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EDITORIAL

Hepatic problems during pregnancy

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The liver is one of many organs affected by the physiological changes occurring during gestation. Hepatic excretion of bilirubins may be impaired in the second half of normal pregnancy. However, the liver tests are usually normal. Abnormal liver tests can be found in about 10% of pregnancies.[1] Severe liver disease complicates pregnancy in only 0.1% of the cases.[2] Among the serious liver enzyme abnormalities occurring during pregnancy, acute viral hepatitis is the most common cause, accounting for 40%.[2,3]

Liver diseases found during pregnancy can be categorised into four groups:[4-7]

1. Diseases unique to pregnancy that occur in the context of and exclusively during the pregnancy, such as liver involvement in patients with hyperemesis gravidarum, intrahepatic cholestasis of pregnancy (obstetric cholestasis), liver disorders associated with pre-eclampsia including HELLP syndrome (haemolysis, elevated liver enzymes, and low platelet count), hepatic infarction, intrahepatic haemorrhage/liver rupture and acute fatty liver of pregnancy (AFLP).
2. Liver diseases that are pre-existing in the pregnant patient, including a broad spectrum of liver disorders such as autoimmune hepatitis, Wilson's disease, focal nodular hyperplasia and non-cirrhotic portal hypertension. Worsening of chronic hepatitis B and C has been described; while some women with liver cirrhosis can sustain a normal pregnancy without deterioration of hepatic dysfunction, others develop liver failure. However, it is important to note that women with liver cirrhosis or with chronic liver disease are generally less fertile and have a higher rate of both stillbirths and premature infants.
3. Liver diseases that tend to arise or be exacerbated during pregnancy: cholelithiasis and Budd-Chiari syndrome are more prevalent in pregnant women. Viral infections involving the liver that are usually benign, such as viral hepatitis E and herpes simplex, are more likely to be exacerbated and more likely to develop into fulminant hepatic failure in pregnant women.
4. Liver disease incidental to pregnancy. Pregnant women can have the same liver problems as any one else, such as viral hepatitis or toxic hepatitis.

All liver disorders need to be recognised during pregnancy, because they can affect the wellbeing of the mother and baby. Although an unequivocal diagnosis of hepatic problems during gestation is often difficult to make, it should be attempted to determine the nature of the disease at an early stage so that optimal treatment can be given. Certain disorders such as acute viral hepatitis in pregnancy, AFLP, and intrahepatic haemorrhage or rupture associated with pre-eclampsia should be considered as medical emergencies and delay in diagnosis and treatment will adversely affect maternal and foetal outcomes.
A careful clinical history, physical examination, appropriate laboratory tests including serological investigation and imaging methods should allow a diagnosis to be made within 24 to 48 hours of presentation. Liver biopsy for analysis of hepatic disorders during pregnancy is rarely required.

In this issue of the journal there are three articles devoted to the hepatic problems in pregnancy: Tan *et al.* describe AFLP in a woman during the 39th week of gestation.[8] Labour was induced and she gave birth to a healthy boy.

However, the liver test abnormalities persisted for several weeks after birth. A liver biopsy taken 17 days after birth showed microvesicular steatosis. These findings are indeed consistent with those found in AFLP. AFLP is a late gestational complication with clinical similarities to fulminant hepatic failure. According to recent findings,[9,10] this maternal liver disease is associated with an isolated deficiency of long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) or a complete deficiency of a trifunctional protein that catalyses the last three steps of mitochondrial fatty acid oxidation in the foetus. Liver disease in pregnant women occurs most often when the deficiency of enzymatic activity in the foetus is severe. However, not all women who are carrying a foetus with LCHAD deficiency or trifunctional protein deficiency have liver disease. Moreover, at this moment it is not clear what the molecular mechanism is of the disease that develops in mothers not carrying a foetus with the above-mentioned deficiency, as described in this case report. AFLP may result in marked hypoglycaemia, hyperammonaemia and an increased clotting time. AFLP never resolves before delivery. Therefore induction of labour or caesarean section is the penultimate therapeutic intervention. Nevertheless, maternal mortality is still approximately 12.5% with a corresponding perinatal mortality rate of 9%.[11] The only indication for liver transplantation in patients with AFLP is neurological deterioration in the presence of increased intracranial pressure. In most cases, however, with intensive support patients recover within the first post-partum week.

In the second article, den Dulk *et al.* describe a pregnant woman with severe persisting pruritus which developed in the 22nd week of gestation after a short course of treatment with amoxicillin for an upper respiratory tract infection.[12] Although a diagnosis of intrahepatic cholestasis of pregnancy was made by the authors and treatment with ursodeoxycholic acid was given with success, alternative diagnoses (gallstone disease or amoxicillin drug reaction) were possible, since this patient had constant epigastric pain with several attacks of colicky-like pains, with a past history of cholelithiasis and cholecystectomy. Although no bile duct stones were visualised, the stones could have passed to the intestine before the ultrasonography and MRI were performed. Cholestatic liver disease and pruritus can also be caused by a reaction to a drug such as amoxicillin. Moreover, pruritus in this patient developed at 22 weeks of gestation after an episode of abdominal pain, while in intrahepatic cholestasis of pregnancy, pruritus usually occurs during the third trimester of gestation without abdominal pain. Pruritus generally occurs before any abnormalities are seen in the liver tests.[13] It recurs in 40 to 60% of future pregnancies. Consequently, past history of pregnancy is important for diagnosis. The disease has no serious consequences for the mother, but it is associated with an increased risk of foetal distress, causing premature deliveries and stillbirths. The cause of the disease is unknown. Nevertheless sex hormones, mainly oestrogen and progesterone, appear to be involved in the pathogenesis.[14] Genetic factor(s) may play an important role.[15] Its prevalence is high in Latin America (American Indians) and in Scandinavian countries (Sweden and Finland).[16] According to recent studies,[17] treatment with ursodeoxycholic acid could indeed provide a significant improvement in pruritus and in the biochemical abnormalities with no side effects in the mother and child, as described in this case report.[12]

In the third article, Conchillo *et al.*[18] reported liver involvement in three patients with hyperemesis gravidarum. Although the pathogenesis of the liver injury is still unknown, hypovolaemia, malnutrition and lactic acidosis as the result of hyperemesis are believed to play an important role. The disease is usually benign and occurs in the first trimester of pregnancy in primigravidae.[19] Upon rehydration, enteral or parenteral nutrition, the abnormal liver tests normalise. Symptomatic therapy with antiemetics is, however, required although in most cases the nausea and vomiting would resolve spontaneously after 20 weeks of gestation.

With increasing awareness, especially the early recognition of hepatic problems during pregnancy and prompt appropriate management including early termination of the pregnancy by induction of delivery or by caesarean section, the maternal and child outcomes are improving.

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REVIEW

Clinical practice guidelines in infectious diseases

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INTRODUCTION

Clinical practice guidelines have been defined as 'systematically developed statements to assist practitioner and patient decisions about appropriate healthcare for specific clinical circumstances'.[1] They are intended to help practitioners to assimilate and implement the ever-increasing amount of scientific evidence and opinion on best current medical practice. Clinical practice guidelines should aim to improve the quality and appropriateness of care and possibly increase the cost-effectiveness of care. Furthermore, they can serve as an educational tool and as an instrument to reduce unjustified variations in clinical practice.[2-4] For infectious diseases and antimicrobial therapy, another major goal of clinical guidelines is the control, or even reduction, of the level of antibiotic resistance.[5-7]
Clinical practice guidelines for infectious diseases were recommended by the European Union – initiated conferences in Copenhagen (1988) and Brussels (2001) – as an essential part of the measures to combat the problem of antimicrobial resistance. Scientific societies also supported the development of clinical practice guidelines.[8-10]
Clinical practice guidelines preferentially rely upon the principles of evidence-based medicine, i.e. the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. Hereby, they integrate individual clinical expertise with the best available external evidence and patient's values and expectations.[11] This implies that professional autonomy remains of the utmost importance in the application of a guideline to an individual patient. A guideline can never be specific enough to be applied in all situations and frequently a practitioner will be able to motivate the non-adherence to a guideline. Practice guidelines cannot be used as a cookbook, but merely offer a framework that is adjusted by clinical judgement. Therefore, guidelines are no substitutes for clinical evaluation and expertise nor can they replace expert witness opinions in litigation issues.[12-14]
A high-quality evidence-based guideline requires a rigorous guideline development process by a multidisciplinary team of experts based upon a thorough systematic review of the literature with explicit grading of the guideline according to the level of the scientific evidence, and is followed by an external validation and quality appraisal.
Such a systematic and transparent consensus development process linked directly to evidence from clinical studies guaranties both the validity of the guideline and a clarity in communication, so that the users of the guideline can act as responsible and informed practitioners. It also limits the risk of potential harm and non-intended use of the guideline.[4,12-14]

GUIDELINE DEVELOPMENT

When analysing guideline development processes three main methods can be distinguished: informal consensus development, formal consensus methods and guidelines explicitly linked to evidence.
Informal consensus development is the oldest and most common approach, coined by James Petrie, the former president of the Royal College of Physicians of Edinburgh, with 'GOBSAT' (Good Old Boys Sat Around the Table). Many guidelines are in fact developed by a group of experts that gather to write a guideline based upon their own opinion and experience. The composition of the group strongly

influences the outcome of the development process. The group members (some may even be self-appointed members) often share the same profession, read the same journals, regularly meet at other conferences and mutually reinforce their opinions. Moreover, small group processes may influence decisions when strong personal opinions dominate the development process, possibly resulting in ego-based guidelines instead of evidence-based guidelines. Thus, there are major sources of bias and guidelines produced in this manner are frequently of poor quality and lack adequate documentation of methods. This critique does not necessarily mean that these guidelines (or guides) are worthless. They can be very instructive and useful for clinicians and can be perceived as practical, familiar and realistic. Their flaws, mainly lack of methodological rigour and transparency, however, must be taken into account and it is often difficult for the user to assess how and on what basis the recommendations were derived.[15,16] Practice guidelines developed by speciality societies are particularly vulnerable to these weaknesses. A survey of 431 guidelines from speciality societies showed that only 28% included a professional with another speciality (86% epidemiologist), 13% contained information on the systematic literature search and 18% used a grading of the strength of evidence.[17] A typical example of the confusion these guidelines may create is given by the comparison of the practice guidelines on chronic obstructive pulmonary disease published by scientific societies from 15 countries.[18]

Formal consensus development uses a more systematic approach to assess expert opinion. In a consensus conference a broad-based panel listens to scientific data presented by experts, weighs the information and then composes a consensus statement that addresses a set of questions previously posed to the panel.[16] This methodology to develop guidelines has several limitations as well. First, it is important to recognise who took the initiative for the conference, since it may reflect the overt or hidden goals, for example cost-containment instead of quality of care when organised by insurers or indirect marketing of a drug when organised by the industry. Other sources of bias are the composition of the panel, the selection of the literature and the experts, and the type of questions that were raised. The participants tend to be advocates of a certain view and participation in the conference allows them to be judge and jury to their advocacy.[4,16] Small group processes within the panel again may have an influence on the final decision. The nature of a consensus conference may lead to imperfect scientific recommendations. Other sometimes quite sophisticated and complex methods, such as the Delphi method and the nominal group technique, have attempted to minimise the risk of major biases in the guideline development process, but so far it is unclear which formal consensus method is superior.[19,20]

Another type of a more or less formal consensus method, sometimes used for antibiotic prescription guides, is the organisation of a closed meeting of highly regarded experts that present and review previously raised questions. At the end they vote anonymously on the answers that were derived from the discussion. Finally, all participants approve a manuscript containing the literature review and the formal voting results. An example of this type of conference is the consensus paper on the management and prevention of severe Candidal infections.[21] When looking at the voting results one realises that for the majority of the questions they reflect expert opinion not always supported by solid scientific data, for example differences in the choice of the antifungal agent or dosing according to underlying disease and severity of the inflammatory response. This type of consensus paper has its merits for guiding clinicians and raising unresolved issues, but its recommendations are less solid than evidence-based-type guidelines.
Evidence-based guideline development links guidelines directly to scientific evidence of effectiveness and appropriateness. A structured and transparent development process by a multidisciplinary development group based upon a thorough preferentially systematic review of the literature with a grading according to the strength of the scientific evidence is used. A consensus in the development group based on solid evidence is emphasised over expert opinion. The development process always includes an external validation with quality appraisal of the final guideline draft. The methodology such as described by the Scottish Intercollegiate Guidelines Network (SIGN, www.sign.ac.uk), and similar approaches by other scientific organisations, fulfils these requirements.[3,22,23]

GUIDELINE DEVELOPMENT PROCESS

An evidence-based guideline development process typically distinguishes five steps:[22,23]

1. The identification and refinement of the subject area.
2. The selection of the development group.
3. The search for and critical appraisal of the evidence in the literature.
4. The translation of the evidence into a clinical practice guideline and grading of the strength of the recommendation according to the level of evidence.
5. External review and quality appraisal of the guideline and scheduling of the updating.

To identify the subject for a guideline, priority is usually given to topics that are clinically relevant (volume, variation in practice, risks for patient, costs) and for which sufficient evidence is available. The subject must then be narrowed (e.g. not 'treatment of pyelonephritis' overall but 'antibiotic therapy of uncomplicated community-acquired pyelonephritis in immunocompetent women', excluding patients

with underlying disease, obstruction, surgery, urinary catheters, etc.). The purpose of the guideline must be clearly stated. Next and most critical, a guideline development group is selected. This multidisciplinary group consists of at least six and, for practical reasons, not more than 12 to 15 methodological and mostly clinical experts from related disciplines including delegates from scientific and professional societies. The roles of the group leaders – i.e. to supervise the process and keep in mind the final goal, and the group members to interpret the evidence, add clinical expertise and watch practicalities – are well understood from a first introductory meeting.
A systematic literature search using preappraised sources (such as existing evidence-based guidelines, systematic reviews in e.g. the Cochrane Library, meta-analyses, etc.) and databases with the original primary literature (such as Medline or Embase) is done and the search strategy is described explicitly. Randomised controlled trials are preferred in therapy guidelines, but if unavailable or insufficient the search can be extended to observational studies.
Critical appraisal of the methodological quality of the evidence and an appreciation of its relevance, validity and applicability is performed using standardised checklists.
An evidence table incorporating a description of the validated studies is then compiled and the evidence is summarised in a first guideline draft in which the levels of evidence are clearly categorised. The members of the development group evaluate the volume and consistency of the evidence and integrate it with their clinical expertise and with feasibility issues to create a graded recommendation in multiple rounds.

A distinct and specific feature of infectious diseases guidelines is that local epidemiology and resistance data must be taken into account. This may lead to a different appraisal of the evidence in foreign guidelines on the treatment of the same infectious disease.
The grade of the recommendation reflects the strength of the evidence on which it is based. Unfortunately, different grading systems are being used (e.g. revised SIGN grading; Infectious Diseases Society of America – United States Public Health Service grading system, etc.) leading to confusion for the inexperienced reader. When strong evidence from clinical trials is lacking expert opinion and extrapolation from other patient groups, pathophysiological insights or animal experiments can be used. This must be clearly recognisable for the guideline users as such in the grading.
Once the final guideline manuscript is approved by the development group, an external review with quality appraisal is initiated to ensure validity, clarity and applicability.
This review process should include an expert in the clinical context, an expert in systematic review and guideline development, and potential users of the guideline. More and more evidence-based guideline developers include further peer review via an open meeting where all medical societies involved are invited. Frequently, guidelines are pretested by a limited group of practitioners before general dissemination and implementation of the guideline. Finally an updating of the guideline must be scheduled.

