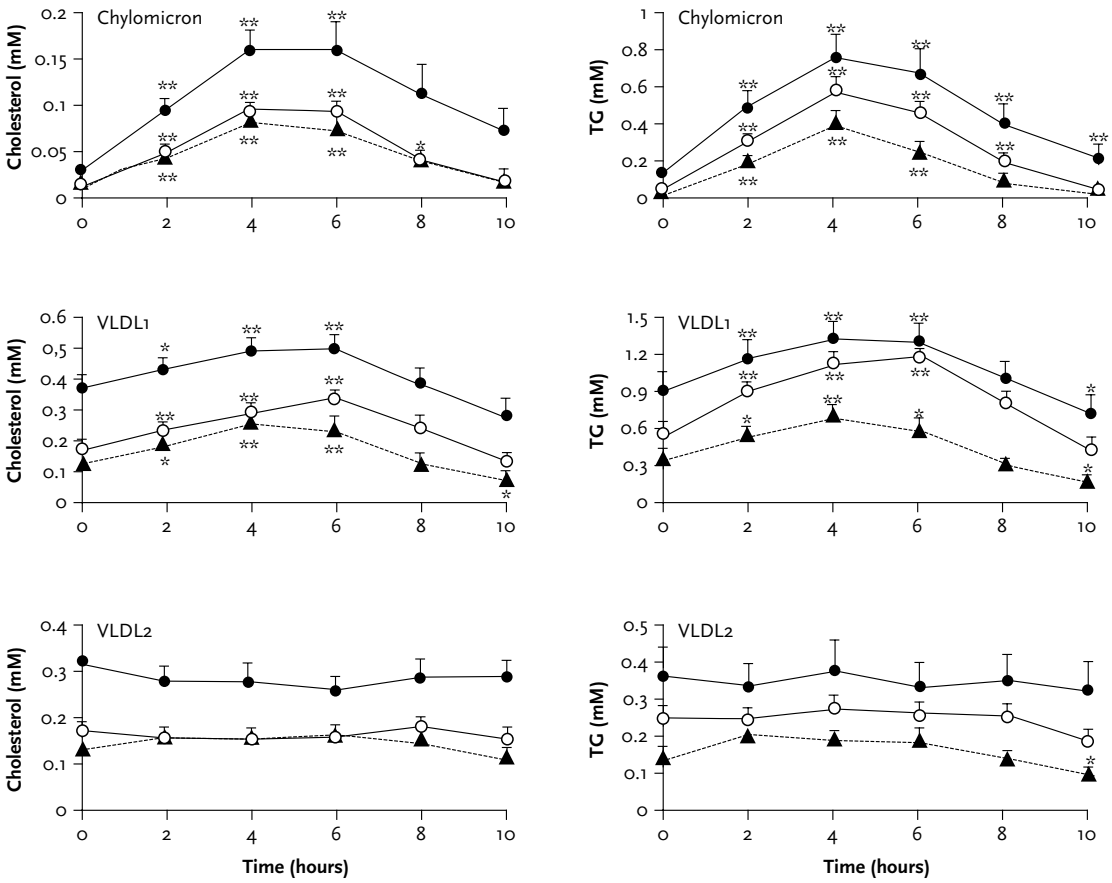
In the present study postprandial lipaemia was investigated by conventional metabolic ward testing and in a real-life situation by ambulant capillary TG self-measurement. The former makes it possible to perform lipoprotein quantification and measurement of RLP-C under controlled circumstances, since diet is known to affect lipid metabolism.[7,11,13] Usually, postprandial lipaemia is estimated by TGp-AUC. In the present study TGp-AUC showed strong associations with all other AUCs, but was best predicted by RLP-C-AUC. Furthermore, RLP-C-AUC was the best predictor of TGc-AUC. However, from all postprandial lipid parameters, the real-life TG

Table 4. *Univariate regression analysis (Spearman's correlation coefficients) with postprandial lipaemia parameters from table 3 in controls and untreated patients (n=40)*

	TGp-AUC	TGc-AUC	RLP-C-AUC	Chylo-chol-AUC	VLDL1-chol-AUC	VLDL2-chol-AUC	Chylo-TG-AUC	VLDL1-TG-AUC
TGp-AUC								
TGc-AUC	0.82							
RLP-C-AUC	0.92	0.82						
Chylo-chol-AUC	0.71	0.65	0.78					
VLDL1-chol-AUC	0.84	0.76	0.81	0.81				
VLDL2-chol-AUC	0.86	0.74	0.84	0.78	0.89			
Chylo-TG-AUC	0.69	0.68	0.72	0.83	0.85	0.74		
VLDL1-TG-AUC	0.70	0.70	0.65	0.54	0.84	0.69	0.92	
VLDL2-TG-AUC	0.70	0.66	0.73	0.62	0.79	0.85	0.83	0.91

RLP = remnant-like particle; Chylo = chylomicron; chol = cholesterol; AUC = area under the curve. For all associations p<0.001.

Figure 2. *Mean±SEM postprandial plasma cholesterol (left panels) and triglycerides (right panels) in chylomicron, VLDL1 and VLDL2 fractions after a standardised oral fat load in 20 CAD patients off lipid-lowering medication (closed bullets) and after rosuvastatin 40 mg/d (open bullets) in comparison with matched controls (n=20, dotted line)*



*p<0.05, **p<0.005 *vs* T=0 h.

load (TGc-AUC) differentiated best between untreated patients and controls and also between patients on and off-treatment. According to our data, the predictive power of TGc-AUC was better than metabolic-ward-derived parameters but also stronger than fasting lipid parameters such as LDL-C.

It was remarkable that upon treatment TGc-AUC did not reach control levels, whereas all postprandial parameters after the OFLT were reduced to levels not different from those of controls. Dietary intake, a predictor of daylong TGc,[10,11] was not different from controls and did not change during the study. A possible explanation for the discrepancy may be that daylong TGc measurements are not performed under standardised settings. For that reason, fasting TGc, the strongest predictor of TGc-AUC in the present study and in previous reports,[10] was not measured after a strict overnight fast, which may explain higher baseline TGc when compared with fasting TGp. In addition, TGc-AUC is based on averages of two or three days, which may have decreased intra-individual variability as has previously been demonstrated in healthy subjects and patients with familial combined hyperlipidaemia.[21] Finally, in some cases the detection range of TGc has caused an overestimation of TGc. In our opinion, TGc-AUC is the most realistic determination of postprandial lipaemia, since it takes into account that in real life, subjects are exposed to repetitive food intake with prolonged stressing of the lipoprotein clearance pathways. Furthermore, TGc-AUC does not exclude effects of different food components and moderate exercise on lipoprotein clearance. When compared with the OFLT, diurnal TGc is less expensive, since hospitalisation of study subjects is not necessary and laboratory techniques are cheaper; furthermore, the test is more tolerable for the subjects. We have to underline that the use of postprandial tests to identify patients at risk of CAD is still a matter of debate, since postprandial lipaemia is strongly associated to fasting lipaemia. In agreement with previous reports, we showed a strong association between fasting TGc

and diurnal TGc and also between fasting TGp and TGp-AUC.[2,3,10,22] Furthermore, when fasting TGc and TGp were included in the regression models, postprandial TG lost its power to differentiate patients from controls. Thus, fasting plasma TG identify CAD strongly. However, the aim of our study was to evaluate which postprandial variable differentiated best between CAD patients and healthy subjects.