The AGREE instrument (Appraisal of Guidelines Research and Evaluation in Europe collaboration), supported by the 5th framework of the European Union and published in June 2001 (www.agreecollaboration.org), offers an internationally accepted methodology to evaluate the development process (not the content). It is a checklist that allows the individual practitioner or organisation to make an informed judgement about the methods that were used to develop a guideline and to assess the overall quality of the guideline and the recommendations it contains. Six domains are covered by 23 key items: scope and purpose, stakeholder involvement, rigour of development, clarity and presentation, applicability, and editorial independence.
A review of 279 guidelines published from 1985 to 1997 shows that 51% of the guidelines adheres to the methodological standards for guideline development and format, 34% for identification and summary of the evidence and 46% for the formulation of recommendations. There was improvement over time but the identification, evaluation and synthesis of the scientific evidence remained weak.[24]
The clinical guideline development project of the Belgian Antibiotic Policy Coordination Committee (BAPCOC) section hospital medicine, which started two years ago, strictly adheres to an evidence-based methodology.[25] It engaged two part-time scientific collaborators to act as group leaders, perform the systematic literature search and summarise the evidence in a first guideline draft.
Active involvement of several of the national scientific societies concerned, according to the topic, was obtained. The selection of the topics was decided by a steering committee taking into consideration priorities that arose from the analysis of the linked minimal clinical data (using All Patients Refined Diagnostic Related Groups; APR_DRGs) and minimal financial data. This reporting is legally imposed in all Belgian hospitals and provides detailed data on the use of resources (including choice and duration of medication) and the precise diagnosis for each hospitalisation. Great variation in clinical practice, overuse and underuse, inappropriateness and unjustifiable costs and differences between hospitals are striking features of this type of analysis.
This minimal data analysis will also allow evaluation of the impact of a future guideline on daily clinical practice.
The 'Stichting Werkgroep AntibioticaBeleid' (SWAB) in the Netherlands started in October 1996. It has a similar structure (scientific staff, steering committee and extended expert group) and aims to develop national guidelines for antibiotic use in hospital practice.[7] SWAB also moves towards a more explicit grading of the evidence and the recommen-

dations and has departed from the more narrative style of previous publications.

DISSEMINATION, IMPLEMENTATION AND EVALUATION

The methodology for guideline development was gradually developed and adopted by the medical scientific community over the last decade. Much more research is still needed in the dissemination, implementation and evaluation processes of well-conceived guidelines.[5,26] Passive methods of dissemination and implementation of guidelines, such as publication in a scientific journal or mailings, rarely lead to changes in professional behaviour.[26,27] Multifaceted interventions are supposed to be more successful and the choice of the strategies depends upon available resources, perceived barriers and research evidence about effectiveness and efficiency of different approaches. Academic detailing (educational outreach), local adaptation by a multidisciplinary antibiotic management team, small-group interactive sessions, computer-assisted care and audit and feedback appeared to be useful implementation methods (Cochrane review).[26-30] The Belgian experience concerning the change in the reimbursement of perioperative antibiotic prophylaxis indicates that, apart from professional, organisational, social and regulatory interventions, financial incentives can have a strong impact on achieving guideline implementation.[31]
It is of utmost importance to identify the reasons why physicians do not follow clinical practice guidelines. Three domains of barriers to physician's adherence are recognised in general: lack of knowledge, attitude and external barriers.[5,32] Lack of awareness, of accessibility or of familiarity with guidelines are often cited by physicians as a main reason for non-adherence.[32] An assessment of the knowledge of guidelines for the prevention of infective endocarditis underscored this deficiency.[33] Lack of agreement with guidelines in general or with a specific guideline and lack of outcome expectancy (risk for patient) also determine physician's attitudes.[32,34] Physicians with a longer clinical experience and possibly more settled attitudes may be more likely not to follow a guideline.
Patient factors and environmental factors (general practitioner involvement, financial barriers, hospital bed management policy, etc.) also may compromise adherence.[32,33] A survey of Italian physicians illustrates the traditional perception of practice guidelines.[35] They declared that personal experience, opinions of colleagues and other sources of information were more useful, that guidelines are not transferable to the individual patient or local situation and threaten the doctor's autonomy, that guidelines are externally imposed for cost-containment reasons only and that they are administrative rather than informative or educational. Furthermore, there was no enthusiasm for multidisciplinary involvement. Other surveys, however, revealed that physicians are generally positive and confident in guidelines developed by physicians and that acceptability of the format and medium for guideline presentation should be pretested.[36] Finally, evaluation of the impact of the guideline must be planned. It assesses the efficacy of the guideline to ensure that the intended changes in practice and outcome were produced.[5,26] Audit is the most effective way of doing this. Feedback to practitioners enhances the impact of the evaluation process.

GUIDELINES IN INFECTIOUS DISEASES

Guidelines are considered an essential part of the measures to combat antimicrobial resistance.[6-10] There is general agreement that they can improve both the quality and the cost-effectiveness of care.[37] Many types of interventions to implement changes in antibiotic prescriptions have been proposed.[10,38] A distinction is made between educational, supportive and more restrictive methods. To a lesser or greater extent, all of them can have an influence on prescription behaviour, but good data that determine the best type of intervention for specific purposes are scarce. An EPOC (Effective Practice and Organisation of Care) evaluation protocol for systemic review of the literature on this topic is currently being performed.
During a workshop at the European Union Conference in Brussels it was concluded that a large body of evidence from databases such as the Cochrane database indicates that the following interventions have a significant effect on healthcare provider behaviour: education, guidelines, outreach visits and academic detailing, audit and feedback. Of these, outreach visits and academic detailing appear to be the most consistently effective and several studies confirm their feasibility and safety in the infectious diseases setting.[39-42] The infectious diseases consultation service enhanced the appropriateness of the clinical management and favourably influenced the outcome of patients with severe infections. The European Study Group on Antibiotic Policy recommended the establishment of a rational antibiotic policy as the key issue for better care of patients and the combat of antimicrobial resistance.[43] A national expert committee on antibiotic policy (such as BAPCOC in Belgium)[25] should be established in each country to establish national strategies for creating and auditing national antibiotic policies, including the development of regional clinical practice guidelines. Each healthcare institution should have a therapeutics committee to develop a local antibiotic policy based on national guidelines. The Belgian government is now planning to start with the funding of local antibiotic policy committees in the Belgian hospitals. Such a multidisciplinary antimicrobial management team is considered

to be the most appropriate structure to adapt, implement and evaluate infectious diseases guidelines and interventions in hospitals according to local epidemiology, antibiotic consumption patterns and antibiotic resistance data, as was stated in the workshop report of the 2001 European Union-initiated Brussels Conference.

CONCLUSION

High-quality clinical practice guidelines rely upon a rigorous guideline development method using a systematic review of the scientific evidence and an explicit linkage between the level of evidence and the strength of recommendation. Implementation of practice guidelines requires timely and multifaceted interventions and evaluation of the impact of the guideline should be undertaken. Infectious diseases guidelines must meet the international standards of guideline quality but, most importantly, they also require the integration of local epidemiology and resistance data. A multidisciplinary antimicrobial management team in the local hospital seems essential for interpretation, local translation, and implementation of infectious diseases guidelines.

NOTE

Presented in part by WEP as an invited lecture at the 12th European Congress of Clinical Microbiology and Infectious Diseases, Milan, Italy, 21-24 April 2002.

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Th1/Th2 cytokine imbalance in a family with hyper-IgE syndrome

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ABSTRACT

Background: Hyperimmunoglobulin E (hyper-IgE) syndrome is a rare immunodeficiency characterised by recurrent skin and respiratory tract infections, skeletal and dental abnormalities, chronic eczema, and elevated serum IgE. We describe a family with four hyper-IgE syndrome patients (38, 37, 30 and 7 years old), in which we investigated the cytokine response to both specific and non-specific stimulation.

Methods: Whole blood from patients and volunteers was stimulated for either 24 or 48h at 37°C with heat-killed *Staphylococcus, C. albicans* or a combination of IL-12 and IL-18. Cytokine concentrations in the plasma were measured by specific radioimmuno-assays or ELISA.

Results: Serum IgE ranged from 5000 to 16,670 IU/ml, and neutrophil chemotaxis was normal in all four patients. Tumour necrosis factor, interleukin (IL)-1β, IL-6 and IL-8 production after stimulation of whole-blood cultures with lipopolysaccharide or heat-killed *S. aureus* did not differ between the adult patients and four healthy controls. In contrast, when blood from patients and controls was stimulated with heat-killed *S. aureus* or *C. albicans*, a severe imbalance towards a Th2 phenotype was found, with 10- to 30-fold reduction in the IFNγ/IL-10 ratios in the hyper-IgE syndrome patients. The IFNγ production in the patients was less severely impaired when blood was non-specifically stimulated with a combination of IL-18 and IL-12.

Conclusion: In this family with hyper-IgE syndrome, the imbalance in the Th1/Th2 cytokine production may have been involved in the pathogenesis of the recurrent infections and/or chronic eczema characteristic of this disease.

INTRODUCTION

Hyperimmunoglobulin E syndrome is a rare immunodeficiency characterised by recurrent staphylococcal skin abscesses, respiratory tract infections such as pneumonia with pneumatocele formation, mucocutaneous candidiasis, chronic eczematous dermatitis, and extremely elevated circulating IgE concentrations.[1,2] The recent publication of the clinical characteristics of a large series of patients revealed important non-immunological features such as dental abnormalities (retained primary teeth, non-eruption of permanent teeth, double rows of teeth), anomalies in midline facial development, and skeletal abnormalities (bone fractures, hyperextensible joints, scoliosis).[3] Most cases are sporadic, but the description of several families with clustering of hyper-IgE syndrome patients suggests an autosomal dominant transmission with variable expressivity.[3] Although the precise genetic abnormality is not yet known, linkage of hyper-IgE syndrome to the proximal q region of the chromosome 4 has been demonstrated.[4]

It has been hypothesised that the increased susceptibility to infections and the skin hypersensitivity could be the result of an altered cytokine production pattern in hyper-IgE syndrome patients. IFNγ as a prototype of the Th1 cytokines activates the cellular immune responses, whereas IL-4 and IL-10 as cytokines of the Th2 family induce activation of the

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humoral immune system.[5] A bias towards Th1 responses is associated with autoimmune diseases, whereas a Th2 imbalance induces allergic reactions through hypereosinophilia and hyper-IgE production. A defective IL-12/IFNγ axis leads to high susceptibility to infections.[6] Several studies have tried to document a defective production of IFNγ by cells from hyper-IgE syndrome patients, with variable results.[7-11] However, these studies have used mitogenic stimulation with phytohaemagglutinin (PHA), and not more relevant stimuli derived from microbes for which the hyper-IgE syndrome patients are highly susceptible. In a recent study, Borges and colleagues used *Staphylococcus aureus* as a cytokine stimulus, and they reported a defective IL-12/IFNγ pathway in patients with hyper-IgE syndrome.[12] Very limited information is available regarding production of Th2 cytokines by cells of hyper-IgE syndrome patients, with a production of IL-4 upon PHA stimulation which is not altered compared with controls.[7,11]

In the present study we investigated the Th1/Th2 cytokine balance in three adult patients with hyper-IgE syndrome by using whole-blood stimulation with either heat-killed *S. aureus* or *C. albicans*. The patients belong to a family in which another seven-year-old patient was diagnosed, and in which five other individuals also have clinical and/or laboratory abnormalities suggestive of hyper-IgE syndrome. In addition, we also have studied the capacity of the blood collected from the patients to produce proinflammatory cytokines such as TNF, IL-1β, IL-6 and the chemokine IL-8.

PATIENTS AND METHODS

Patients

One patient under control for essential hypertension at the outpatient clinic of Bosch MediCentrum, 's-Hertogenbosch, reported recurrent skin abscesses, recurrent respiratory tract infections since childhood (but no pneumatocele or bronchiectasis), and chronic eczematous dermatitis. From the family history it became apparent that the patient's brother and sister, as well as a nephew, had similar clinical manifestations (*figure 1*). Other manifestations in the four patients included chronic candidiasis of the nail beds (all patients), late loss of primary teeth (in two of them), and skeletal abnormalities (one patient cheilopalatoschisis, and one patient three fractures of the left wrist). The characteristic coarse facies was present in a mild form in two of the patients. When the scoring system proposed by Grimbacher *et al.*[4] was used, all these four individuals scored higher than 14 (17, 23, 24 and 33), consistent with the diagnosis of hyper-IgE syndrome. In addition, five other members of the family scored between 10 and 14, being classified as 'unknown' patients (*figure 1*). The deceased mother of the three adult patients had severe recurrent skin abscesses and it was recalled to be said that 'she was like Job'. It is of note that hyper-IgE syndrome used to be known as Job's syndrome.[1] The other 'unknown' patients had features of chronic eczema, recurrent skin infections, and moderately increased IgE concentrations (in two of them). The cause of death of patients I.1 and II.1 was malignancy and a ruptured aortic aneurysm, respectively. All hyper-IgE syndrome patients were evaluated during an infection-free interval. Informed consent was obtained from all subjects before the studies were performed.

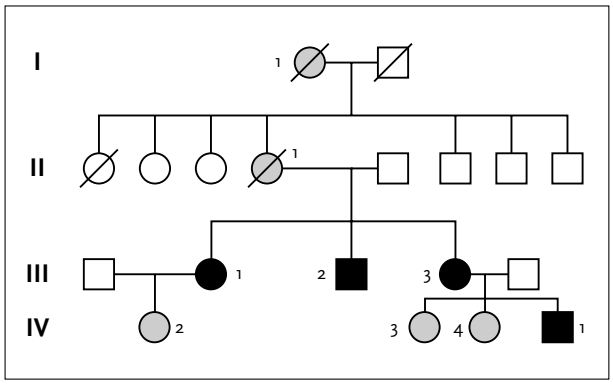

Figure 1

Pedigree of a family with hyper-IgE syndrome

The squares represent male family members, and the circles represent female family members. A slash indicates that a person has died. Open symbols represent healthy persons, closed symbols represent patients with hyper-IgE syndrome and hatched symbols represent persons with clinical and/or laboratory characteristics associated with hyper-IgE syndrome and uncommon in the general population. Generations are labelled with Roman numerals and patients with Latin numerals.

Cytokine studies

The three adult patients (aged 27, 30 and 38 years) and four normal subjects (two men and two women), without atopic diseases or chronic infectious complications, ranging in age from 26 to 47 years, were investigated as study and control groups for cytokine production.

Blood was collected by venous puncture from the cubital vein in heparinised tubes (Monoject, 's-Hertogenbosch, the Netherlands). Whole-blood cultures were performed as described previously.[13] Briefly, 200 μl blood was added to 100 μl stimulus and 700 μl RPMI 1640, and incubated in 24-wells plates (Greiner, Alphen aan den Rijn, the Netherlands) at 37°C. As stimuli we used 10^7 CFU/ml heat-killed (30 minutes at 100°C) *S. aureus* (clinical isolate from the patients) or *C. albicans* (ATCC 10231), or 10 ng/ml lipopolysaccharide (LPS, *E.coli* serotype O55:B5, Sigma, St. Louis), or a combination of 1 ng/ml rhIL12 (Peprotech) + 10 ng/ml rhIL-18 (kindly provided by Dr. Masashi Kurimoto, Hayashibara Laboratories, Okayama, Japan). The plasma was isolated for 24 hours (for TNF, IL-1β, IL-6 and IL-8 measurements) or 48 hours (for IFNγ and IL-10

determinations) after incubation, and the samples were kept at -70°C until cytokine measurements had been completed.

TNFα and IL-1β concentrations were determined by specific radioimmunoassays as described.[14] IL-6, IL-8, IL-10 and IFNγ were measured by commercial ELISA kits (Pelikine Compact, CLB, Amsterdam, the Netherlands), according to the instructions of the manufacturer.

RESULTS

Immunological characteristics

The laboratory characteristics of the patients are presented in *table 1*. The patients had normal leucocyte and neutrophil counts, with normal CD4, CD8 and CD4/CD8 ratios. Chemotactic response of the neutrophils towards IL-8 was approximately 60% of the normal in two patients, and almost normal in the other two. Similar data were obtained when chemotaxis towards C5a or PAF was performed (not shown). The NK-cell population was in the low-normal range, and the B-cell population was normal. Complement factors C3 and C4 were normal, and Ig fractions A, M, and G were present in normal concentrations. Eosinophil counts ranged from 250 to 900 eosinophils/μl, and the serum IgE concentrations from 5000 to 16,700 UI/ml. *S. aureus* was isolated from skin abscesses and nose of all four patients, and after binary strain typing[15] it appeared that they all had the same strain.

IFNγ and IL-10 production

Stimulation of whole blood from hyper-IgE syndrome patients with heat-killed *S. aureus* led to 13- to 36-fold lower IFNγ production compared with control volunteers (*table 2*). Similarly, *C. albicans* stimulation induced 16- to 180-fold lower IFNγ synthesis in blood cultures of hyper-IgE syndrome patients compared with controls. In contrast, production of IL-10 was in the same range in the hyper-IgE syndrome patients and the healthy volunteers. IFNγ/IL-10 ratios were severely reduced in the patients compared with controls (*table 2*). IL-12 and IL-18 synergistically stimulates T cells for the production of IFNγ. When whole blood from hyper-IgE syndrome patients was stimulated with a combination of IL-12 +IL-18, 1.5 to 9-fold less IFNγ was produced compared with the blood from healthy controls (*table 2*). As expected, no IL-10 was produced in response to IL-12/IL-18 stimulation (all samples below detection limit).

Production of the proinflammatory cytokines TNF, IL-1β, IL-6 and IL-8

When whole blood from hyper-IgE syndrome patients was stimulated with either *S. aureus* (*table 3*) or *C. albicans* (not shown), no differences compared with control volunteers could be found. Stimulation of blood with a non-related stimulus, the LPS component of Gram-negative bacteria, also led to a similar proinflammatory cytokine profile in patients and healthy subjects (*table 3*).

DISCUSSION

The results of the investigations we performed suggest that patients with hyper-IgE syndrome have a strong bias towards a Th2 response, expressed as reduced IFNγ/IL10 ratios, especially when specifically stimulated with heat-

Table 1
Clinical and laboratory characteristics of four patients with hyper-IgE syndrome

	PATIENT III.1	PATIENT III.2	PATIENT III.3	PATIENT IV.1	NORMAL
Age (years)	27	30	38	7	
Sex	Female	Female	Male	Male	
Leucocytes (x 10^9/l)	8.3	6.2	7.4	9.7	4.3-10.8
Lymphocytes (x 10^9/l)	2.1	1.7	1.7	2.9	1.0-2.9
CD3+ (x 10^9/l)	1.7	1.3	1.3	2.2	0.7-2.1
CD3+CD4+ (x 10^9/l)	1.1	0.9	0.9	1.2	0.3-1.4
CD3+CD8+ (x 10^9/l)	0.6	0.4	0.4	0.8	0.2-0.9
CD3-CD16+CD56+(x 10^9/l)	0.08	0.12	0.09	0.1	0.09-0.6
CD19+ (x 10^9/l)	0.2	0.2	0.2	0.5	0.1-0.5
PMN chemotaxis (% norm)	88	55	89	62	100
C3 (g/l)	1.5	1.5	1.0	1.6	0.8-1.7
C4 (g/l)	0.27	0.31	0.29	0.42	0.14-0.45
IgG (g/l)	10	12	10	10	6-15
IgA (g/l)	0.9	2.3	1.7	0.8	0.7-4.6
IgM (g/l)	1.4	1.5	1.1	0.7	0.3-2.3
IgE (UI/ml)	7210	12,140	16,700	5000	<150
Eosinophils (x 10^9/l)	410	900	500	250	50-350

Table 2

IFNγ and IL-10 production in hyper-IgE syndrome patients and healthy controls after stimulation of whole-blood cultures with either heat-killed S. aureus, C. albicans or a combination of IL-12 + IL-18

		PATIENT III.1	PATIENT III.2	PATIENT III.3	CONTROLS*
IFNγ (pg/ml)	*S. aureus*	150	290	230	3895 ± 1228
	C. albicans	20	225	37	3697 ± 1046
	IL-12/IL-18	130	640	97	953 ± 329
IL-10 (pg/ml)	*S. aureus*	129	159	441	210 ± 97
	C. albicans	306	228	96	97 ± 32
	IL-12/IL-18	<5	<5	<5	<5
IFNγ/IL-10	*S. aureus*	1.2	1.8	0.5	21.3 ± 8.4
	C. albicans	0.07	1.0	0.4	36.3 ± 9.1

** Mean ± standard deviation of four volunteers.*

Table 3

Proinflammatory cytokine production in hyper-IgE syndrome patients and healthy controls after stimulation of whole-blood cultures with either heat-killed S. aureus or LPS

		PATIENT III.1	PATIENT III.2	PATIENT III.3	CONTROLS*
TNF (ng/ml)	*S. aureus*	8.2	6.6	7.6	7.7 ± 2.4
	LPS	0.9	3.6	2.7	2.6 ± 1.1
IL-1β (ng/ml)	*S. aureus*	6.3	7.2	9.1	6.7 ± 2.1
	LPS	0.4	1.7	2.1	0.9 ± 0.3
IL-6 (ng/ml)	*S. aureus*	24.8	7.6	17.8	12.9 ± 3.1
	LPS	6.2	12.5	17.2	10.5 ± 2.7
IL-8 (ng/ml)	*S. aureus*	153	31	77	47.5 ± 9.2
	LPS	7.7	8.1	13.1	8.8 ± 1.8

** Mean ± standard deviation of four volunteers.*

killed *S. aureus* or *C. albicans*. Whereas Th1 cytokines are important for stimulating the defence against intracellular micro-organisms, Th2 cytokines activate the humoral arm of the immune system and are involved in the defence against parasites and protozoa.[5] The recurrent infections of the skin and the respiratory tract in patients with hyper-IgE syndrome fit a Th1/Th2 imbalance, with low IFNγ production. In contrast, relatively high IL-4 and IL-10 concentrations could explain the high IgE concentrations, eosinophilia and allergic reactions. Our data are sustained by the study by Borges *et al.*, who also observed impaired IFNγ production in cells isolated from hyper-IgE syndrome patients stimulated with *S. aureus*.[12] Although the IFNγ/IL-10 ratios were strongly reduced, it is remarkable that IL-10 concentrations were normal.

IFNγ is mainly produced by T cells and NK cells, and its synthesis can be induced through different mechanisms. Firstly, an antigen can stimulate a monocyte/macrophage to produce proinflammatory cytokines such as IL-12, IL-18, IL-23 and to a lesser extent IL-15 or IL-1β, which in turn stimulate IFNγ synthesis from T cells and NK cells.[16,17] Secondly, T cells can be directly stimulated to produce IFNγ either by mitogens, or by components of bacteria directly binding to the T-cell receptor and acting as superantigens.[18] Finally, when antigen-specific T cells bind the antigen presented by the MHC complex, they also respond with IFNγ production. When the blood of hyper-IgE syndrome patients was stimulated with heat-killed *S. aureus* or *C. albicans*, the production of IFNγ was lower compared with controls. Because stimulation with a combination of IL-12/IL-18 (which can directly stimulate T cells) also led to a lower IFNγ production in the hyper-IgE syndrome patients, it is likely that the defect leading to this skewed Th1/Th2 balance is located at the level of T cells, and not the monocytes/macrophages. This conclusion is sustained by the normal monocyte function as expressed by normal production of TNF, IL-1β, IL-6 and IL-8 in our study, and of normal IL-12 synthesis observed by others.[11,12] Chehimi and colleagues have demonstrated a decreased IL-12Rβ2 expression in cells from hyper-IgE syndrome patients,[11] which could explain the decreased response upon IL-12/IL-18 stimulation in our study. Another argument in favour of a T-cell defect in hyper-IgE syndrome patients comes from studies showing a defective IFNγ synthesis upon mitogenic stimulation with PHA.[7,8,10] In addition, Rodriguez and colleagues observed higher IL-4 production in cells of hyper-IgE syndrome patients compared with controls, which would also argue for a Th2 bias.[9] We could not confirm this, however.

No defect in neutrophil chemotaxis was found in two of our patients, whereas in two others a 40% decreased chemotaxis was measured when IL-8, C5a or PAF were used as chemoattractants. The chemotactic defect in the syndrome is a controversial issue. Some reports show a marked defect in neutrophil chemotaxis in hyper-IgE syndrome patients, whereas others do not.[19,20] It has been proposed that an increased production of granulocyte-macrophage colony-stimulating factor might be responsible for this effect.[20] The reason for the discrepancy between these data is not clear. No other immune abnormalities, in terms of lymphocyte numbers and phenotype, NK-cell numbers, complement factors, immunoglobulin classes (except of course IgE), have been found in our patients.

In conclusion, hyper-IgE syndrome is a complex multisystem disease with both immunological and non-immunological abnormalities. Although our study investigated a small number of patients, all from one affected family, the data obtained strongly suggest that the increased susceptibility to infections and the chronic allergic dermatitis may be the result of an altered Th1/Th2 cytokine profile towards a Th2 bias. We are currently performing a larger study of hyper-IgE syndrome patients to try to confirm and extend the data presented here. These findings could have important therapeutic implications, as it has been shown that recombinant IFNγ can enhance neutrophil chemotactic responses in hyper-IgE syndrome patients[21] and a small pilot study has suggested beneficial effects of treatment with recombinant IFNγ in these patients.[22]

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ORIGINAL ARTICLE

Doxazosin and hydrochlorothiazide equally affect arterial wall thickness in hypertensive males with hypercholesterolaemia (the DAPHNE study)

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ABSTRACT

Background: Observational studies suggest a synergistic effect of hypertension and hyperlipidaemia on the progression of atherosclerosis. The α-blocker doxazosin has favourable effects on plasma lipids, insulin resistance and blood pressure, while the diuretic hydrochlorothiazide (HCTZ) principally affects blood pressure and increases insulin resistance.

Methods: A randomised double-blind study over 36 months was performed to compare the effects of doxazosin and HCTZ on fasting lipids and on progression of peripheral atherosclerosis. Eighty males (45 to 70 years) with peripheral atherosclerotic disease and increased cholesterol levels (5.2-8.0 mmol/l) were treated for essential hypertension with either doxazosin (n=41) or HCTZ (n=39). Main outcome measures were arterial intima-media thickness (IMT) of the carotid and femoral arteries and fasting lipid parameters.

Results: In the doxazosin-treated group, significant changes were observed in the concentration of triglycerides (-13.7%, p<0.01), HDLc (+25.7%, p<0.05) and IDLc (-30.1%, p<0.05). In the HCTZ-treated group no significant changes in plasma lipid levels were observed. On follow-up visits systolic blood pressure in the doxazosin-treated group was 6 mm higher than in the HCTZ group. Nevertheless, the groups treated with doxazosin or HCTZ showed no differential effect on IMT after three years of treatment (p=0.81). A significant reduction of the IMT of combined carotid and femoral arterial walls was shown in both treatment groups (p<0.005).

Conclusions: Hypertension treatment with doxazosin or HCTZ resulted in a comparable change in arterial IMT after three years, in spite of differences in effect on plasma lipids. The study emphasises the importance of blood pressure control in patients with peripheral vascular disease and hypercholesterolaemia.

INTRODUCTION

Hypertension and hyperlipidaemia are major risk factors for atherosclerotic disease. Observational studies have demonstrated a synergistic effect of risk factors for vascular disease:[1] the individual with moderate hypertension without other risk factors is at lower risk for atherosclerosis than a person with other risk factors such as hypercholesterolaemia.[2] Major clinical trials have demonstrated that treatment with diuretics reduces the risk of cardiovascular disease.[3-5] These drugs are effective in the treatment of hypertension, but their use has been questioned since they exert adverse effects on lipids and lipoproteins.[6,7] It is not known to what extent these changes in plasma lipids affect the atherosclerotic risk in hypertensive patients. Doxazosin, a selective α_1-antagonist, is as effective as diuretics in the treatment of hypertension.[8,9] Additionally doxazosin has favourable effects on serum lipid concentrations, lowering triglycerides (TG) and increasing high-density lipoprotein cholesterol (HDLc).[10-12] If cardiovascular risk and hyperlipidaemia in patients with hypertension are

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associated, doxazosin could be an important agent to prevent vascular disease in patients with hypertension. Doxazosin has antiatherogenic properties in animal models.[13,14] As cardiovascular risk reduction is the ultimate goal of antihypertensive therapy, the effect of doxazosin on various forms of cardiovascular disease needs to be studied.
The Doxazosin Atherosclerosis Progression study in Hypertensives in the Netherlands (DAPHNE) is a three-year study in males with mild hypertension, hypercholesterolaemia and peripheral atherosclerosis designed to compare the effects of doxazosin and the diuretic hydrochlorothiazide (HCTZ) on lipid profile and atherosclerosis progression. For the detection of atherosclerosis progression quantitative B-mode ultrasound measurements of the arterial intima-media thickness (IMT) were used.[15,16]

PATIENTS AND METHODS

Study population and design

Eighty male patients, 45 to 70 years of age, with essential hypertension were recruited from the vascular surgical outpatient facilities of the University Hospital Dijkzigt Rotterdam and the St. Antonius Hospital Nieuwegein, in the Netherlands. Besides the presence of essential hypertension, the major inclusion criteria were the presence of peripheral atherosclerosis and mild hypercholesterolaemia. To be included, the diastolic blood pressure (DBP) readings had to be between 95-115 mmHg, measured twice in supine position on three separate occasions after five minutes rest. Mild hypercholesterolaemia was defined as a plasma cholesterol concentration between 5.2-8.0 mmol/l while on a cholesterol-lowering diet for at least six weeks. Peripheral artery disease was defined as intermittent claudication or peripheral vascular surgery because of atherosclerosis. The major exclusion criteria were systolic blood pressure (SBP) above 200 mmHg, secondary hypertension, symptomatic coronary heart disease or myocardial infarction within three months prior to the study, diabetes mellitus, and apolipoprotein E_2/E_2 genotype. The medical ethics committee of the University Hospital Dijkzigt approved the study; all participants gave written informed consent. All patients were given single-blind placebo medication for the first six weeks of the study, followed by a double-blinded treatment. Patients were randomised to one of two treatment arms: doxazosin or HCTZ, and were started on the lowest possible dose, which was increased in two weekly intervals, until the goal DBP of 90 mmHg was achieved. Dose adjustment was allowed during the rest of the study when DBP was consistently above 90 mmHg. For doxazosin the regimen was 1 mg, 2 mg, 4 mg, 8 mg and 16 mg once a day; for HCTZ the dosing was 12.5 mg, 25 mg, 50 mg and 100 mg once a day. At each visit compliance with treatment was checked by tablet count. At randomisation (baseline) 6, 12, 24 and 36 months the following investigations were carried out. Fasting (≥8 hours) blood samples were taken and carotid and femoral artery walls were assessed by means of B-mode ultrasound examinations. Electrocardiograms were carried out to identify left ventricular hypertrophy (LVH).
The primary objective of DAPHNE was to determine the effectiveness of doxazosin compared with HCTZ on the change in the maximum arterial wall thickness of twenty combined segments, over three years. The effect of doxazosin versus HCTZ on plasma lipids and lipid parameters was studied as a secondary objective.