Upon treatment with rosuvastatin all fasting lipid values were improved to levels below the latest ATP III guidelines.[23] These effects were more or less comparable with previous studies, despite the fact that our study group only showed moderate fasting hyperlipidaemia.[24,25] In line with total cholesterol, fasting and postprandial cholesterol content of individual lipoproteins was markedly reduced to levels not different from those of controls. There was a shift towards a relatively higher content of cholesterol in the HDL fraction than in the LDL fraction. By contrast, the effects of the statin on fasting and postprandial TG in lipoprotein subfractions were less pronounced. After rosuvastatin 40 mg/day, the total TGp response after a standardised oral fat load was markedly reduced to reference levels. However, of all postprandial lipaemia markers, TGc-AUC differentiated best between the pre- and post-treatment situation, again indicative of the strength of this parameter. The postprandial effects of rosuvastatin have not been reported before and are difficult to compare with other statins due to different patient groups and study meals.[26,27]

Plasma RLP-C has been shown to be a predictive marker of CAD risk and reduction of fasting RLP-C has been described upon treatment with various statins including rosuvastatin. We have shown a 40% reduction in fasting and postprandial RLP-C by rosuvastatin 40 mg/day in the present report, which is in line with two other rosuvastatin 40 mg/day studies in hyperlipidemic non-CAD patients[28] and with an atorvastatin 80 mg/day study.[29]

It is known that type 2 diabetes and the prediabetic conditions of insulin resistance and the metabolic syndrome are characterised by a disturbed TG metabolism and reduced HDL-C rather than elevated LDL-C.[30,31] The present report in subjects with reduced insulin sensitivity showed benefit of postprandial lipaemia testing, diurnal TGc in particular, when compared with determination of fasting lipid parameters. Future studies should emphasise whether other patient groups show this benefit as well.

CONCLUSION

Self-determined diurnal capillary TG seems the best and easiest method to test postprandial lipaemia to identify patients with premature CAD and to study effects of statin treatment.

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ORIGINAL ARTICLE

Colorectal adenomas in patients presenting with inflammatory bowel disease

R.J.L.F. Loffeld

Department of Internal Medicine, Zaans Medisch Centrum, Zaandam, the Netherlands,
tel.: +31 (0)75-650 27 79, fax: +31 (0)75-650 23 79, e-mail: loffeld.r@zaansmc.nl

ABSTRACT

Background: Adenoma is the precursor of colorectal carcinoma (CRC). Patients with longstanding active ulcerative colitis (UC) are at risk of developing CRC. Every patient with UC can also develop adenomas, which is an extra risk factor.

Aim: A large retrospective cross-sectional study was conducted to identify patients with UC and polyps.

Material and methods: All consecutive lower intestinal endoscopies carried out in a period of 16 years were searched for the presence of inflammation and concomitant polyps.

Results: Inflammatory bowel disease was diagnosed in 1029 patients. Forty-seven (4.5%) patients had concomitant polyps. The patients with polyps were divided in two groups: group 1 consisted of 34 patients in whom active inflammation was seen with coinciding polyp(s), and group 2 consisted of 12 patients in remission, in whom polyps were detected. One patient had had adenomas in the past and presented with active inflammation and a new adenoma.

In group 1, four patients had a history of active inflammation, and adenomas were seen in 29 patients, while seven patients showed hyperplastic polyps. Two patients had adenomas as well as hyperplastic polyps. In group 2 nine patients had adenomas and three had hyperplastic polyps.

Conclusion: Patients with different phenotypic expressions of inflammatory bowel disease can have concomitant adenomas in the colon. Hence, it is plausible to assume that these patients have an increased risk of developing CRC because of adenomas.

KEYWORDS

Adenoma, cancer risk, colorectal cancer, inflammatory bowel disease, ulcerative colitis

INTRODUCTION

Colorectal cancer (CRC) is one of the most prevalent cancers in the Western world. The adenoma has been established as the precursor of CRC in the general population. The adenoma-carcinoma sequence is generally accepted. Every individual has a certain, albeit unknown, risk of developing adenomas. Since detection and removal of this precursor lesion can prevent cancer developing later in life, screening colonoscopy is advocated in people above the age of 50. Initial screening in asymptomatic individuals can be done with faecal occult blood testing. The estimated increase in colonoscopy workload in normal practice is minor, given the results of a recent Dutch study.[1]

A well-known fact in the literature is that patients with longstanding active ulcerative colitis (UC) are at risk of developing CRC. Among patients with UC, dysplasia is associated with, and precedes, invasive carcinoma. Colonoscopic surveillance is recommended after eight to ten years of extensive colitis or after 15 to 20 years for left-sided colitis. Surveillance does not prolong survival.[2]

CRC in patients with UC is always thought to be the result of inflammation-associated dysplasia. Suchy *et al.* studied a series of polymorphisms in six different genes active in inflammation, and found that there are genetic changes capable of influencing risk of CRC especially in older persons.[3] Many of the molecular alterations responsible for CRC, namely chromosomal instability, microsatellite instability, and hypermethylation, also play a role in colitis-associated colon carcinogenesis.[3] Apart from this risk, every patient with UC also has the potential, just as individuals without UC, to develop adenomas. It can be hypothesised that CRC in UC can also be the result of an adenoma not associated with chronic inflammation.

Despite very many studies in the literature on adenomas and CRC, and cancer occurring in patients with UC, there is little data on the simultaneous occurrence of adenomas in patients with UC. For this reason, a large cross-sectional

retrospective study was carried out to identify patients with UC and concurrent adenomas. Also the CRCs occurring in these patients were identified.

MATERIAL AND METHODS

In this retrospective study, all consecutive lower intestinal endoscopies, sigmoidoscopies as well as colonoscopies, performed over a period of 16 years at the Department of Gastroenterology of the Zaans Medical Centre, the regional hospital for the Zaanstreek region in the Netherlands, were included.

The endoscopies were done with fibre-optic Olympus endoscopes in the beginning of the 1990s. From 1993 to 2000, the EVIS 100 system was used and after 2000 the Exera 160 and 180 systems of Olympus were used. Two experienced endoscopists performed the procedures. The result(s) of the endoscopy/endoscopies were hand-written in standardised reports. From the beginning of 2003 the Endobase™ computerised system from Olympus was used. The results of all endoscopies are entered in a prospective database system.

For the present study, all endoscopy reports in which inflammation in the colon or rectum and concomitant polyp(s) was noted, were included. The medical records of the patients were retrieved in order to obtain demographic data, as well as details on the colitis or proctitis. The histological features of the detected polyps were noted. Patients with inflammatory bowel disease and inflammatory polyps were excluded.

Statistical analysis was done with the χ^2 test for contingency tables. A value below 0.05 was considered statistically significant.

RESULTS

In 16 years, 17,780 consecutive endoscopies were performed (90.4% colonoscopies).

In 1029 patients inflammatory bowel disease was diagnosed (164 cases of Crohn's disease, 183 of ulcerative colitis, distal colitis (procto-sigmoiditis) and left-sided colitis in 231 patients, proctitis in 212, segmental colitis (inflamed small segment, usually of the sigmoid without specific signs pointing to Crohn's disease) in 44, and indeterminate colitis in 195 patients).

Forty-seven (4.5%) patients presented signs of inflammation and concomitant polyps. Biopsies and/or polypectomy specimens, for confirmation of the inflammation and type of polyps, were available from all patients.

The patients with polyps were divided in two groups: group 1 consisted of 34 patients in whom active inflammation was seen with coinciding polyp(s), and group 2 consisted of 12 patients with documented active inflammation of colon and/or rectum in the past, in whom polyps were detected during the most recent endoscopy. Finally, there was one patient in whom adenomas in the sigmoid were removed and who presented four years later, one year prior to regular follow-up, with active inflammation and a new adenoma in the sigmoid (tubulo-villous adenoma with moderate dysplasia).

In group 1, four patients had a history of active inflammation. This diagnosis was made 10 to 17 years earlier (mean 13 years). They underwent an endoscopy because of symptoms pointing to an exacerbation. During the present colonoscopy, active inflammation and concomitant polyp(s) were present.

Table 1 shows more details of the patients in both groups. In group 1 adenomas were seen in 29 patients, while seven patients showed hyperplastic polyps. Two patients had adenomas as well as hyperplastic polyps. In group 2 nine patients had adenomas and three had hyperplastic polyps. These polyps were detected during a follow-up endoscopy at a mean of 10.3 years after the endoscopy showing active inflammation (median 10 years, range 1 to 28 years).

Table 2 shows the histological classification, as well as the level of dysplasia in the polyps in both groups of patients.

Table 1. *Details on patients in the two groups*

	Group 1	Group 2
Number	34	12
Men/women	23/11	10/2
Mean age (SD)	59.7(15)	56.4(14)
Site and extent of inflammation:		
• Proctitis	5	2
• Distal colitis or left-sided colitis	16	5
• Ulcerative pancolitis	2	2
• Crohn's disease of the colon	1	3
• Indeterminate colitis	10	-
Localisation of the polyp:		
• Sigmoid	21	7
• Ascending colon/caecum	3	4
• Descending colon	1	-
• Unknown	4	-
Number of polyps	1.6	1.25
Range	1-5	1-2

Table 2. *The classification of the polyps in the two groups of patients*

	Group 1	Group 2
Hyperplastic	7	3
Tubular adenoma	25 (86%)	7 (78%)
Tubulo-villous adenoma	4 (14%)	2 (22%)
Villous adenoma	-	-
	p=ns	
Low-grade dysplasia	20 (69%)	2 (22%)
Moderate dysplasia	7 (24%)	7 (78%)
High-grade dysplasia	2(7%)	-
	p=0.03	

Moderate dysplasia was significantly more often present in the polyps of patients in group 2 (p=0.03).
Three patients with IBD developed CRC in the course of their disease (two sigmoid cancers and one rectal cancer). None of these patients had concurrent or previous adenomas.

DISCUSSION

A specific group of patients who have an increased risk of developing CRC is patients with UC. The risk increases with the duration and extent of UC. The incidence is reported to be 2% after ten years of active colitis, 8% after 20 years and 18% after 30 years.[5,6] Also in population studies, the incidence of CRC was higher when compared with a control population.[7] In more recent studies a much lower risk was calculated.[8,9] In a large population study in Italy mortality due to CRC did not appear to be different in patients with UC or patients with sporadic cancer.[10] Lutgens *et al.* assessed the time interval between onset of IBD and CRC.[11] It appeared that 22% of patients developed cancer before the recommended starting points of surveillance. They concluded that the diagnosis of CRC is delayed or missed in a substantial number of patients when conducting surveillance strictly according to formal guidelines. However, the study was done in seven university hospitals, hence investigator bias is present.
An adenoma usually precedes a sporadic cancer, while flat dysplastic lesions or dysplasia-associated lesion or mass (DALM) precedes cancer associated with UC. DALMs are a heterogeneous population of lesions with different endoscopic features (adenoma-like and non-adenoma-like).[12] Distinguishing a polyp from a mass associated with non-adenoma-like disease can be difficult. In an internet-based study significant inter-observer variability was noted between investigators.[13] An important observation is the fact that dysplasia is detected in inflamed mucosa.[14] On the other hand, dysplasia can occur because of active inflammation; it will disappear if the inflammation goes into remission. Aggressive medical treatment will result in long-standing remission and a decrease in risk of CRC.[15] The risk for development of CRC in normal non-inflamed mucosa does not appear to be higher, compared with the normal population.[16]
The present study shows that adenomas can be present in patients with either active inflammation or remission. Just as the normal population, patients with UC are also at risk of developing adenomas. Hence, it is also plausible that cancers detected in patients with UC can arise from adenomas and have nothing to do with chronic inflammation. Despite all the studies on adenomas and UC, little data are known on the occurrence of adenomas in patients with UC. Jess *et al.* studied colorectal dysplasia in inflammatory bowel disease (IBD). They used a large database of patients with documented inflammatory bowel disease. In 4% of patients, dysplasia was detected. Of these, 18 had adenoma-like lesions in regions with active inflammation, and two had an adenoma outside the region with inflammation.[8]
In another study, the prevalence of adenomas in patients with IBD was compared with local age-matched controls who participated in the national screening trial for CRC. Of 106 patients, 80 suffered from UC, 20 from Crohn's disease, and six from indeterminate colitis. Distal adenomas were found in three patients with UC compared with 67 of 749 controls (2.8 *vs* 8.9%, p=0.03). This result suggests that distal adenomatous polyps are rare in patients with IBD compared with a control population. The authors state that this finding supports the hypothesis that lesions other than polyps are important for the development of CRC in patients with IBD.[17]
Torres *et al.* studied 89 benign polypoid epithelial neoplasms from 59 patients with IBD (51 with UC, eight with Crohn's colitis). Patients were categorised arbitrarily as having a probable sporadic adenoma if the polypoid epithelial neoplasm was not located within areas of histologically proven colitis and a probable IBD-associated polypoid dysplasia if the lesion developed within an area of colitis. Sixty-six percent had active disease at the time of presentation. Nearly 70% of patients had only one polyp; the majority occurred in either the left colon or the rectum (66%). At follow-up evaluation a further neoplastic lesion developed in 20%; low-grade flat dysplasia was seen in five (12.5%), and adenocarcinoma developed in three (7.5%). However, dysplasia or adenocarcinoma did not develop in the patients who had polyps located outside areas of active colitis. Patients with probable IBD-associated polypoid dysplasia had a statistically significantly (p<0.05) longer disease duration than patients with probable sporadic adenoma.[18] However, sporadic adenomas did occur in this study.
Rubio *et al.* studied the histological phenotype of the dysplastic lesion juxtaposing colorectal carcinomas in 50 consecutive colectomy specimens of patients with inflammatory bowel disease. Adenomatous growths juxtaposing carcinomas were found in 76% (n=38) of the IBD cases: 52.3% were villous, 28.9% were serrated, 5.3% tubular and the remaining 13.2% were mixed.[19]
Given the results of the present study, patients with different phenotypic expressions of inflammatory bowel disease can have concomitant adenomas in the colon. The prevalence is according to the literature. Hence, it is plausible to assume that these patients also have an increased risk of developing CRC because of adenomas. Development of CRC in these patients is not necessarily associated with the inflammation. The presence of adenomas is irrespective of disease activity or extent.
Patients with IBD in remission should undergo screening colonoscopy, as advocated in the normal population, for detection of adenomas.

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CASE REPORT

Disseminated *Rhizopus microsporus* infection in a patient on oral corticosteroid treatment: a case report

P. de Mol[1*], J.F.G.M. Meis[2]

Departments of [1]Internal Medicine and [2]Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands, [*]corresponding author: tel.: +31 (0)24-365 76 57 (pager 2445), e-mail: P.d.Mol@cwz.nl

ABSTRACT

A 71-year-old male with mild steroid-induced hyperglycaemia was diagnosed with a lethal invasive *Rhizopus microsporus* infection.
Disseminated zygomycosis is a rare entity and is most frequently found in neutropenic patients with haematological malignancies, post-transplants or in patients on deferoxamine therapy. Infection is characterised by tissue infarction and necrosis due to angioinvasive hyphae. Culture of *Zygomycetes* is necessary for species determination but histology is a must to prove the infection. Ante-mortem diagnosis and culture is challenging and therefore mortality approaches 100%. Apart from amphotericine B, most anti-fungals have no activity against *Zygomycetes* but posaconazole might offer new possibilities as a first-line agent. Timely diagnosis, rapid surgery of infected tissue, correction of underlying disorders and correct anti-fungal therapy might be life-saving. Due to the increasing use of potent immunosuppression, stem cell and organ transplants and possibly selection for *Zygomycetes* by prior treatment with broad-spectrum antifungal therapy, the incidence of zygomycosis is rising. Therefore, clinicians might encounter an increasing number of zygomycosis cases in the near future.

KEYWORDS

Corticosteroids, disseminated zygomycosis, non-neutropenic, *Rhizopus microsporus* var. *microsporus*

INTRODUCTION

Very few cases of zygomycosis in adults due to *Rhizopus microsporus*, a fungus belonging to the class of *Zygomycetes*, have been reported in English literature.[1-6] Disseminated zygomycosis caused by *Rhizopus microsporus* var. *microsporus* has only been reported once.[6] Disseminated zygomycosis is almost exclusively diagnosed in patients with major risk factors for development of zygomycosis such as neutropenia, (post) transplantation, haematological malignancies, diabetic keto-acidosis and corticosteroid treatment.[7,8]
We present a case of disseminated *Rhizopus microsporus* infection in a non-neutropenic patient with corticosteroid therapy and mild steroid-induced hyperglycaemia.