Lipids and lipoprotein analysis

Fasting blood samples were drawn into tubes containing 1.5 mg/ml Na_2EDTA. Plasma was prepared from blood within two hours and immediately stored at -80°C until analysis. For the separation of lipoproteins, plasma was stored at 4°C and used within 24 hours. Cholesterol and triglycerides were determined by enzymatic methods (Boehringer test kit combinations, Mannheim, Germany). Plasma HDL was measured after precipitation of VLDL and LDL by addition of manganese chloride.[17] Apolipoprotein B was estimated by an immunoturbidimetric method using commercially available kits from DAKO (Glostrup, Denmark). Lipoproteins were separated by flotation during sequential ultra-centrifugation at densities of 1006 g/ml (VLDL), 1019 g/ml (IDL) and LDL 1063 g/ml. All runs were for 18 hours at 15°C and 40,000 rpm in a Ti 50.3 Beckman rotor in quick seal poly-alomere tubes. The recovery of cholesterol after centrifuge was >90% and of triglycerides >8%. As described, Apo E isoforms were determined by genotyping.[18]

B-mode ultrasound imaging, off-line video image analysis

B-mode ultrasound scans were taken using an ACUSON 128 ultrasound system equipped with an L7384 7 MHz linear array transducer (ACUSON Corporation; Mountain View, CA). At each ultrasound visit, six carotid and four femoral arterial segments were investigated. The B-mode ultrasound imaging and analysis includes the right and left common carotid (CCA) and the right and left bulb (BUL) arterial segments, the right and left internal carotid (ICA), the left and right common femoral (CFA) and the right and left superficial femoral (SFA) arterial segments. The length of measurement along the arterial wall is kept as close to 1 cm as practically possible, but is prone to differ between sites and patients due to individual anatomy and interference in the ultrasound image. Each arterial segment has a near wall (the wall proximate to the transducer) and a far wall (the wall distant from the transducer). Consequently, 20 longitudinal arterial wall segments were investigated per patient in each of the five ultrasound visits. A max IMT is defined as the maximum δ between two lines. The max IMT is the average over the 20 maximum IMT values of the segments. Segment images of each arterial

wall were stored on S-VHS tape. The video images were analysed off-line. Licensed PROSOUND image analysis software as developed in cooperation with Robert Selzer was used.[19-21] The study was quality controlled by repeated measurement procedure.[22] On a per subject basis, the SD of the means of the paired intrasonographer IMT measurements calculated according to Bland and Altman was 0.04 mm.[23] The co-efficient of variation of IMT measurements, defined as {(SD of the mean difference √(2) x 100}/ pooled means of IMT values), was 3%.[24]

Statistical analysis and definitions

The analysis was performed on an intention-to-treat basis. For the missing values in the ultrasound measurements the strategy as described by Espeland was used.[25] The significance of the change from baseline within a group was assessed using paired t-tests. To estimate the difference between doxazosin and HCTZ in treatment effect for an outcome variable, all patients with a baseline measurement and at least one follow-up measurement available were used in a repeated measurement analysis (SAS PROC MIXED).[26] We used an appropriate covariance structure, the follow-up data as outcome and the baseline measurement, treatment, visit number (categorical) and, if significant, the interaction between visit number and treatment as covariables. If the interaction was not significant, the mean difference between doxazosin and HCTZ during the total follow-up period was regarded as the difference in treatment effect. Variables with a skewed distribution were log-transformed, and then the corresponding treatment effect is expressed as the ratio of doxazosin/HCTZ. For the ultrasound measurements the interaction term was always included in the model and the mean difference between the treatments at the last visit was regarded as the treatment effect. Data are presented as mean ± standard deviation, unless stated otherwise.

RESULTS

Baseline characteristics

Eighty male patients were selected to participate in the study. The mean age was 59.1 ± 7.2 years, 24 patients were between 44 to 54 years, 35 patients between 55 to 64 years and 21 patients older than 65 years of age. The mean body mass index was 26 ± 3 kg/m². Diastolic blood pressure (DBP) was 101 ± 5 mmHg ranging from 95-117 mmHg, systolic blood pressure (SBP) 163 ± 17 mmHg ranging from 130-200 mmHg. Left ventricular hypertrophy was present in 11% of the patients. At randomisation 37 (46%) of the patients admitted smoking. During the trial 40 (50%) patients smoked cigarettes, with a median of ten cigarettes a day. The mean total cholesterol concentration was 6.0 ± 0.9 mmol/l. The median triglycerides concentration was 2.0 mmol/l, varying between 0.6-6.0 mmol/l.

Distribution of the most frequent apolipoproteins E genotype was 75% ApoE_3/E_3 and 17% Apo E_3/E_4.
The distributions of age, blood pressure, body mass index, symptomatic vascular disease, IMT of the arterial walls, plasma lipid concentrations and other metabolic variables at baseline were similar for the two treatment groups.
The major baseline characteristics of the two study groups are provided in *tables 1* and *2*.

Table 1
Description of the DAPHNE population at baseline

	DOXAZOSIN GROUP (n=41)	HCTZ GROUP (n=39)
Demographics		
Age (years)	58.7 ± 6.7	59.4 ± 7.7
LVH on ECG	4 (10%)	5 (13%)
Risk factors		
Current smoking (%)	44	49
Duration of hypertension (years)	5.3 (range 2-12)	6.0 (range 3-8)
Systolic blood pressure (mmHg)	163 ± 16	164 ± 18
Diastolic blood pressure (mmHg)	100 ± 5	101 ± 5
Body mass index (kg/m²)	26.1 ± 2.7	26.6 ± 3.2
Prior history		
Intermittent claudication	33 (81%)	29 (74%)
Peripheral vascular surgery	16 (39%)	19 (49%)
Myocardial infarction	8 (20%)	7 (18%)
Coronary bypass	6 (15%)	2 (5%)
Cerebrovascular disease	3 (7%)	5 (13%)

Data are presented as mean ± SD or as median (25-75%) percentiles.

Table 2
Description of the intima-media thickness at baseline

	DOXAZOSIN GROUP (n=41)	HCTZ GROUP (n=39)
Combined carotid and femoral arteries		
Average of 20 mean far and near walls	1.09 ± 0.13	1.11 ± 0.19
Average of 20 max. far and near walls	1.42 ± 0.19	1.45 ± 0.27
Average of 10 mean far walls	1.11 ± 0.15	1.14 ± 0.21
Average of 10 max. far walls	1.45 ± 0.21	1.47 ± 0.30
Average of 10 mean near walls	1.06 ± 0.16	1.09 ± 0.19
Average of 10 max. near walls	1.39 ± 0.25	1.43 ± 0.29
Carotid arteries		
Average of 12 mean far and near walls	1.05 ± 0.17	1.08 ± 0.19
Average of 12 max. far and near walls	1.39 ± 0.24	1.43 ± 0.30
Femoral arteries		
Average of 8 mean far and near walls	1.14 ± 0.17	1.17 ± 0.21
Average of 8 max. far and near walls	1.46 ± 0.23	1.49 ± 0.29

Data are presented in mm (mean ± SD).

Drug effects on blood pressure and adverse events

Systolic and diastolic blood pressure decreased significantly from baseline in the two treatment groups. In the doxazosin group, the SBP decreased from 163 ± 16 mmHg at baseline to 155 ± 24 mmHg (p<0.001) after three years of follow-up. The DBP decreased from 100 ± 5 mmHg to 87 ± 8 mmHg (p=0.002). In the HCTZ group the SBP decreased from 164 ± 18 mmHg to 148 ± 19 mmHg (p=0.002). The DBP decreased from 101 ± 5 mmHg to 87 ± 7 mmHg (p<0.001). On the follow-up visits the SBP in the doxazosin group was 6 mmHg higher than in the HCTZ group, adjusted for baseline SBP (p<0.01). No difference in treatment effect on DBP was seen between the groups (p=0.62). In both groups eight patients withdraw because of adverse events. The number of patients with major vascular events did not differ significantly between the two treatment groups. In both treatment groups, five patients had peripheral vascular surgery because of progressive peripheral artery disease. In the doxazosin group four patients suffered from a myocardial infarction, while in the HCTZ group two patients had a cerebrovascular event. The number of serious and non-serious events was not significantly different between the treatment groups (p=0.12). One patient discontinued the trial because of poor compliance. Four patients in the doxazosin group and three patients in the HCTZ group were discontinued because of inadequate blood pressure lowering. A total of 29 patients in the doxazosin group and 27 in the HCTZ group completed the study. The baseline SBP in the HCTZ-treated group was slightly higher in the 'non-completers' as compared with the 'completers' at 172 and 160 mmHg, respectively (p=0.04). Comparison of baseline DBP, mean IMT, max IMT, cholesterol, TG, HDLc, VLDLc, VLDLtg, IDLc and LDLc between the group that did and the group that did not complete the study showed no differences.

Drug effects on blood lipids

Within six months of treatment a significant decrease in TG levels occurred in the doxazosin group; this effect remained over three years of treatment (*figure 1*). At the end of the trial, TG levels were decreased by an average of 13.7% (p<0.01) as compared with baseline levels. In the HCTZ group, the TG was slightly increased, but this did not reach the level of significance. The difference in effect on TG levels between the two groups over three years was significant (p<0.005)(*figure 1*). Changes in the total TG concentration over three years paralleled the changes in VLDLtg concentration as shown in *table 3*. In the doxazosin group the total HDLc concentration increased by 25.7% (p<0.001). A substantial 30.1% decrease in the concentration

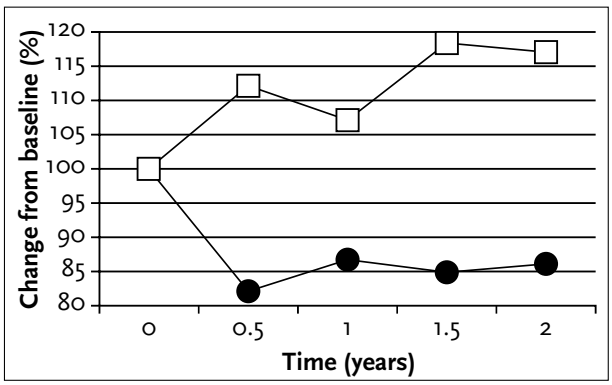

Figure 1

Effects of doxazosin (closed circles) and hydrochlorothiazide (open blocks), on fasting triglycerides concentration during three years of treatment

Table 3

Lipids and lipoproteins at baseline and their change after three years of follow-up

	BASELINE	DOXAZOSIN TREATMENT	CHANGE*	BASELINE	HCTZ TREATMENT	CHANGE*	DIFFERENCE IN EFFECT (CI)†	P VALUE
Lipids								
Cholesterol	6.15 ± 0.76	5.95 ± 0.81	-0.11 ± 0.78	5.83 ± 0.94	5.72 ± 0.89	-0.08 ± 0.72	(-0.41; 0.14)	0.33
TG	2.03 (1.51-2.50)	1.60 (1.14-2.29)	-0.46 ± 0.95‡	1.96 (1.44-2.66)	1.99 (1.25-2.55)	+0.15 ± 0.8	(70%; 92%)	0.002
HDLc	1.08 ± 0.26	1.33 ± 0.26	+0.22 ± 0.24‡	1.14 ± 0.28	1.24 ± 0.25	+0.10 ± 0.30	(-0.06; 0.14)	0.42
Lipoproteins (ultracentrifugation)								
VLDLtg	1.26 (0.90-1.96)	0.90 (0.5-1.6)	-0.41 ± 0.92‡	1.27 (0.82-1.81)	1.38 (0.69-1.81)	+0.23 ± 0.74	(59%; 87%)	0.001
VLDLc	0.53 (0.34-0.77)	0.38 (0.23-0.70)	-0.18 ± 0.35‡	0.49 (0.29-0.72)	0.49 (0.23-0.78)	+0.07 ± 0.33	(57%; 101%)	0.06
IDLc	0.16 (0.07-0.27)	0.13 (0.07-0.18)	-0.05 ± 0.12‡	0.09 (0.05-0.20)	0.09 (0.06-0.17)	-0.02 ± 0.08	(73%; 121%)	0.63
LDLc	4.03 ± 0.83	3.72 ± 0.83	-0.26 ± 0.70	3.72 ± 0.93	3.54 ± 0.82	-0.18 ± 0.90	(-0.34; 0.19)	0.56
HDLc	0.95 ± 0.26	0.98 ± 0.25	+0.03 ± 0.20‡	0.93 ± 0.23	0.95 ± 0.29	+0.0 ± 0.27	(-0.10; 0.07)	0.67

** Due to dropouts, the change is not exactly equal to the difference between the mean after treatment and the mean at baseline, † the 95% confidence intervals, the result from the repeated measurements analysis, being the difference doxazosin – HCTZ (absolute number) or ratio doxazosin/HCTZ*100% (%), ‡ statistically significant difference from baseline value tested by paired Student's t-test. Data are presented as mean SD or as median (25-75% percentiles).*

of IDLc was observed in the doxazosin group (p<0.05), while IDLc was not affected in the HCTZ group. Neither of the two treatment groups showed changes in the total cholesterol or the LDLc concentration over three years of treatment (*table 3*). In both groups a decrease in Apo B concentration was observed in 17.5% and 11.3%, respectively (both p<0.001, data not shown).

Drug effects on arterial wall thickness

Hypertension treatment with either doxazosin or HCTZ resulted in a significant reduction of the IMT of the combined arterial walls of the carotid and femoral arteries over three years (*table 4*). Separate analyses of the combined far wall IMTs and the combined near wall IMTs showed similar results (*table 4*). There was no difference in treatment effect on carotid and femoral IMT (p=0.66 for mean IMT, p=0.83 for maximal IMT). After three years of treatment a significant decrease of the maximal carotid artery IMT was observed in the doxazosin group (p<0.05) and in the HCTZ-treated group (p<0.005), respectively. A significant difference in treatment effect between the two drugs on the femoral or carotid arteries was not observed (*table 4*). On studying the treatment effect without taking the basal IMT into consideration, the same conclusion was reached (data not shown).

DISCUSSION

Hypertension treatment with either doxazosin or HCTZ in hypercholesterolaemic patients with peripheral vascular disease resulted in a comparable effect on the arterial IMT. In the doxazosin-treated group favourable changes in plasma triglycerides, HDLc and IDLc levels were seen within six months of treatment and present throughout the study. In the HCTZ-treated group no such favourable changes in fasting lipids were observed. This did not result in a greater treatment effect on the arterial IMT: in both treatment groups the maximal and the mean thickness of the combined carotid and femoral arterial walls showed a comparable regression over three years. Under the restriction that IMT is only a surrogate for atherosclerosis, this study suggests that in a highly selected population of males with hypertension, hypercholesterolaemia and peripheral atherosclerosis, lowering of blood pressure with either doxazosin or HCTZ is equally effective with regard to progression of atherosclerosis.