CASE REPORT

A 71-year-old male presented to the emergency department with acute, non-colic like, abdominal pain in the lower right quadrant. There was no history of fever, but he was complaining of shortness of breath on exertion for three weeks without chest pain, cough or sputum production. There were no complaints of nausea or vomiting and his defecation and urinary patterns were unremarkable.
He had been treated with 90 mg/day oral prednisolone therapy for idiopathic thrombocytopenic purpura (ITP) for two months. Further medical history was uneventful.
On physical examination the body temperature was 37.4°C, blood pressure 170/88 mmHg, pulse rate 108/min, pulse oxymetry 99% SaO_2 (room air) and respiratory rate was 18 breaths/min. Lung and heart sounds were normal. Abdominal examination showed right flank and lower quadrant pain without rebound tenderness or guarding. No masses or lymphomas were noted. Genital inspection and

rectal examination were unremarkable. Both shins showed brownish discoloured petechiae.

Laboratory findings were as follows (normal reference values in parentheses): haemoglobin 7.8 mmol/l (8.5 to 11.0), mean corpuscular volume 90 fl (80 to 100), white cell count 5.4 x 10^9/l (4.0 to 11.0) with 76% segments and 21% lymphocytes and no bands in the differentiation, platelets 23 x 10^9/l (150 to 400), C-reactive protein 100 mg/l (<5), glucose 10.2 mmol/l (4.0 to 9.0), and creatinine 66 µmol/l (60 to 110). Serum electrolytes and liver biochemistry were normal. Urinalysis: >100 erythrocytes (0 to 5) and no leucocytes/visual field; no protein or casts were noted but glucosuria without ketonuria was present.

Abdominal ultrasound and plain X-ray taken on the day of first presentation were normal and patient was admitted for observation of the abdominal pain, mild steroid-induced hyperglycaemia and haematuria due to thrombocytopenia (*figure 1*). The next day an IV-contrast enhanced abdominal CT was made which showed no abnormalities except for irregular enhancement of the upper zone of the right kidney, suspect for a cyst. Cystoscopy and urine cytology were normal and haematuria receded spontaneously. Infection parameters rose (day 3) and a chest X-ray revealed a large opacity in the left lung. In contrast, a chest X-ray taken three months prior to presentation showed no abnormalities.

Pneumonia and/or a possible malignancy of the lung was suspected and oral moxifloxacin was started and prednisolone dosage was tapered (day 3).

A contrast-enhanced CT thorax showed an opacity in the left lung and an additional mass in the right apex (day 4). Blood, sputum and urine cultures for bacteria and fungi remained sterile (*figure 2*).

Figure 1. *Thorax*

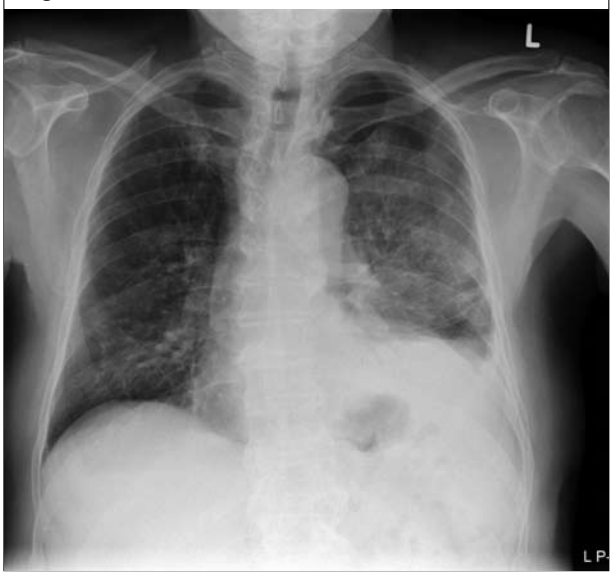
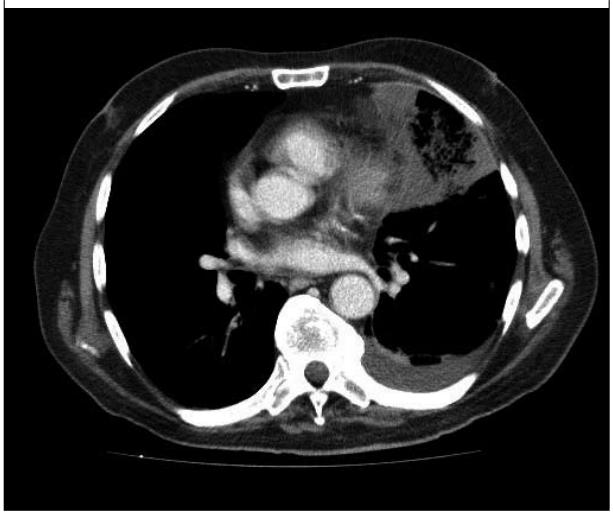


Figure 2. *CT-scan*



Bronchoscopy and broncho-alveolar lavage (BAL) were performed on the 10th day of admission demonstrating a purulent haemorrhagic area in the left upper lobe. Lung biopsy was abandoned because of the patient's low platelets. Lavage analysis demonstrated an inflammatory process, no malignant cells, no *Pneumocystis jiroveci* and no acid-fast bacilli. Culture grew pharyngeal flora including *Candida albicans*. Oral glimepiride was started for mild hyperglycaemia and antibiotics were changed into ciprofloxacin and amoxicillin intravenously. Oral itraconazol 300 mg twice a day was added empirically for systemic mycosis and prednisolone therapy was stopped (day 14).

After 24 days of hospital admission a high-resolution CT thorax showed progression of pulmonary lesions but no halo-sign. The right kidney now demonstrated evidence of infarction. By this time, the glimepride had been stopped and glucose levels remained within the normal range. Invasive aspergillosis and changing antifungal therapy into voriconazole was considered after further deterioration of the patient's clinical state. However, in absence of further diagnostic procedures and normal aspergillus antigen test (Galactomannan), itraconazole therapy was continued.

On the 31st day the patient became febrile, developed respiratory failure and was transferred to the ICU of a tertiary care hospital. The patient died one day after open lung biopsy.

Post-mortem histological examination showed broad, irregular branched, aseptate hyphae in the left lung, and right kidney. Cultures of this material grew *Rhizopus microsporus*.

DISCUSSION

Zygomycosis is the third most common invasive fungal infection after candidiasis and aspergillosis.[9] The incidence of zygomycosis is rising due to multiple factors including

the increasing use of potent immunosuppression, stem cell and organ transplants and possibly selection for *Zygomycetes* by prior treatment with broad-spectrum antifungal therapy, which has no activity against *Zygomycetes*.[10] *Rhizopus*, together with *Mucor, Rhizomucor, Absidia, Apophysomyces, Cunninghamella* and *Saksenaea* are placed in the order of *Mucorales*, class of *Zygomycetes*. The common term zygomycosis is used for infections caused by any of these fungi. The organisms are ubiquitous spore-forming saprophytes growing in decaying organic matter. Inhalation or percutaneous introduction of spores causes infection almost exclusively in patients with underlying immuno-compromising conditions. Diabetes mellitus and/or keto-acidosis, neutropenia, haematological malignancies, deferoxamine therapy, IV drug use, local burn wounds or trauma, severe malnutrition and solid organ transplantation are known risk factors for invasive zygomycosis.[7,8]

Infection is characterised by tissue infarction and necrosis due to angioinvasive hyphae. Once established, the disease is rapidly progressive and often fatal.

Six clinical syndromes caused by *Zygomycetes* are recognised: rhino-orbital-cerebral, pulmonary, cutaneous, disseminated, gastrointestinal and miscellaneous forms.