It cannot be excluded that the favourable effects of doxazosin on lipids contributed to atherosclerosis regression. However, this effect could not be observed by B-mode ultrasound IMT measurements in the relatively small DAPHNE population. We have no indications for methodological or technical factors that could explain the lowering of IMT. We studied whether our data are influenced by a so-called 'regression to the mean' effect. In order to do so we analysed the groups with a low and a high basal IMT, i.e. below and above median (1.114 mm). In both these two groups IMT decreased significantly, 0.045 mm (p=0.03), CI (0.0038-0.0871) and 0.173 mm (p=0.0001), CI (0.1284-0.2173), respectively. This indicates that treatment effect also occurs in subjects with relatively small IMT values.

It cannot be excluded that beneficial changes of plasma lipids on IMT by doxazosin are balanced by the greater reduction of systolic blood pressure by HCTZ. The Systolic

Table 4

Quantitative change of mean and maximal combined or isolated carotid and femoral artery intima-media thickness after three years of follow-up

	IMT CHANGE FROM BASELINE		DIFFERENCE IN 3 YEARS		
	DOXAZOSIN	HCTZ	CHANGE	(CI)	P VALUE
Combined arteries					
Average of 20 mean far and near walls	-0.08 ± 0.13**	-0.12 ± 0.14**	0.013	(-0.044; 0.069)	0.66
Average of 20 max. far and near walls	-0.18 ± 0.14**	-0.21 ± 0.21**	-0.009	(-0.09; 0.073)	0.83
Average of 10 mean far walls	-0.07 ± 0.18*	-0.09 ± 0.19*	-0.011	(-0.093; 0.071)	0.79
Average of 10 max. far walls	-0.16 ± 0.21**	-0.14 ± 0.27*	-0.044	(-0.149; 0.062)	0.41
Average of 10 mean near walls	-0.09 ± 0.14**	-0.15 ± 0.13**	0.034	(-0.021; 0.090)	0.23
Average of 10 max. near walls	-0.20 ± 0.18**	-0.27 ± 0.20**	0.025	(-0.049; 0.010)	0.50
Carotid arteries					
Average of 12 mean far and near walls	-0.05 ± 0.14	-0.08 ± 0.12**	0.007	(-0.054; 0.068)	0.82
Average of 12 max. far and near walls	-0.15 ± 0.16**	-0.18 ± 0.20**	-0.008	(-0.090; 0.075)	0.85
Femoral arteries					
Average of 8 mean far and near walls	-0.13 ± 0.20**	-0.16 ± 0.2**	0.018	(-0.064; 0.099)	0.67
Average of 8 max. far and near walls	-0.23 ± 0.24**	-0.24 ± 0.29**	0.013	(-0.090; 0.116)	0.80

*Data in mm (mean ± SD). * Significantly different from baseline p<0.05, ** p<0.005.*

Hypertension in the Elderly Programme (SHEP) reported a reduction in morbidity from vascular disease in patients treated for systolic hypertension by chlorthalidone.[27] In the SHEP trial, SBP was 13 mmHg lower with active treatment than with placebo. Therefore we can not exclude that the difference in SBP treatment effect in the DAPHNE study of 6 mmHg in favour of HCTZ affected the IMT. To analyse the effect of change in SBP on IMT, we created two groups of patients, one with patients whose decrease in SBP was greater than the average, and a second group with patients whose SBP decreased less than average. Comparison of the three-year treatment effect on changes in IMT between these two groups did not show a statistical difference (p=0.08).

A previous trial, known as the Multicentre Isradipine Diuretic Atherosclerosis Study (MIDAS) with 883 hypertensive patients, compared the effect of isradipine and HCTZ on the progression of early atherosclerosis in carotid arteries over three years.[28] In contrast to the results of the DAPHNE trial, the progression of IMT was not changed by hypertension treatment. There are several important differences between these two trials: in the MIDAS trial fixed doses of medication were used,[28] while in the DAPHNE study the dose was adjusted up to a threshold of diastolic blood pressure. The latter mode of treatment may have a greater effect on blood pressure and consequently on vascular wall thickness. Another difference between the trials is that the DAPHNE population was selected by the presence of peripheral artery disease. Recently the Verapamil in Hypertension and Atherosclerosis Study (VHAS),[29] with four years of follow-up, showed that a difference in treatment effect was only observed in the thicker carotid IMTs. Therefore, the selection of patients with thick IMTs in the DAPHNE study may have added to the observed favourable changes in IMT.

At the start of this millennium, the doxazosin-treatment arm was discontinued in the Antihypertensive and Lipid-Lowering treatment to prevent Heart Attack Trial (ALLHAT), a very large study with 40,000 patients.[30] This decision was based on a significantly higher percentage of patients on doxazosin developing congestive heart failure and on the view that doxazosin was unlikely to be better than the diuretic chlorthalidone in preventing coronary heart disease. In DAPHNE none of the patients were known to have congestive heart failure or developed congestive heart failure. Doxazosin and the diuretic HCTZ appeared to be equally effective in the reduction of the IMT.

EFFECTS ON LIPIDS

This study confirms other studies showing that TG and HDLc are the lipid fractions most affected by doxazosin.[10,11,31] We did not observe the previously described adverse changes of plasma lipid levels in the HCTZ group, which have been attributed to an adverse effect of HCTZ on insulin resistance.[32,33] Several epidemiological and clinical studies have demonstrated a relation between plasma TG levels and risk of cardiovascular disease, with an enhancement of this risk in the presence of a combined elevation of TG and LDLc.[34-36] Additionally it has been shown that a reduction of fasting TG levels could induce a decrease in atherosclerotic disease.[37,38] Triglyceride-rich lipoprotein species may either have direct atherogenic effects or indirect effects via changes in other lipoproteins associated with atherosclerosis, such as IDLc.[39,40] The IDLc levels were shown to be strongly and independently predictive of progression of carotid artery intima-media thickness and coronary disease risk.[39] It has also been shown that a high concentration of IDLc plays an important role in the development and severity of peripheral vascular disease.[41] Favourable changes in lipid levels only occurred in the doxazosin-treated group: TG and IDLc levels decreased and HDLc increased. However, this did not result in a difference in IMT between doxazosin and HCTZ within three years of study. Most studies into the effects of doxazosin consistently report a slight 2 to 3% decrease in calculated LDL cholesterol levels, especially in hypercholesterolaemic patients.[33] In DAPHNE, the measured LDLc concentration was decreased to the same extent (-5%) as has been found previously. However, this change did not reach the level of significance. Lipid intervention trials with HMG-CoA reductase inhibitors, which predominantly lower LDLc levels, showed prevention of atherosclerotic disease as measured by ultrasound of the carotid and femoral arteries,[15,21,42-44] coronary angiography[45,46] and morbidity of vascular events.[47-51] Therefore, the absence of a significant effect by doxazosin or HCTZ on LDLc may add to the absence of a difference in IMT in DAPHNE.

The DAPHNE study was performed in a highly selected population of male patients with signs or symptoms of peripheral atherosclerosis, in combination with hypertension and hypercholesterolaemia, and of whom approximately 50% admitted smoking cigarettes during the study. For this special group of patients we conclude that hypertension treatment with the diuretic HCTZ and the α_1-blocker doxazosin have comparable effects on peripheral vascular parameters as assessed by B-mode ultrasound, despite large favourable differences in lipid variables by doxazosin which may be balanced by a greater reduction of systolic blood pressure by HCTZ.

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A patient with skin and bone lesions

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A 39-year-old nurse was referred for evaluation of bone lesions. Seven months earlier she had undergone a hysterectomy because of severe vaginal discharge and urge incontinence. Two months after the operation, she noticed pain in the mid left clavicula. There was no trauma, fever, redness or swelling. In the same period she developed red, itchy and scaling skin lesions of both hands, left foot, right ear and forehead, as well as recurrent oral aphthae.
Two months later she noticed a painful swelling at the left clavicula. An X-ray of the clavicula showed an old fracture with callus formation. X-rays of the skull, the chest and mammography were normal. Laboratory tests showed an ESR of 16 mm, aspartate aminotransferase 66 U/l, alanine aminotransferase 141 U/l, γGT 23 U/l and lactate dehydrogenase 299 U/l. Histology of fine needle biopsy of the clavicula showed chronic osteomyelitis with extensive fibrosis and perostitis (*figure 1*). Cultures were negative. Bone scintigraphy showed increased uptake in the left clavicula, right clavicula and parasternal region five minutes after injection; three hours after injection increased uptake was seen at the right skull (*figure 2*).
On physical examination there was a slight tenderness over the left clavicula. The skin showed red, non-vesicular, eczematous scaling lesions on hand palms, ears and forehead; there were aphthous ulcers in the mouth and the perianal region.

What is your diagnosis?
See page 379 for the answer to this photo quiz.

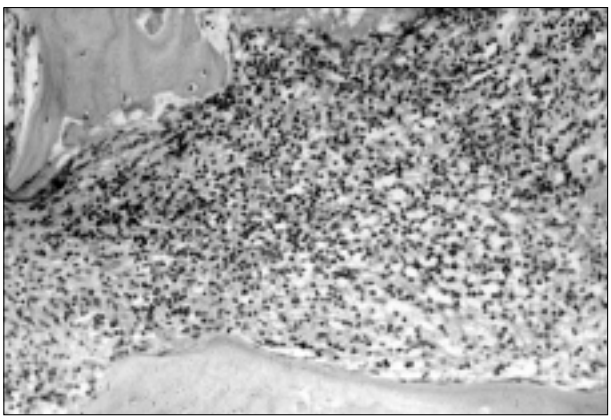

Figure 1

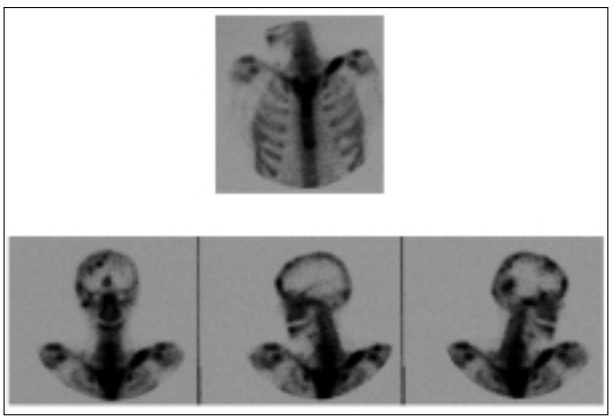

Figure 2

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CASE REPORT

Ruptured giant liver cyst: a rare cause of acute abdomen in a haemodialysis patient with autosomal dominant polycystic kidney disease

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ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) is a common hereditary disorder. Although liver involvement is the most frequent extra-renal manifestation, serious complications due to liver cysts are very rare. We report the occurrence of an acute abdomen caused by massive haemoperitoneum resulting from rupture of a giant liver cyst in ADPKD. Data suggest that chronic anticoagulation therapy should be avoided where possible in the presence of a giant liver cyst.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) has multiple systemic manifestations, including the renal, hepatic, cardiovascular, cerebrovascular and gastrointestinal systems.[1] Therefore, these patients may develop various unusual complications.

Liver involvement is the most frequent extra-renal manifestation of ADPKD. However, unlike renal cysts, which may cause infection or intracystic bleeding, serious complications due to liver cysts are very rare.[2-4] We report the fourth case in the literature of acute abdomen due to rupture of a giant liver cyst.

CASE REPORT

In December 1999, a 76-year-old man was admitted at the emergency department of our hospital with progressive abdominal pain. There was no nausea or vomiting. Micturition and defecation had been impaired for 24 hours. His medical history revealed end-stage renal disease (ESRD) due to ADPKD for which he had been receiving chronic haemodialysis for four hours three times a week since June 1999. His medication included oral anticoagulation (acenocoumarol) because of a polytetrafluoroethylene (PTFE) shunt in the left arm.

Upon physical examination, we saw a severely ill patient. His blood pressure was 75/50 mmHg, pulse rate 100 beats/minute and body temperature 37°C. Abdominal inspection revealed no distension; bowel sounds were sparse and inactive. Severe tenderness was noted in the upper abdomen with diffuse rebounding pain. No masses were palpable. Rectal examination revealed no abnormalities.

Relevant laboratory investigation included the following (normal ranges in brackets): C-reactive protein 210 mg/l (<10); haemoglobin level 6.1 mmol/l (8.5-11.0); platelet count 195 x 10^9 /l (150-400); white cell count 12.2 x 10^9 /l (4-10) with normal differential count. Serum urea amounted to 18.7 mmol/l (2.5-7.5); creatinine 848 mmol/l (75-110); sodium 142 mmol/l (135-145) and potassium 5.5 mmol/l (3.5-5.0). Liver biochemistry tests were abnormal: ALAT 198 U/l (<30); ASAT 137 U/l (<30); LDH 419 U/l (<320); γGT 27 U/l (<40) and alkaline phosphatase 56 U/l (<100). Blood gas analysis revealed: pH 7.34; pCO_2 39.8 mmHg; base excess -4.1 mmol/l; bicarbonate 20.7 mmol/l; pO_2 86 mmHg; O_2 saturation 96%. Serum lactate was 1.70 (<2.4). As the International Standardised Ratio (INR) amounted to 7.9, 20 ml of prothrombin complex (coagulation factors II, VII, IX and X) was administered. Two days prior to admission, the INR had been 2.9.

Abdominal radiography showed no subdiaphragmal air or distension of the bowel. Computed tomography of the abdomen disclosed multiple cysts in both kidneys and a large cyst with a diameter of nine centimetres in the right lobe of the liver (*figure 1a*). As the debris in the lower CT slices was visible on the dorsal site of the cyst (i.e., following gravity with the patient lying down during CT scanning), bleeding was suspected (*figure 1b*). In addition, free fluid was noted in the lower abdomen. No aortic aneurysm was present. Exploratory laparotomy revealed massive haemoperitoneum due to a ruptured giant liver cyst. Placing omentum over the ruptured cyst stopped the bleeding. There were no signs of intra-abdominal infection or infection of (other) liver cysts. The postoperative course was eventful with several serious complications (persistent haemodynamic instability, arrhythmias, bacterial pneumonia) eventually leading to the patient's death four weeks after admission.