This case represents a non-neutropenic, immuno-compromised patient on oral corticosteroid therapy with mild, steroid-induced hyperglycaemia who developed a disseminated *Rhizopus microsporus* infection, proven by histological analysis at autopsy. Although diabetes mellitus is a major risk factor for rhinocerebral disease, disseminated zygomycosis is a rare entity and is most frequently found in neutropenic patients with haematological malignancies, post-transplants or in patients on deferoxamine therapy.[11,12] In a large series, *Rhizopus* species accounted for 218 of 465 (47%) patients but *R. microsporus* has only been isolated from 11 of 465 (2%) patients.[12]

R. microsporus is a thermophilic zygomycete with a worldwide distribution, and is saprophytic, living in soil, air and decaying matter and growing well on substrates such as fruits, cereals and bread. Among the four known varieties, only *R. microsporus* var. *microsporus* and *R. microsporus* var. *rhizopodiformis* have been reported to cause infections in humans.[3]

So far only five cases of *R. microsporus* var. *microsporus* have been reported in English literature of which only one disseminated case.[1-5,6]

As this case illustrates, ante-mortem diagnosis of disseminated zygomycosis is a challenge and many patients are often co-infected with other pathogens. Typically, zygomycosis is only considered after precious time has been lost unsuccessfully treating suspected bacterial or other fungal infections with broad-spectrum antibiotics. Due to easy inhalation of spores, pulmonary zygomycosis and rhino-orbital forms of zygomycosis are common; as in this case, the airway was probably the primary site of infection. Negative culture and analysis of bronchalveolar lavage fluids do not safely exclude zygomycosis.[13] Radiological findings such as halo-signs, consolidation and necrosis on (high-resolution) CT scans might support diagnosis but are not specific for zygomycosis.[14] As a result, mortality is high, approaching 100% in disseminated cases.[12] Culture of *Zygomycetes* is necessary for species determination but histology is necessary to prove the infection.

Therefore we stress the importance of tissue biopsy for histological confirmation of zygomycosis. Although at-risk patients might have thrombocytopenia or other conditions making biopsy complicated, timely histological confirmation of zygomycosis could be life-saving. Exogenous transfusion of platelets and tissue biopsy earlier in the course of this case might have provided earlier recognition of the causative pathogen followed by the appropriate treatment.

Furthermore, the immuno-compromised patient population is significant and growing in size as chemotherapy, potent immunosuppressive therapy and transplant procedures are increasing.[15] Therefore, clinicians might start encountering cases of zygomycosis more frequently in the near future.

As noted earlier incidence of zygomycosis is rising. An additive cause might be empirical treatment of at-risk patients with voriconazole causing selection of nonsusceptible fungi such as *Zygomycetes*. Decreased incidences of aspergillosis together with breakthrough cases of zygomycosis are reported in literature. In the same study Kontoyiannis and co-workers marked voriconazole therapy as an independent risk factor for zygomycosis.[16]

For treatment of zygomycosis, amphotericin B in the highest tolerable dose is still the drug of choice together with resection of infected tissue and correction of underlying risk factors. In this case, immunosuppressive therapy was tapered and mild hyperglycaemia treated. Typically, due to the post-mortem diagnosis the patient had not been treated with surgery and treatment with antifungal therapy directed against *Zygomycetes*.

Triazoles, such as itraconazole, fluconazole and voriconazole, have little activity against *Zygomycetes* and therefore are ineffective for empirical treatment when zygomycosis is suspected. However, posaconazole, a new broad-spectrum triazole, has proven successful as salvage therapy in treatment of breakthrough zygomycosis.[17] Furthermore, Peel *et al*. have recently reported a case of disseminated zygomycosis due to *R. microsporus* var. *microsporus* successfully treated with posaconazole as first-line therapy.[6]

In vitro susceptibility testing showed superior activity for a wide range of fungi including *Zygomycetes* for posaconazole compared with fluconazole, itraconazole and voriconazole.[18] Interestingly, the different species of *Rhizopus* show different susceptibilities in murine models to posaconazole where *R. microporus* var. *rhizopodiformis* is susceptible at higher minimal inhibitory concentrations

and *R. oryzae* is non-susceptible. *R. microsporus* var. *microsporus* susceptibility to posaconazole has not been tested yet.[19] Considering this together with difficulties in appropriate dosage for amphotericin B due to toxic side effects, there might be a new place for posaconazole as first-line therapy for zygomycosis.[20]

CONCLUSION

We present a case of disseminated *R. microsporus* infection in a non-neutropenic patient with mild, steroid-induced hyperglycaemia. Timely diagnosis of invasive zygomycosis, preferably by culture and histological confirmation together with surgery and correction of underlying conditions, is a prerequisite for successful treatment. Posaconazole might offer new possibilities for first-line therapy against zygomycosis although amphotericin B remains the cornerstone of antifungal therapy.

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CASE REPORT

A diagnostic difficulty: two cases of haemophagocytic syndrome in adults

C.A. Wijsman[1], J.E. Roeters van Lennep[1*], P.A. von dem Borne[2], A.J. Fogteloo[1]

Departments of [1]Internal Medicine and [2]Haematology, Leiden University Medical Center, Leiden, the Netherlands, [*]corresponding author: tel.: +31 (0)70-526 20 85, e-mail: J.E.Roeters@lumc.nl

ABSTRACT

Haemophagocytic syndrome is a rare and life-threatening disease, which often goes unrecognised in adults, with high mortality as a consequence. Here we present two adult patients who were diagnosed with haemophagocytosis of distinct underlying causes which, despite treatment, led to fatal outcomes. Measuring ferritin is an easy and cheap resource in diagnosis.

KEYWORDS

Ferritin, haemophagocytic syndrome, haemophagocytic lymphohistiocytosis

INTRODUCTION

Haemophagocytic syndrome, or haemophagocytic lymphohistiocytosis (HLH), is a rare, potentially life-threatening disease characterised by an inappropriate activation of lymphocytes and histiocytes, which leads to an uncontrolled systemic inflammatory response. Two forms of HLH can be distinguished: primary or familial HLH, which usually occurs at a young age because of underlying genetic immunodeficiencies, and a secondary form, which may occur at all ages and is associated with infection, malignancies (mostly lymphomas) and different types of rheumatological disease.[1,2]

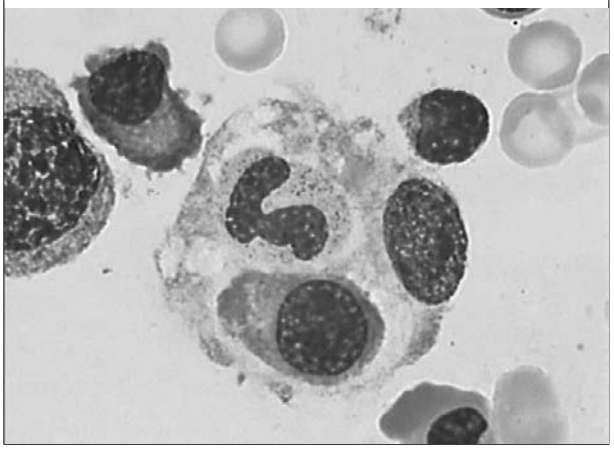
Clinically, the two forms are indistinguishable, characterised by a sepsis-like presentation with splenomegaly, cytopenias, hyperferritinaemia, coagulopathy and haemophagocytosis. Consequently, multi-organ failure often develops, leading to high mortality.[2]

We describe two adult patients with HLH of different underlying aetiologies, illustrating both the severity of the disease, and the need for prompt recognition, diagnostics and adequate treatment. In both patients, among nonspecific clinical and laboratory findings, markedly increased ferritin levels were found, which facilitated the difficult diagnosis.

CASE REPORTS

Case 1, a 35-year-old man was diagnosed with an aggressive cutaneous T-cell lymphoma in 2005, for which he received chemotherapy and finally myeloablative stem-cell transplantation from a related donor. Two months after transplantation, relapse of the cutaneous T-cell lymphoma was diagnosed, for which a donor lymphocyte infusion was scheduled. A few weeks thereafter, the patient developed unremitting high fever without signs of an underlying cause. Laboratory investigation revealed pancytopenia (haemoglobin 6.2 mmol/l, white blood cell count (WBC) 1.4 x 10^9/l, platelets 6 x 10^9/l), abnormal liver enzymatic function (alkaline phosphatase 189 U/l, gamma-glutamyl-transferase (γGT) 172 U/l, asparate aminotransferase (ASAT) 171 U/l, and alanine aminotransferase (ALAT) 67 U/l), elevated triglycerides at 3.36 mmol/l and elevated ferritin at >18,000 µg/l. On polymerase chain reaction (PCR), no active Epstein-Barr virus (EBV), cytomegalovirus (CMV) or herpes simplex infection were found. Bone marrow aspirate and biopsy revealed a strong increase in macrophages and haemophagocytosis, without lymphoma localisation (*figure 1*). HLH was diagnosed and treatment with etoposide 150 mg/m^2 twice weekly, and dexamethasone 10 mg/m^2 daily initiated. After three weeks of treatment, no clinical improvement ensued and haemophagocytosis prevailed, undiminished. Because of elevated liver enzymes, the etoposide dose was reduced by half. Ultimately, clinical improvement followed and the ferritin concentrations diminished to 12,000 µg/l. One week later, the patient was re-admitted because of high fever. The ferritin concentration proved to be >80,000

Figure 1. *Bone marrow showing macrophages with haemophagocytosis*



µg/l. Sepsis caused by a pneumococcal infection followed, and the patient died of respiratory failure.

Case 2, a 63-year-old man with systemic lupus erythematosus (SLE) was admitted because of suspected exacerbation of SLE. His complaints consisted of malaise, weight loss, loss of muscle strength and diminished concentration and memory. Upon physical examination, the patient proved to be a moderately ill man, with slow responses and fever (39.2°C). Laboratory evaluation revealed anaemia and thrombocytopenia (haemoglobin 6.2 mmol/l, platelets 109 x 10^9/l, and WBC 4.5 x 10^9/l), abnormal liver enzymes (ASAT 331 U/l, ALAT 123 U/l, lactate hydrogenase 1619 U/l, alkaline phosphatase 148 U/l, γGT 213 U/l) and a strongly elevated ferritin of 19,000 µg/l, without coagulopathy.

No abnormalities were seen on the chest X-ray, ultrasound, CT abdomen, and MRI of the cerebrum. Lumbar puncture showed no signs of infection. Under suspicion of neuro-SLE, treatment with methylprednisolone was initiated. A few days later, spontaneous bleeding from the jaw occurred, caused by diffuse intravasal coagulation. The patient was transferred to intensive care, and because of respiratory failure he was intubated and artificial ventilation was started. Bone marrow biopsy revealed increased iron-containing macrophages and haemophagocytosis. HLH was diagnosed and treatment with prednisolone and cyclophosphamide was started. After a brief improvement and detubation, the patient developed respiratory failure and haemodynamic insufficiency due to peritonitis based on perforated diverticulitis. A laparotomy was performed but, postoperatively, fulminant sepsis lead to his death.

DISCUSSION

Haemophagocytic syndrome is a rare disease. The primary or familial form has an autosomal recessive inheritance with an incidence of approximately 1 in 50,000 live-born children.[3] The disease usually occurs in the first two years of life, although cases in adulthood have been described.[4,5] The incidence of secondary HLH is unknown; it probably exceeds the incidence in children. Because of clinically overlapping presentations, it is probable that HLH is overlooked in patients considered to have severe inflammatory response syndrome (SIRS) on the ICU.[1]

Secondary HLH is associated with several diseases in a more or less convincing way (*table 1*).[1] Most case reports describing HLH in adults concern patients with haematological malignancies or EBV infections as the underlying disease.[1,6-8]

HLH is characterised by lymphohistiocytosis with marked proliferation of histiocytes, T lymphocytes and natural killer (NK) cells. Analysis of families with primary HLH has revealed markedly low NK and T-cell mediated cytotoxicity, with distinctive genetic abnormalities in genes encoding proteins involved in NK and T-cell induced cytotoxicity. Perforin deficiency is often found. Deficient cytotoxicity could lead to continuous immune activation and a cytokine storm, with concomitant phagocytosis of erythropoietic cells due tot proliferation and activation of macrophages.[2,7,9] Consequently, a severe inflammatory reaction is seen with fever, pancytopenia, coagulopathy and hyperferritinaemia due to release of ferritin by the reticulo-endothelial system.[10] The pathogenetic background

Table 1. *Diseases associated with secondary HLH*

Infections
Viral (mostly Epstein-Barr virus)
Bacterial
Parasitic (mostly visceral leishmaniasis)
Fungal
Rheumatological disease
Rheumatoid arthritis (mostly Still's disease)
Systemic lupus erythematosus
Sarcoidosis
Systemic sclerosis
Dermatomyositis
Autoimmune diseases
Glomerulonefritis
Inflammatory bowel disease
Vasculitis
Hashimoto thyreoiditis
Malignancy
Haematological malignancy (mostly lymphoma of T and NK cell type)
Solid tumours
Secondary immunodeficiency
HIV/AIDS
Transplantation
Chemotherapy
Immunosuppressive therapy
Dermatological disease
Pyoderma gangrenosum

of secondary HLH remains unclear. As in primary HLH, the dysregulation of the immune system probably leads to an ongoing immune activation and cytokine storm. HLH associated with malignant lymphoma could possibly be triggered by tumour-induced cytokine secretion.[1,11]

Diagnostic criteria for HLH, developed by the Histiocyte Society, are described in *table 2*.[12] Haemophagocytosis can be established not only in bone marrow, but also in other lymphoid tissue such as liver, spleen and lymph nodes. While histologically proven haemophagocytosis is regarded as the gold standard for HLH, it cannot be found on the first bone marrow biopsy in 20% of patients.[13] As localisation of haemophagocytosis may differ during the course of the disease, repetitive biopsies are sometimes required for diagnosis.[11] Therefore, an important diagnostic parameter is ferritin; mildly elevated ferritin is seen in many inflammatory illnesses, and therefore nonspecific. Nonetheless, ferritin concentrations greater than 10,000 µg/l are only seen in HLH, Still's disease, malignant histiocytosis, and after multiple blood transfusions. For HLH, research in children has shown a specificity of 96% for ferritin concentrations greater than 10,000 µg/l, increasing to 98% when fever is present.[14] Therefore, in combination with clinical and laboratory findings, extremely high ferritin levels are highly specific for HLH.[1,15] As in our cases, finding markedly elevated ferritin concentrations could lead to prompt recognition of the disease.

Table 2. *Diagnostic criteria (≥5) for HLH according to the Histiocyte Society*

Fever (>38.5°C for at least 7 days)
Splenomegaly
Cytopenia (at least 2 of 3 cell lines)
Hypertriglyceridaemia and/or hypofibrinaemia
Haemophagocytosis in bone marrow, spleen or lymph nodes, without signs of malignancy
Ferritin ≥500 µg/l
Soluble CD25 ≥2400 U/ml*
Low or absent NK-cell activity*

*No routine measurement.

Treatment of primary HLH is, because of its low incidence, done in multicentre trials coordinated by the Histiocyte Society, (HLH 94 and HLH 2004 studies). The aim of treatment is the induction of remission with etoposide 150 mg/m² twice weekly, dexamethasone 10 mg/m² daily, and cyclosporine-A 6 mg/kg/day in combination with maximum supportive care with antibiotics, antiviral therapy and intravenous immune globulins. Ultimately stem cell transplantation should follow.[12] In secondary HLH, treating the underlying cause is essential. In infection-induced mild HLH, this is possibly sufficient. However, without intensive treatment, severe HLH is fatal without exception. Early intensive treatment in adults could possibly increase the chances of survival.[7] Therefore, treatment according to HLH protocols is recommended.[12]

CONCLUSION

The patient histories described above illustrate the severity of the secondary form of HLH in adults. Diagnosis is difficult, as many symptoms are nonspecific and often present in severely ill patients. Although rare, HLH should be considered in patients with unexplained fever and signs of SIRS. For prompt diagnosis and treatment, ferritin can be used as an easy and cheap diagnostic clue.