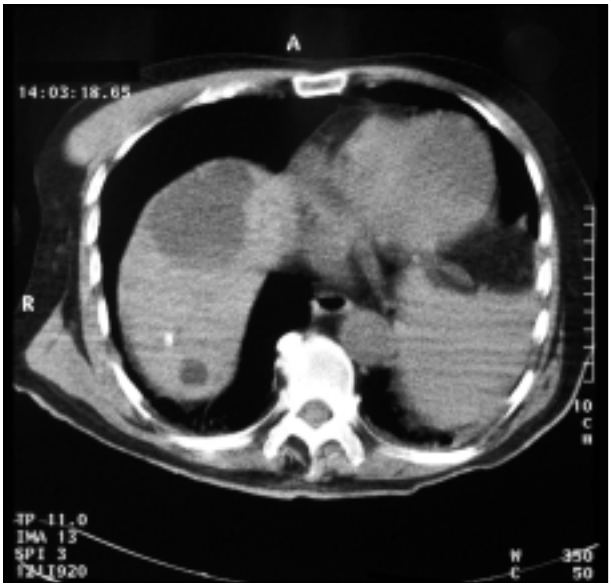

Figure 1a
Abdominal computed tomography revealing a massive subcapsular cyst with a diameter of 9 cm in the right upper lobe of the liver. The medial wall of the cyst contains a dense configuration, which obliterates the border of the cyst

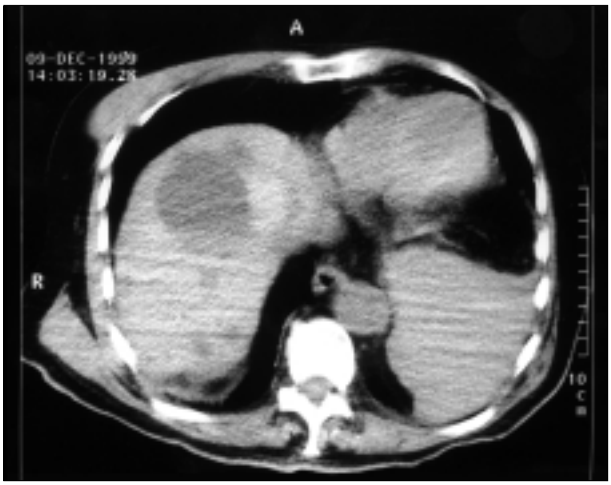

Figure 1b
In a lower coupe the borders of the cyst are clearly shown. The dense configuration caused by debris was visible on the dorsal site of the cyst (i.e. following gravity with the patient lying down during CT scanning), which is suggestive of intracystic bleeding

DISCUSSION

ADPKD is one of the most common hereditary disorders. It is characterised by cyst formation in ductal organs, particularly in the kidney.[5] Patients with ADPKD represent 3 to 10% of patients treated for end-stage renal disease (ESRD).[6,7] Cyst formation may occur in the liver, pancreas, ovary, spleen and central nervous system.[5] However, contrary to the present case, these patients usually develop complications associated with renal cysts, such as complaints related to the mass effect or more seriously intracystic infection or bleeding with gross haematuria and abdominal pain.[8] The prevalence of hepatic cysts in ADPKD increases with age: 10% of patients 20 to 29 years of age compared with 75% of patients over the age of 60.[9] Therefore, liver cysts are more likely to be present in the age group on haemodialysis. Women have more extensive hepatic cysts than men and pregnancy is strongly associated with extensive liver cysts.[10] Female steroid hormones appear to influence liver cyst formation.[10]

Only 5 to 15% of patients with liver cysts develop symptoms related to the mass effect of cysts such as abdominal fullness, swelling, intermittent or continuous abdominal pain, dyspnoea due to elevation of the diaphragm, heartburn and change in bowel movements.[1,11] Liver cysts can also become infected, presenting with fever and right upper quadrant tenderness. The incidence and complications of hepatic cyst haemorrhage in ADPKD patients on haemodialysis has not been well assessed but is probably rare. Patients with liver cyst bleeding may present with signs and symptoms similar to those in cystic infection: right abdominal pain and fever.[12] To date, only three cases with haemoperitoneum due to rupture of a liver cyst have been described.[2-4] In one case, the cyst was 11 cm, in the other two cases the size of the ruptured cyst was not mentioned. As in the present case, one patient was also on chronic anticoagulation therapy. Surgical treatment, i.e. marsupialisation of the cyst, was undertaken in two cases, whereas in the third case no surgery could be performed because of rapid clinical deterioration due to complicating abdominal sepsis and death.[2-4] Of note, other serious complications

related to liver cysts have also been reported in anecdotal case reports, such as portal hypertension[13] and obstructive jaundice.[14]

There is no accurate assessment of the influence of hepatic complications on the prognosis of haemodialysed patients. However, a review of 50 ADPKD patients on haemodialysis suggests limited influence of hepatic manifestations on their outcome,[11] but death related to liver cyst complications has been reported.[15] Our patient also died after he presented with acute abdomen and hypovolaemic shock due to massive haemoperitoneum.

Although not readily recognised by the authors as such, the size of the liver cyst may have been a risk factor for cyst bleeding.[3] However, it remains unclear whether regular ultrasound monitoring of the size of *asymtomatic* liver cysts should be performed. Several surgical options for significantly enlarging liver cysts are available (i.e. cyst aspiration, cyst fenestration or marsupialisation, enucleation or partial hepatic resection). However, the associated risks with these surgical interventions are probably higher than the risk of spontaneous life-threatening bleeding from a large liver cyst.[12,13,16]

Another risk factor for bleeding in chronic haemodialysis patients is uraemic bleeding tendency, repeated systemic anticoagulation during dialysis procedures and/or the use of chronic oral anticoagulation therapy to prevent thrombosis of (graft) fistulas. On reviewing the patient's charts, no careful evaluation was noted of the benefits and risks of the use of oral anticoagulation with regard to the presence of large liver cysts. Therefore, in the cases of large or enlarging liver cysts, we suggest that the need for oral anticoagulation should be weighed carefully against the risk of bleeding in these patients. The presence of a giant liver cyst, if not treated surgically as described above, may perhaps be regarded as a relative contraindication for chronic anticoagulation treatment. As such, Cimino-Brescia fistulas should probably be preferred in these patients because of a significantly lower risk of thrombosis as compared with graft fistulas.[17] In addition, heparin-free or regional anticoagulation haemodialysis should be considered in patients with giant liver cysts.

In conclusion, we describe a rare but life-threatening complication of ADPKD, i.e. rupture of a giant liver cyst with massive haemoperitoneum. Data suggest that asymptomatic giant liver cysts may be at significant risk of rupture. Therefore, if no surgical treatment of the cyst is performed, we feel that chronic anticoagulation therapy should be avoided if possible in these patients.

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CASE REPORT

Intrahepatic cholestasis of pregnancy

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ABSTRACT

Intrahepatic cholestasis of pregnancy (ICP) is a rare disease occurring mainly during the last trimester of pregnancy. Pruritus, often accompanied by excoriation of the skin but without other skin lesions, and elevated concentrations of bile acids are characteristic for this disorder. We present a 30-year-old woman with pruritus, elevated bile acids, ASAT and ALAT in the 22nd week of pregnancy. Treatment with ursodeoxycholic acid resulted in complete disappearance of the pruritus and normalisation of the bile acids, ASAT and ALAT. A healthy child was born at term. In the differential diagnosis of liver function abnormalities during pregnancy, ICP should be included. ICP responds very well to treatment with ursodeoxycholic acid, with no detrimental effects for mother and child.

INTRODUCTION

Intrahepatic cholestasis of pregnancy (ICP) is a rare disease of unknown aetiology, usually occurring during the third trimester of pregnancy in otherwise healthy women. ICP is characterised by skin pruritus and secondary excoriation but without other evidence of skin lesions, abnormal – cholestatic – liver function tests and a characteristic elevation of the concentration of serum bile acids.
Except for the, at times extreme, pruritus the disease is benign for the mother and disappears rapidly after delivery.[1-3] However, ICP has been associated with an increased risk of premature deliveries and stillbirth.[2,4,5] Furthermore, it usually leads to an extensive diagnostic work-up to exclude other liver diseases. Ursodeoxycholic acid administration is an effective and safe treatment for early onset ICP, according to a randomised, double-blind study controlled by placebo.[6] It reduces pruritus, normalises biochemical abnormalities and most importantly improves the outcome of pregnancy.
We describe a case of early onset ICP that was successfully treated with ursodeoxycholic acid.

CASE REPORT

The patient is a 30-year-old female, with a history of cholelithiasis followed by a cholecystectomy in 1987, pregnancy-related hypertension and tachycardia during her first pregnancy in 1996 and hypothyreoidism substituted with levothyroxine since the beginning of 2000. This patient was seen during her second pregnancy in the 22nd week of gestation with severe complaints of pruritus, which developed after a short course of treatment with amoxicillin for an upper respiratory tract infection. The pruritus did not diminish after the amoxicillin had been stopped. She also complained of constant epigastric pain, sometimes accompanied by attacks of colicky-like pains. The pain disappeared after a few days. She complained of nausea, vomiting and anorexia. There was no fever, the urine was not darkly coloured and the faeces were not discoloured. She had not travelled outside the country in the last three months. On physical examination blood pressure was 135/80, confirmed by automatic blood pressure measurement (mean 135/77). The skin showed many effects of scratching, but no other abnormalities were noticeable. The pregnant uterus was conform gestational age. Palpation of the epigastric

area caused diffuse epigastric pain. Liver and spleen were neither palpable nor tender. Lungs were clear and heart sounds were normal.

Laboratory evaluation showed normal haematological parameters, thrombocytes 191 10^9/l (150-400 10^9/l), bilirubin 6 µmol/l (<10 µmol/l) alkaline phosphatase 298 U/l (normal 40-120, during pregnancy 60-200 U/l), γ-glutamyl transpeptidase 71 U/l (5-35 U/l), aspartate aminotransferase (ASAT) 85 U/l (12-35 U/l), alanine aminotransferase (ALAT) 73 U/l (8-40 U/l), lactate dehydrogenase 429 U/l (200-450 U/l), bile acids 43 µmol/l (0-10 µmol/l). Other laboratory values were creatinine 65 µmol/l (55-95 µmol/l), urea 2.5 mmol/l (2.6-7.5 mmol/l), albumin 37 g/l (43-53 g/l) , APTT 30 seconds (27-33 seconds), PTT 10.2 seconds (9.5-12.3 seconds), INR 1.0 and no proteinuria. Antimitochondrial antibodies, antibodies against hepatitis A, B and C were not demonstrable. Ultrasound imaging and MRI scan did not show any dilatation of intrahepatic or extrahepatic bile ducts.

After ten days, as the itching increased in severity, both ASAT and ALAT increased to more than 500 U/l, and the concentration of bile acids rose to 199 µmol/l. The diagnosis of intrahepatic cholestasis of pregnancy was made and we decided to start treatment with ursodeoxycholic acid administration at a dosage of 900 mg/day. Seven days after initiating ursodeoxycholic acid, the pruritus diminished and after two weeks it disappeared completely. After two weeks of treatment both ASAT and ALAT were only slightly above normal. The bile acid level also returned to normal. At 37 weeks and 5 days a healthy boy was born vaginally after induction with oxytocin drugs, weighing 3570 g, with an Apgar score of 10 after five minutes. Directly after delivery the treatment with ursodeoxycholic acid administration was stopped since all biochemical values remained normal.

The time course of clinical signs and symptoms and the liver function abnormalities in relation to the treatment with ursodeoxycholic acid are shown in *figure 1*. The rapid clinical and biochemical response to treatment is evident.

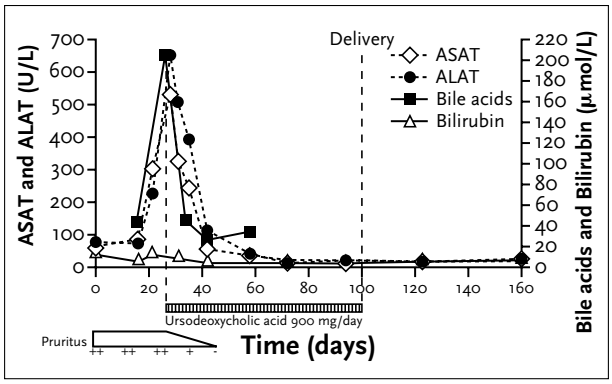

Figure 1

Time course of clinical signs and symptoms and the liver function abnormalities in relation to the treatment with ursodeoxycholic acid

DISCUSSION

We present a case of intrahepatic cholestasis of pregnancy successfully treated with ursodeoxycholic acid. ICP has been identified all over de world, but the prevalence varies greatly according to the country and ethic origin. The prevalence varies between 0.1 to 1.5% in Europe, to 16% of pregnancies in Chile.[1,2,7] In native Araucanian Indians in Chile, almost 28% of pregnancies have ICP.[7,8] Typically, intrahepatic cholestasis of pregnancy is a disease of the third trimester of pregnancy.[9-11] Our case is a report of very early onset intrahepatic cholestasis of pregnancy, early in the second trimester.

The differential diagnosis of liver function abnormalities during pregnancy is extensive and includes diseases that are not primarily associated with pregnancies, such as hepatitis and (obstructive) biliary tract diseases and pregnancy-related liver disorders. Diseases with liver function abnormalities related to pregnancy include hyperemesis gravidarum (usually first trimester), toxaemia of pregnancy and HELLP syndrome (usually third trimester), acute fatty liver of pregnancy (often late third trimester or after delivery) and intrahepatic cholestasis of pregnancy. In our case an extrahepatic origin of the liver function abnormalities was excluded by magnetic resonance cholangiopancreatography (MRCP). The epigastric pain followed by colicky-like pain is not characteristic of ICP. Although the MRCP did not show any abnormalities, the possibility of a passed gallstone, even after cholecystectomy, should still be considered.

Typical for intrahepatic cholestasis of pregnancy are the pruritus, classically starting at the palms and soles, worsening during the evening and night, and the high concentration of serum bile acids with increased levels of ALAT, ASAT and normal bilirubin as was the case in our patient.[6,9,11] Bile acids are abnormally high in 90% of ICP patients. However, ALAT and ASAT values are abnormal in 55 and 60% of patients, respectively. Alkaline phosphatase is abnormal in 70% of patients and bilirubin value in 25%.[2,12] Liver biopsy is not generally necessary. If a biopsy is taken for differential diagnostic purposes, the histology shows mild focal irregular intrahepatic cholestasis with bile plugs in the canaliculi and small amounts of bile pigment in centrilobular hepatocytes and macrophages.[2,13] What is difficult in the differential diagnosis is that about 10% of ICP patients develop jaundice.[2,4,7] Some patients may develop steatorrhoea with decreased absorption of fat-soluble vitamins. Increased rates of postpartum haemorrhage have been reported,[3,14] possibly related to vitamin K deficiency.[2,15]

The aetiology of ICP is not completely elucidated. Historically, ICP has been associated with the cholestatic effects of oestradiol metabolites and progesterone.[1,2,16-18] Recent findings in other cholestatic liver diseases such as progressive familial intrahepatic cholestasis (PFIC) and benign recurrent intrahepatic cholestasis (BRIC) suggest malfunction of

biliary canalicular transporters.[1,2,19,20] PFIC is a disorder characterised by the onset of cholestasis in early childhood which can progress to cirrhosis and liver failure before adulthood.[19,21] PFIC can be classified in three subclasses (PFIC1-3). PFIC1 and 2 have low concentrations of biliary bile acids and low to normal γ-GT values in the serum,[19] whereas PFIC3 patients have high serum levels of γ-GT and bile acids which lack phospholipids but have a normal biliary bile acid concentration.[19,22] Homozygote mutations of multidrug resistant 3 (MDR-3) gene have been described in three pedigrees with PFIC3.[22,23] MDR-3 is a protein responsible for secretion of phospholipids.[1] The clustering in families[24,25] and the endemic occurrence of ICP[2,8] suggest there is also a genetic basis in this disease. ICP is more common in families with PFIC3.[19,23] In some ICP patients with a raised serum γ-GT heterozygous MDR-3 missense mutations have been found.[19] However, some patients have normal γ-GT values. It is possible that other biliary canalicular transporters are involved in these patients. For example, ICP is also more common in BRIC patients,[20,26] in whom mutations in the familial intrahepatic cholestasis 1 (FIC1) gene have been described.[27]

ICP is considered a benign disease for the mother, but has been associated with an increased risk of premature delivery and stillbirth.[2,4,5] The pathophysiological mechanisms have to be defined, but some evidence suggests that the foetal consequences might be caused by the increased maternal and foetal[28-31] bile acid concentration. High bile salt levels were associated with more frequent occurrence of foetal distress.[2,30,32] Moreover, high bile acid levels are reported to worsen cardiac rhythm[33] and to induce vasoconstrictive effects on isolated human placental chorionic veins.[34] Various treatments, such as antihistamines, anion exchange resins (e.g. cholestyramine), phenobarbitol and corticosteroids, have been tried for ICP, however with disappointing results.[2,35-38] S-adenosylmethionine (SAM) has also been used. In two studies[39,40] beneficial effects have been shown, but negative results were found in a double-blind, placebo-controlled study.[41] Ursodeoxycholic acid (UDCA) is a naturally occurring hydrophilic bile acid[42] that improves clinical and biochemical indices in a variety of cholestatic liver diseases.[43,44] At present, ursodeoxycholic acid seems to be the best treatment option for ICP.[2,6,45-47] In a placebo-controlled double-blind trial of ursodeoxycholic acid in the treatment of ICP, all infants born to the eight women treated with ursodeoxycholic acid were born near or at term, and no stillbirths occurred.[6] However, in the placebo-treated group five out of seven women delivered prematurely, including one stillbirth. No toxicity was observed up to three months after delivery in mother or child. In another small double-blind randomised study, treatment with UDCA was associated with improved clinical and biochemical results.[48] However, the literature about the effectiveness is still not conclusive.[15,32] It has been demonstrated that treatment with UDCA does not increase meconium levels of potentially toxic metabolites of UDCA.[31] It seems to have no adverse effects on the baby.[6,31,32,46]

The mechanism of action of UDCA is not completely clear. It might be that the hydrophilic UDCA protects against injury by hydrophobic bile acids and stimulates the excretion of these and other potentially hepatotoxic compounds.[2,43] Furthermore, UDCA is able to reverse the impairment of bile acid transport across the trophoblast, which might contribute to favourable foetal outcome.[49] Our patient was successfully treated with ursodeoxycholic acid and a healthy child was born. After delivery, treatment was stopped and liver functions remained normal during follow-up for one year. In affected mothers the symptoms classically disappear within two days.[1-3] In patients in whom ICP develops very late in the third trimester, delivery might be awaited without treatment. However, this decision should be taken with due care, as different groups conclude that ICP is associated with adverse perinatal outcome not predicted by conventional foetal surveillance.[47,50] It should be reminded that patients with a past history of ICP are at risk for recurrence during subsequent pregnancies in 60 to 70%, or rarely during the use of oral contraceptives.[6,18]

In conclusion, intrahepatic cholestasis of pregnancy has to be included in the differential diagnosis of patients with pruritus and liver function abnormalities during pregnancy, especially when concentrations of bile acids are increased. ICP can be treated successfully with ursodeoxycholic acid.

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CASE REPORT

Acute fatty liver in pregnancy

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ABSTRACT

When confronted with liver abnormalities during the third trimester of pregnancy, one should consider acute fatty liver of pregnancy. The differential diagnosis with (pre-)eclampsia and HELLP syndrome is sometimes difficult. In these cases a liver biopsy is helpful though rarely performed during pregnancy. After delivery of the child the liver test abnormalities will ultimately disappear. Recent publications reveal that a dysfunction in the β-oxidation of mitochondrial fatty acids may contribute to the aetiology of this rare disorder. We describe a case of acute fatty liver in pregnancy, with liver dysfunction (decreased albumin, prolonged prothrombin time) slowly returning to normal after delivery. Testing for disorders in β-oxidation of mitochondrial fatty acids did not reveal abnormalities in mother or child.

INTRODUCTION

Liver function abnormalities during pregnancy can be due to pre-existent liver diseases or to newly developed liver diseases. In the latter group, liver diseases occurring coincidentally during pregnancy (i.e. viral hepatitis) have to be distinguished from liver diseases occurring exclusively during pregnancy,[1] such as pre-eclampsia or the HELLP (haemolysis elevated liver enzymes low platelet count) syndrome. Other less frequent causes should also be kept in mind,[2] including acute fatty liver in pregnancy and intrahepatic cholestasis of pregnancy. The timing of the onset, specific clinical symptoms and if necessary a liver biopsy can differentiate between these disorders. We describe a patient with a rare disorder of liver failure during pregnancy.

CASE REPORT

A 32-year-old woman was admitted to hospital with anorexia, tiredness and right upper quadrant pain. She was a mother of one healthy child and was in the 39th week of her second pregnancy. She was not on any medication. Prior history was unremarkable. Physical examination did not reveal anything significant; there were no signs of chronic liver disease (ascites, venous collaterals, encephalopathy, spider naevi). Blood pressure was 130/75 mmHg. Laboratory results revealed alarmingly disturbed liver function tests: aspartate aminotransferase (ASAT) 295 U/l (normal 5-25), alanine aminotransferase (ALAT) 447 U/l (normal 5-35), alkaline phosphatase 576 U/l (normal 30-90), γ-glutamyl transpeptidase (γGT) 43 U/l (normal 5-25) and a total bilirubin of 154 μmol/l (normal 2-17). Albumin was decreased at 18.9 g/l (normal 31-39), prothrombin time was prolonged to 23 seconds (normal 12-14) and uric acid was 0.51 mmol/l (normal 0.15-0.35). Haemoglobin content was 8.6 mmol/l and platelet count was 161 x 10^9/l. There were no fragmentocytes. Initially renal failure was present (creatinine 289 μmol/l), which improved upon rehydration (97 μmol/l). Urine analysis did not reveal proteinuria. The CTG (cardiotocogram) showed some decelerations. In view of the severe liver disturbances at 39 weeks of pregnancy, labour was induced and she gave birth to a healthy boy of 4070 g. After birth she received amoxicillin/clavulanic acid because of fever. The fever subsided but the liver function abnormalities persisted and she was transferred to our university hospital. Bilirubin increased to 218 μmol/l (direct 166 μmol/l) and other liver function tests were: alkaline phosphatase 366 U/l, ASAT 147 U/l, ALAT 168 U/l and γGT 97 U/l. Albumin decreased to 16 g/l, prothrombin time was 22 seconds and factor V 64%, which are signs

of liver insufficiency, although glucose was borderline normal (3.6 mmol/l). A direct Coombs test was negative, again there were no fragmentocytes. Fibrinogen was 1880 (normal 1600-3200 E) and D-dimer count was 900 (normal <500 U). Viral serology (hepatitis B,C, E, CMV, EBV) was negative. There were no signs of autoimmune or metabolic liver diseases (ANA, antismooth muscle antibody, antiliver kidney cell antigen, antimicrosomal antibody were all negative, γ-globulin was 8.4 g/l, α-1-antitrypsin, ceruloplasmin, serum copper and iron were normal). Clinically there were no signs of encephalopathy although the ammonia content was initially 67 μmol/l, and later decreased to 45 μmol/l. Echography of the liver showed normal liver parenchyma and a normal flow in both the portal and hepatic veins. Blood pressure was normal (130/80 mmHg) and repeat urine analysis did not reveal proteinuria. Puncture of ascites fluid showed a leukocyte count of 100 x 10^6/l, albumin 1 g/l and lactic dehydrogenase of 88 U/l. Cytologically there was no suspicion of malignancy. The liver abnormalities gradually improved. Using diuretics, the ascites disappeared. Seventeen days after birth a liver biopsy was performed showing microvesicular steatosis (*figures 1* and *2*). This fits into the diagnosis of acute fatty liver during pregnancy. Later, the child was investigated for disturbances of the mitochondrial β-fatty acid oxidation: urine analysis for organic acids did not show any abnormalities. Acylcarnitine analysis was normal, as was the enzyme activity of the long chain fatty acid dehydrogenase. Acylcarnitine and tetradecenic acid blood levels were also found to be normal in the mother.

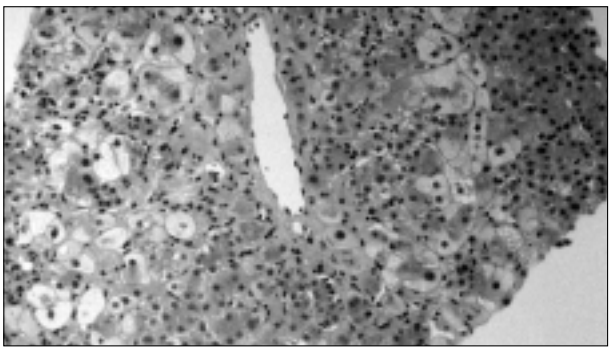

Figure 1
Liver biopsy: an overview showing a central hepatic vein surrounded by regular hepatocytes with partial steatosis
There are no signs of fibrosis or inflammation.

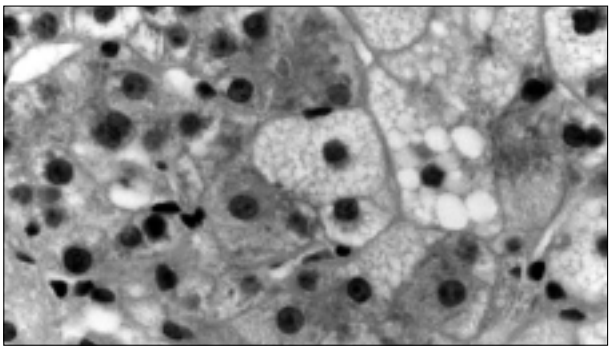

Figure 2
Liver biopsy showing a larger image of the same area as seen in figure 1
In the cytoplasm of the hepatocytes, a micro and macrovesicular steatosis is shown. In some hepatocytes the yellow-green bile is seen as a sign of cholestasis. There are only few lymphocytes and acidophilic bodies.

DISCUSSION

Liver diseases occurring exclusively during pregnancy are intrahepatic cholestasis of pregnancy, pre-eclampsia, the HELLP syndrome, and acute fatty liver.[1,2] When pain is a predominant symptom, rupture of the liver capsule should be considered. Towards the end of pregnancy pre-eclampsia, the HELLP syndrome and acute fatty liver of pregnancy are most probable. After delivery of the child, liver function abnormalities return to normal. *Table 1* on page 372 shows some clinical characteristics of liver diseases in pregnancy. In our patient, there were no signs or symptoms of a pre-existing liver disease. Laboratory results were negative for viral hepatitis or autoimmune liver disease. The occurrence during late third trimester, the absence of itching and the relatively low levels of serum bilirubin did not point to intrahepatic cholestasis of pregnancy. There were no signs of hypertension, proteinuria, persisting renal failure or thrombopenia (suggesting pre-eclampsia or HELLP syndrome). Plasma glutathione S-transferase alpha values (often elevated in these conditions) were normal.[3] In view of these findings, the clinical diagnosis in our patient was acute fatty liver of pregnancy. This diagnosis was confirmed by the results of the liver biopsy, which showed predominantly steatosis. There was mild ballooning of liver cells, cholestasis and cell death. These features are fairy typical for fatty liver and argue against autoimmune hepatitis (no plasma cells, too much steatosis) or viral hepatitis (too little inflammation and necrosis). There are several causes for fatty liver and the clinical history suggests here a diagnosis of fatty liver of pregnancy rather than a drug-induced fatty liver; moreover, more severe cholestasis and inflammation often accompany drug effects. The clinical course and the complete recovery of liver function abnormalities are in agreement with this diagnosis.

Acute fatty liver during pregnancy is a poorly understood disease that occurs during the third trimester of pregnancy.[4] The frequency varies from 1:6600 to 1:13,000 pregnancies.[5] In comparison, the incidence of the HELLP syndrome is 0.1 to 0.6%. Sheehan first described acute fatty liver of pregnancy in 1940.[6] It can occur during the first as well as during subsequent pregnancies. Its predominant symptoms

Table 1
Clinical characteristics of liver diseases specific to pregnancy and viral hepatitis

	TRIMESTER	SYMPTOMS	TYPICAL LABORATORY ABNORMALITIES
Hyperemesis gravidarum	1 or 2	Nausea, vomiting	Slight APh and ALAT increase
Intrahepatic cholestasis of pregnancy	2 or 3	Pruritus	Bile acids ↑ ALAT ↑ Bilirubin ↑
Acute fatty liver of pregnancy	3	Upper abdominal pain, nausea, vomiting	ALAT ↑ APh ↑ Thrombopenia Bilirubin ↑ Glucose ↓ Liver insufficiency
Pre-eclampsia/ eclampsia	3 (late 2)	Upper abdominal pain, oedema, hypertension	Urine protein ALAT ↑ APh ↑ Uric acid ↑ Thrombopenia
HELLP syndrome	3 (late 2)	Upper abdominal pain Nausea, vomiting Hyper-tension ±	ALAT ↑ Thrombopenia Haemolysis LDH ↑ Sometimes DIC
Viral hepatitis	All	Nausea, vomiting, fever, jaundice Increased mortality with hepatitis E	ALAT ↑ Bilirubin ↑ Liver insufficiency

APh = alkaline phosphatase, DIC = diffuse intravascular coagulation. In normal pregnancy, a slight increase in APh is normal.

are nausea and vomiting, epigastric pain, anorexia and jaundice. Itching is a rare symptom. The liver is usually small. Ascites can occur due to portal hypertension. Elevated liver transaminases are the predominant liver abnormalities. Liver synthesis can be severely disturbed as shown in our patient. Hepatic encephalopathy and diffuse intravascular coagulation can occur.[4] Echography of the liver can show increased echogenicity due to steatosis. Histologically, a microvesicular fatty infiltration of the hepatocytes mainly in the central part of the liver lobules is observed. Often a mild inflammation with cholestasis is seen. Severe hepatocellular necrosis (as in fulminant viral hepatitis) is seldom seen. Analysis of the intrahepatic lipids shows an accumulation of mainly free fatty acids.[7]
The differential diagnosis with pre-eclampsia and the HELLP syndrome can be very difficult on clinical symptoms. In acute fatty liver a slight hypertension and oedema with proteinuria and intravascular coagulation may occur. In some cases only liver biopsy can solve the differential diagnosis. Liver biopsy, however, is seldom performed during pregnancy because of its associated morbidity. Furthermore, treatment for these syndromes is symptomatic and mainly aimed at stabilising the condition of the mother until delivery can be safely performed. If necessary, liver biopsy can be carried out, preferably under ultrasonographic control (after checking and correction of coagulation disorders).
The final treatment is induction of labour. Liver abnormalities return to normal, though this may take some time[4] as was the case in our patient. There is no known medication for acute fatty liver. Conservative measures should be taken to minimise liver damage and prevent further maternal morbidity. These include correction of coagulation and electrolyte disorders and treating reflux oesophagitis (due to the intense vomiting) with proton pump inhibitors. Blood sugar levels need to be monitored and corrected. Encephalopathy and seizures should be treated appropriately (lactulose and antibiotics, magnesium or benzodiazepines and phenytoin). Acute fatty liver can deteriorate suddenly and progress rapidly to fulminant hepatic failure requiring admission to an intensive care unit. Once the diagnosis is made likely, immediate delivery should always be considered to reduce maternal mortality and morbidity, even if this means sacrificing the foetus. Rare cases have been reported of patients whose condition did not improve after delivery and liver transplantation was necessary.[8]
Before 1970, maternal mortality was very high (70 to 80%). As a result of early diagnosis and termination of pregnancy the mortality is now less than 15%.[9]
The aetiology of acute fatty liver is still unknown. The histological abnormalities have similarities with disorders of the intramitochondrial oxidation of fatty acids. Recent literature shows a predominance of acute fatty liver in mothers who are heterozygous for a genetic abnormality in long chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD),[10] which is an important enzyme in the β-oxidation of fatty acids in mitochondria.[11] In children with LCHAD deficiency, the mother more often experienced an acute fatty liver or HELLP syndrome during pregnancy.[12] All of these children had a specific mutation in the gene coding for LCHAD. This association between the heterozygous mother and the specific mutation in the child probably somehow contribute to the development of acute fatty liver syndrome. The co-occurrence of the HELLP syndrome may point to a common pathophysiological pathway. In view of these abnormalities, both mother and child should be screened for disorders in the mitochondrial β-oxidation.[9-12] In our patient, however, neither mother nor child had these abnormalities.
In conclusion, when encountering liver function abnormalities during the third trimester of pregnancy, acute fatty liver should be considered. This rare disorder has a benign course when the diagnosis is considered at an early stage and delivery is initiated.