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LETTER TO THE EDITOR

Budd-Chiari syndrome

Dear Editor,

I read with interest the article by Hoekstra and Janssen.[1] The authors do not mention some important causes of Budd-Chiari syndrome. Carcinoma, trauma, surgery, immobilisation, sarcoidosis, inflammatory bowel disease, and dacarbazine therapy are also risk factors for hepatic outflow tract obstruction.[2,3]

W. Kittisupamongkol
Hua Chiew Hospital, Bangkok 10100, Thailand, tel.: +66-8799 896 70, e-mail: weekitti@gmail.com

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RESPONSE TO LETTER TO THE EDITOR

Dear Editor,

We thank Dr Kittisupamongkol for his response to our article. He mentions a number of potential causes of Budd-Chiari syndrome that were not specifically addressed in our review. We agree that the list of potential risk factors for Budd-Chiari syndrome, as given in *table 1* of the review, is not complete. However, the aetiological factors mentioned in our article represent the most important causes of this disease in patients from Western countries.[1,2] The causes of hepatic vein obstruction cited by Dr Kittisupamongkol are only encountered in a very small number of cases.

In patients with malignancy, development of Budd-Chiari syndrome can be the result of tumoral invasion of the hepatic veins. As we mentioned in our article, this is a secondary form of Budd-Chiari syndrome which is rarely seen. Furthermore, the main focus of our review was on primary Budd-Chiari syndrome, as caused by thrombosis of the hepatic veins or inferior vena cava. Immobilisation, to our knowledge, has never been identified as an independent risk factor for Budd-Chiari syndrome. Trauma, surgery and sarcoidosis have been reported in the literature as aetiological factors of hepatic vein thrombosis but this has been limited to a few case reports.[3,4] Inflammatory bowel disease is present in some patients with Budd-Chiari syndrome.[5] However, this is also an infrequent cause of hepatic vein thrombosis and in many of these patients another risk factor for thrombosis is present. The last factor referred to by Dr Kittisupamongkol, dacarbazine therapy, is a known cause of sinusoidal obstruction syndrome (SOS), previously known as veno-occlusive disease.[6] This is a separate clinical entity that should be distinguished from Budd-Chiari syndrome.

H.L.A. Janssen*, J. Hoekstra
*Department of Gastroenterology and Hepatology (Ha 206), Erasmus MC, University Medical Center Rotterdam, the Netherlands, *corresponding author: tel.: +31 (0)10-703 59 42, fax: +31 (0)10-436 59 16, e-mail: h.janssen@erasmusmc.nl*

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Docetaxel-induced skin toxicity

Dear Editor,

Chemotherapy has an increasing potential for cure and palliation of most forms of cancer in different stages. However, its use is often limited by side effects. We observed two patients with skin toxicity after docetaxel treatment.

Both men were known with irresectable prostate cancer and presented with erythema on their hands after docetaxel treatment. They were treated with androgen-deprivation therapy, but started docetaxel (Taxotere®, 35 mg/m^2 every week) for hormone-refractory disease with metastases. The first patient presented with a painful well-demarcated erythema of the right hand after four administrations of docetaxel. After two days similar lesions appeared on the fingers of the left hand with vesicles and crustae (*figure 1*). Blood cultures showed no growth. Skin biopsy showed hyperkeratosis, loss of the stratum granulosum and acanthosis (*figure 2*). Intra-epidermal ballooning with degeneration, loss of nucleolar basophilia and basal pleomorphism of keratinocytes was seen. Reactive proliferation of small blood vessels in the upper dermis was accompanied by some mononuclear infiltrate. Based on the histological findings and after exclusion of infectious disease the diagnosis of a drug-induced skin toxicity was likely. After permanent discontinuation of docetaxel all skin lesions resolved completely.

Figure 1. *Erythema and desquamation observed after 4 cycles of weekly docetaxel*

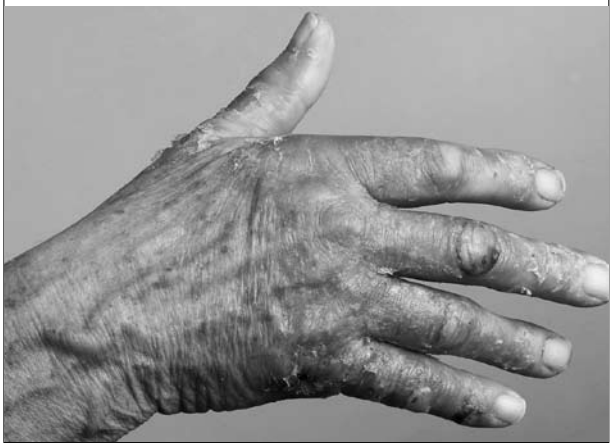
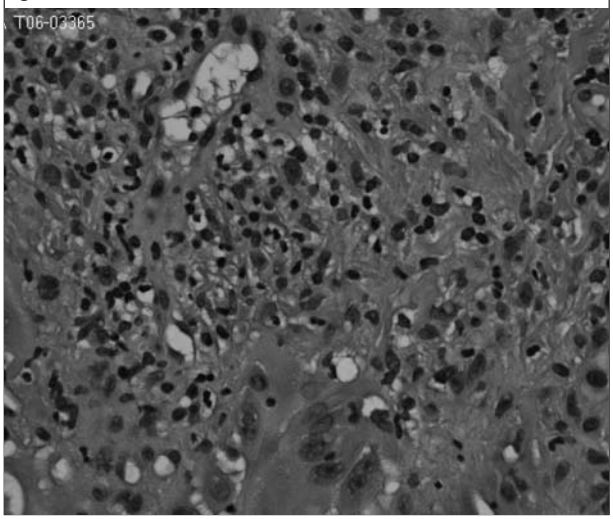


Figure 2. *Microscopic examination of a lesion of the right hand showed hyperkeratosis, loss of the stratum granulosum and acanthosis*



The second patient presented with multiple painful red plaques on both hands after 22 administrations of docetaxel. Docetaxel was discontinued promptly, which resulted in complete resolution of the skin eruptions within two weeks.

Chemotherapy with docetaxel is a palliative option for patients with hormone-refractory prostate carcinoma. Skin toxicity due to docetaxel (erythema and exfoliation to diffuse desquamative dermatitis) has been described in studies in breast and ovarian cancer.[1] In a dose-finding study of weekly docetaxel in patients with breast or ovarian cancer, skin toxicity was observed in 10 out of 32 patients (31%) at a dose level of 80 mg/m^2 or higher.[2]

Our observations resemble the palmar-plantar erythrodysesthesia (PPE), also known as hand-foot syndrome, a syndrome of painful dermatitis of the palms and soles following administration of chemotherapy. It is a well-known side effect of 5-fluouracil and capecitabine,[3] but has also been associated with docetaxel,[4] sunitinib and sorafenib. In contrast to PPE, our patients did not report tingling pain in the fingers, and lacked any symptoms of the feet. The underlying mechanism of

PPE and the reason for its particular distribution are unknown. Pyridoxine may decrease the number of dermatological reactions with docetaxel[4] and has been effective in delaying the onset and severity of doxorubicin-associated PPE.

L. Arwert
Department of Internal Medicine, Medical Center Alkmaar, the Netherlands, tel.: +31 (0)72-548 44 44, fax: +31 (0)72-548 21 65

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Netherlands
The Journal of Medicine

PHOTO QUIZ

Progressive trichomegaly

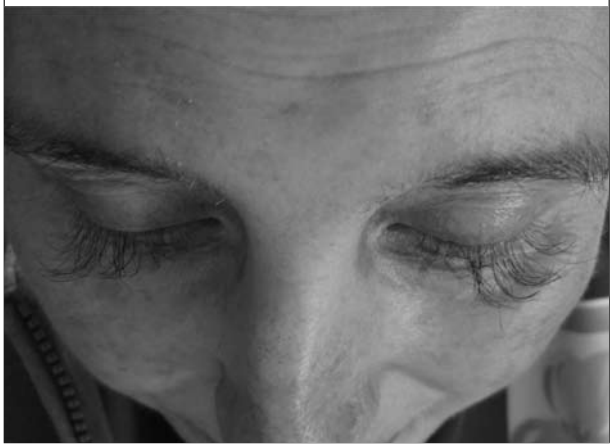
S. Vano-Galvan[1*], P. Moreno-Martin[2], P. Jaén[1]

[1]Dermatology Service, Ramon y Cajal Hospital, University of Alcala, Madrid, Spain, [2]Ophthalmology Service, La Princesa University Hospital, Madrid, Spain, [*]corresponding author: tel.: +34 67662 77 42, fax: +34 9137735088, e-mail: sergiovano@yahoo.es

CASE REPORT

A 62-year-old woman presented with a four-week history of progressive growth of her eyelashes that induced visual discomfort and inconvenienced her while blinking. Her medical history consisted of hypertension, hyperuricaemia and obesity. The diagnosis of T3N2CM0 squamous cell carcinoma of the oropharynx had been made in this patient and she was treated with radiotherapy plus concomitant cetuximab. Radiotherapy was delivered with concomitant boost technique, lateral parallel opposed fields to the primary tumour and upper neck (70 Gy/40 fractions/42 days). Cetuximab was administered one week before radiotherapy at a 400 mg/m^2 loading dose, followed by 250 mg/m^2 weekly during radiotherapy. She noted a change in her eyelashes within several weeks of starting the drug. Examination of her ocular adnexa demonstrated coarse, brittle, irregular and aberrant bilateral eyelash growth (*figure 1*) without associated hypertrichosis. The rest of physical examination was within normal limits. She had not had any other recent changes in her medications and denied application of cosmetic products. Laboratory tests did not reveal thyroid hormones abnormalities.

Figure 1. *Extraordinarily long eyelashes, 25 to 35 mm, after cetuximab therapy*



WHAT IS YOUR DIAGNOSIS?

See page 36 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 35)

DIAGNOSIS

The recent development of progressive trichomegaly during treatment with epidermic growth factor receptor (EGFR) inhibitor led us to make the diagnosis of acquired trichomegaly of the eyelashes induced by cetuximab.

Trichomegaly, the excessive growth of eyelashes, is a relatively rare cosmetic disorder that has been described as part of congenital syndromes, in human immunodeficiency virus 1 infection, associated with an autoimmune disease, or after certain drugs such as latanoprost, phenytoin, zidovudine, penicillamine, cyclosporine and interferon alpha. Other described causes of acquired trichomegaly include porphyria, malnutrition, anorexia nervosa, hypothyroidism, and pregnancy.

Recent communications have reported that EGFR inhibitors used in the treatment of certain malignancies can also lead to symptomatic adnexal and ocular surface changes.[1-4]

EGFR inhibitors are a new class of biologically targeted anticancer agents recently introduced in the management of cancers unresponsive to standard chemotherapeutic agents. Cetuximab has been recently approved by the US FDA for head and neck squamous cell carcinoma, in association with radiotherapy. During treatment with EGFR inhibitors, changes of the hair can be noticed.

Trichomegaly of the eyelashes has been described in association with cetuximab,[1-3] erlotinib[4] and gefitinib. In pathogenesis, EGFR inhibition results in dysregulated keratin gene expression within the hair follicle and results in premature maturation (terminal differentiation) of the epithelial cells of the hair follicle causing the observed trichomegaly.[2]

In our case, trichomegaly of the eyelashes was reported after starting cetuximab with complete regression of these symptoms two months after stopping administration of cetuximab; the patient did not receive other trichomegaly-inducing drugs. These arguments are in favour of the relationship between cetuximab and trichomegaly. Cetuximab can be added to the list of drugs responsible for trichomegaly of the eyelashes.

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PHOTO QUIZ

A patient with prickling boils

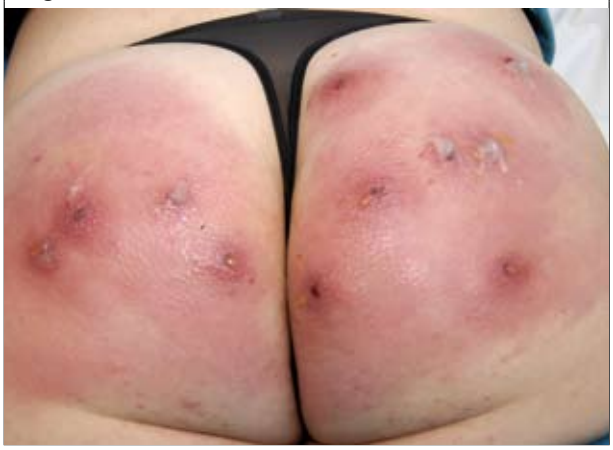
R.A. Douma[1*], M. ten Wolde[1], P.P.A.M. van Thiel[2], M. van Vugt[2]

Departments of [1]Internal Medicine and [2]Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)20-566 82 74, fax: +31 (0)20-696 88 33, e-mail: r.a.douma@amc.uva.nl

CASE REPORT

A 35-year-old woman presented with itchy, painful and 'prickling' boils on her buttocks eight days after a one-week stay in rural Tanzania, where she had worked in a voluntary project building a school. The red nodules had developed over a couple of days, starting two days after her return. Her general practitioner had started oral doxycycline treatment four days earlier without any result. She had no fever or other symptoms. Each boil had a reddish-brown crust with an extensive surrounding inflammatory reaction (*figure 1*). She had no previous medical history and was not on any medication.

Figure 1. *Buttocks with boils*



WHAT IS YOUR DIAGNOSIS?

See page 38 for the answer to this photo quiz.

DIAGNOSIS

After the removal of one of the crusts, a black central punctum was visible, with a moving larva in the central opening. Suspecting cutaneous myiasis, we removed all the crusts and applied Vaseline to each punctum to asphyxiate the larvae and lure them out of the nodules. We managed to extract all larvae (*figure 2*). They were identified as the larvae of *Cordylobia anthropophaga* or Tumbu-fly, endemic in sub-Saharan Africa. The female Tumbu-fly deposits her eggs (100 to 300 per lay) in soil polluted with animal excrement or on wet clothing hanging out to dry in the sun. After hatching, the larvae can stay alive for 7 to 20 days. They can easily penetrate the skin, where they develop into mature larvae in the dermis.[1,2]

The major differential diagnosis is furunculosis. Patients may complain of non-healing boils of the skin, with symptoms of pruritus, a sensation of movement under the skin and/or pain. A history of recent travel to an endemic area may also point into the direction of myiasis. The extraction and subsequent identification of the larvae confirms the diagnosis.

Because the larvae need air to develop, asphyxiation is an effective part of the treatment. Vaseline, but also several substances such as adhesive tape, butter, make-up creams and bacon have been successfully used for this purpose. Alternatively, lidocaine can be injected under the nodule (to paralyse the larva, while the pressure of the injection pushes the larva out) or the larvae can be surgically evacuated. Caution should be taken to extract the larva in its entirety, since remnants may prompt an inflammatory response.[2,3] Travellers to endemic countries are advised to iron all clothes that have been dried outside.

How our patient was infected remains unclear. She denied wearing un-ironed clothes. When sitting outside, she always wore light cotton pants and could not remember sitting on wet or possibly contaminated soil. To prevent secondary infection, our patient was additionally treated with oral amoxicillin-clavulanic acid and povidon-iodine ointment. Her symptoms quickly improved and the nodules resolved completely.

With the increase in travel to the tropics, cutaneous myiasis can be expected more frequently in returning travellers. Familiarity with the clinical presentation and treatment by physicians in non-tropical countries will avoid delayed diagnosis and unnecessary use of antibiotics.

Figure 2. *Larvae of Cordylobia anthropophaga, or Tumbu-fly*

ACKNOWLEDGMENTS

We thank Leny Nieuwendijk-de Bruin from the Section of Parasitology for determining the larval species and Winfried Barendregt, photographer, for taking the pictures.

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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