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Liver enzyme elevation induced by hyperemesis gravidarum: aetiology, diagnosis and treatment

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ABSTRACT

Three primigravidae were admitted during the first trimester of pregnancy with nausea, vomiting, ketonuria and liver enzyme elevation of varying severity. A 29-year-old woman had elevated aminotransferase values, at levels described in the literature (ASAT 112 U/l, ALAT 214 U/l). The second patient, a woman aged 26 years, had undergone *in vitro* fertilisation and showed higher liver enzyme elevation, including the total bilirubin level (ASAT 250 U/l, ALAT 474 U/l, total bilirubin 59.8 µmol/l). A 30-year-old woman had extremely high aminotransferase values (ASAT 705 U/l, ALAT 1674 U/l) and she is the first reported patient with ALAT values exceeding 1000 U/l in connection with hyperemesis gravidarum. Gallstone disease, viral and drug-induced hepatitis were excluded in all of these patients. Treatment was symptomatic and the abnormal liver tests returned to normal promptly when the vomiting resolved, independent of the severity of liver enzyme elevation. The pregnancies proceeded normally and all three patients delivered healthy babies.

INTRODUCTION

Nausea and vomiting are very common in early pregnancy, occurring in 50% of pregnant women until 16 weeks of gestation.[1] Hyperemesis gravidarum is characterised by intractable nausea and vomiting during the first trimester of pregnancy, which in severe cases may lead to dehydration and require hospitalisation. There is no definite delimitation between 'morning sickness' and hyperemesis gravidarum: the latter is considered as the severe end of a gradation of symptoms. The incidence of hyperemesis gravidarum varies from 0.3 to 1% of pregnancies.[2-4]

Liver enzyme elevation occurs in 50% of patients who are hospitalised with hyperemesis. An increase in serum aminotransferases is the most common abnormality and alanine aminotransferase (ALAT) is usually higher than aspartate aminotransferase (ASAT).[5-6] This elevation is usually two to three times the upper limit of normal; it may be in the low hundreds and rarely as high as 1000 U/l.[7-9]

In this article we describe three patients with hyperemesis gravidarum and liver enzyme elevation of varying severity. We also discuss the aetiology, differential diagnosis and recommended diagnostic approach and therapy.

CASE A

A 29-year-old primigravida was admitted for the fourth time because of nausea and vomiting at 13 weeks of gestation. Her medical history revealed appendectomy, cholecystectomy and nephrolithiasis. She occasionally took ipratropium for asthmatic bronchitis. There were no signs of dehydration on physical examination. A transvaginal ultrasonography showed a normal foetus conform the gestational age. Laboratory findings on admission included increased ketones in the urine as well as increased levels of aminotransferases: ASAT 112 U/l, ALAT 214 U/l (*table 1*). Total bilirubin, alkaline phosphatase (ALP), γ-glutamyltransferase (γGT) and serology for hepatitis B were normal. Treatment consisted of intravenous fluids, meclozine/pyridoxine suppositories and occasional omeprazole for heartburn. During admission an abdominal ultrasono-

graphic examination showed no abnormalities of the liver, bile ducts and spleen. After a few days the patient recovered well and she was discharged after reintroducing a normal diet. Blood controls showed improvement of aminotransferase levels: ASAT 50 U/l and ALAT 145 U/l.
At 17 weeks of gestation, she visited the outpatient clinic for nausea, vomiting and abdominal pain. Hospitalisation followed again because of insufficient intake and high ketonuria. Routine liver tests revealed values similar to those at discharge: ASAT 53 U/l, ALAT 96 U/l. An abdominal ultrasonography and a gastroscopy showed no abnormalities. She was treated in the same way as before and discharged after nine days. Control of serum aminotransferases showed an improvement again: ASAT 31 U/l and ALAT 55 U/l (*table 1*).
The pregnancy then progressed uneventfully and she delivered a healthy daughter of 3690 g at 40 weeks gestation.

CASE B

A 26-year-old primigravida visited the outpatient clinic for nausea, vomiting and itching. Her medical history revealed primary subfertility, and she was at six weeks of gestation after undergoing *in vitro* fertilisation.
Blood tests showed elevated liver tests: ASAT 107 U/l, ALAT 330 U/l, total bilirubin 27.3 µmol/l, ALP 456 U/l. Serology to hepatitis A, B, C and cytomegalovirus was negative and there was no evidence for autoimmune hepatitis, haemochromatosis or hyperthyroidism. The β-hCG level was 87,000 U/l, which is very high at week 6 of gestation. Ultrasound examination showed, besides ovarian follicular cysts, no abnormalities and there was no evidence of ovarian hyperstimulation syndrome. Hyperemesis gravidarum was diagnosed and she was treated with metoclopramide and meclozine/pyridoxine.
One week later she was admitted for persistent vomiting, itching and a loss of 3 kg in weight. Liver tests revealed higher levels than before: ASAT 250 U/l, ALAT 474 U/l, total bilirubin 59.8 µmol/l, conjugated bilirubin 47.8 µmol/l (*table 1*). Urine test showed ketosis (2+). Treatment consisted of intravenous fluids and chlorpromazine. She left the hospital without symptoms and medication after two weeks. At week 12 she was readmitted because of vomiting, a weight loss of 2 kg, jaundice and ketosis. Serum aminotransferase levels were no higher than previously, but there was a persistent hyperbilirubinaemia of 47.8 µmol/l. The abdominal ultrasound revealed normal liver and bile ducts. She followed the same treatment as before for two weeks. Symptoms resolved at 14 weeks of gestation and she recovered the lost weight.
The pregnancy proceeded normally until week 31, when she was admitted because of premature contractions. Delivery was postponed using ritodrine and she delivered two healthy neonates of 900 g at 35 weeks gestation.

CASE C

A 30-year-old primigravida was hospitalised three times for intractable nausea, vomiting and liver enzyme elevation, between weeks 13 and 17 of pregnancy. Medical history only revealed hiatal hernia with mild reflux oesophagitis. She had no history of alcohol use or hepatitis. Before the last admission she had been vomiting for four days and initial treatment with meclozine/pyridoxine had had no effect at home.
On admission she had slight signs of dehydration but no signs of jaundice or hepatomegaly. Routine blood samples showed elevated liver tests: total bilirubin 44.9 µmol/l, ASAT 614 U/l, ALAT 1065 U/l and γGT 87 U/l (*table 1*). There were no signs of hyperthyroidism or haemochromatosis. Serology to hepatitis A, B, C and to cytomegalovirus were negative, as were antinuclear and antimitochondrial antibodies. Urine samples showed increased ketones. An abdominal ultrasonography showed no abnormalities of the liver, gallbladder, bile ducts and spleen.
Following admission she was treated with metoclopramide and oral fluids. Within a few days the symptoms had

Table 1
Serum aminotransferase levels of the three cases

	TEST*	ADMISSION	HIGHEST VALUE	AT DISCHARGE	AFTER DISCHARGE
Case A	ASAT	112	112	50	31
	ALAT	214	214	145	55
Case B	ASAT	250	250	49	37
	ALAT	474	474	110	87
Case C	ASAT	614	705	78	19
	ALAT	1065	1674	641	33

* *Normal values: ASAT 5-35 U/l, ALAT 5-35 U/l.*

resolved and the liver tests stabilised (*table 1*). The patient was discharged at day 8, and one week later all the liver tests except the ALAT (141 U/l) had normalised. Three weeks later, she remained symptom-free and all liver tests were normal. The pregnancy proceeded normally and she delivered a healthy child of 3700 g at 39 weeks gestation.

DISCUSSION

There are several classic features of hyperemesis gravidarum. It presents early in the first trimester of pregnancy, usually in the fourth to tenth week of gestation, with intractable vomiting and associated pytialism (excessive salivation). Abdominal pain is infrequent. Patients may present with dehydration, liver test abnormalities, ketosis, electrolyte disturbances and weight loss.
The syndrome is associated with nulliparous women, obesity and multiple gestations. Advanced maternal age (>35 years) and cigarette smoking seem to be protective factors.[1,10,11] Case A showed typical features of this syndrome regarding presentation – nulliparity, classic symptoms and ketosis – liver test abnormalities and spontaneous recovery after admission.

Several causal factors for hyperemesis gravidarum have been described, but the aetiology remains uncertain. Some proposed theories in the pathogenesis of hyperemesis gravidarum include psychological factors (emotional instability, conversion),[12,13] hyperthyroidism,[14] liver abnormalities and changes in gestational hormone levels (especially hCG peak during the first trimester).[15,16] In case B, β-hCG levels were very high for the gestational age, possibly related to the twin pregnancy. On the other hand, Goodwin *et al.* described a significant difference in total hCG and β-hCG between women with hyperemesis and healthy pregnant women.[16]
Other theories to explain hyperemesis include abnormal gastric motility,[17] autonomic nervous system dysfunction,[8] alterations in lipid levels[18] and nutritional deficiencies. On examination, no single theory seems to provide an adequate explanation for hyperemesis gravidarum.[5]
In most cases, nausea and vomiting resolve by 20 weeks of gestation. Only seldom do these symptoms persist into the second half of the pregnancy. A recurrence of hyperemesis with subsequent pregnancies is uncommon.[19]

Hyperemesis gravidarum and liver enzyme abnormalities

There are very few reports about abnormal liver tests in patients with hyperemesis gravidarum.[7,8,18-21] In a prospective study, liver tests were more frequently abnormal in 12 patients with hyperemesis than in healthy controls. Elevations in either ASAT or ALAT occurred in 50% of patients with hyperemesis.[8]
Aminotransferase values can rise to 200 U/l (six times the upper limit of normal)[7] and exceptionally as high as 1000 U/l.[8] Case C developed extremely high serum aminotransferase values: as far as we know, this is the first reported patient with ALAT values higher than 1000 U/l induced by hyperemesis gravidarum.
Mild hyperbilirubinaemia (less than 68 μmol/l) with elevation of both direct and indirect fractions has been found in half the women hospitalised for this condition.[7,19] ALP may be elevated to twice the normal value, but this could also be a physiological increase of placental origin. However, it is known that ALP levels increase gradually during the first seven months of pregnancy and then rise rapidly to peak at term.[9] Abnormal liver tests in connection with hyperemesis gravidarum return to normal promptly when the vomiting resolves.
Although the pathogenesis of the liver injury is unknown, the combined effects of hypovolaemia, malnutrition and lactic acidosis are believed to play a role.[9] Hyperbilirubinaemia is probably related to malnutrition and impaired secretion of bilirubin.[7]

Diagnosis

The differential diagnosis of liver enzyme elevation presenting in the first trimester of pregnancy includes hyperemesis gravidarum – as the only pregnancy-related syndrome – gallstone disease, viral and drug-induced hepatitis (*table 2*). The diagnosis of hyperemesis gravidarum is a clinical one that rests on the exclusion of other causes

Table 2
Differential diagnosis of common causes of elevated serum aminotransferase levels and/or jaundice in pregnancy[9]

TRIMESTER	DIFFERENTIAL DIAGNOSIS*
First	*Hyperemesis gravidarum*
	Gallstones
	Viral hepatitis
	Drug-induced hepatitis
Second	*Intrahepatic cholestasis of pregnancy*
	Gallstones
	Viral hepatitis
	Drug-induced hepatitis
	Pre-eclampsia/eclampsia†
	HELLP syndrome†
Third	*Intrahepatic cholestasis of pregnancy*
	Pre-eclampsia/eclampsia
	HELLP syndrome
	Acute fatty liver of pregnancy
	Hepatic rupture
	Gallstones
	Viral hepatitis
	Drug-induced hepatitis

* *Diseases unique to pregnancy in cursive,* † *uncommon in this trimester.*

in combination with spontaneous recovery of liver test abnormalities when the symptoms resolve.[5]
Regarding other disorders unique to pregnancy, *intrahepatic cholestasis of pregnancy* usually begins in the second trimester and very rarely in the first trimester. The disorders associated with *(pre-)eclampsia* and *HELLP syndrome* (haemolytic anaemia, elevated liver tests and low platelets) occur in the second half of pregnancy, usually in the third trimester toward term. *Acute fatty liver of pregnancy* and *hepatic rupture* generally occur in the third trimester as well as in the immediate postpartum period.[9]

There are certain things to bear in mind when a patient complains of nausea and vomiting in combination with liver test abnormalities. Arguments for hyperemesis gravidarum are: first pregnancy, young age, overweight, non-smoker and onset of vomiting before 15 weeks.
In patients with hyperemesis gravidarum, hospitalisation is prompted by dehydration and ketosis; physical examination only reveals excessive salivation. Laboratory test findings can be normal but often ketonuria is found. In case of liver test abnormalities, viral and drug-induced hepatitis as well as gallstones should be excluded. For this purpose, viral serologies and abdominal ultrasonography should be performed.[5] A liver biopsy is rarely needed to exclude other causes for the laboratory findings. In those cases where a liver biopsy was performed, it was either normal or showed non-specific findings such as steatosis.[6]
On the other hand, further investigation is required in patients with severe serum aminotransferase elevation (ASAT/ALAT >500 U/l), as well as in those patients with persistent liver enzyme abnormalities after the symptoms resolve.

Treatment
The treatment of hyperemesis gravidarum is symptomatic; hospitalisation for intractable vomiting, dehydration or malnutrition may be necessary.[9] Rehydration with intravenous fluids and treatment with antiemetics are often required. No comparative trials exist, but antiemetics such as promethazine, odansetron or droperidol have been reported to be useful.[22]
Further therapy in selected patients may include enteral and parenteral nutrition.[5] Corticosteroids have been reported to be effective, although the mechanism of action is not well understood.[23-25]

CONCLUSION

Hyperemesis gravidarum is a poorly understood disorder in the first trimester of pregnancy. Increased levels of aminotransferases, especially ALAT, are found in 50% of patients who are hospitalised with hyperemesis. Although the pathogenesis of liver abnormalities is unknown, the combined effects of hypovolaemia and malnutrition are believed to play a role.
Gallstone disease, viral and drug-induced hepatitis should be considered in the differential diagnosis of patients with elevated liver tests and hyperemesis. Management is symptomatic and abnormal liver tests return to normal promptly when symptoms resolve.

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Rectification

In the review 'Autoimmunity in Addison's disease' by P. Martín Martorell, B.O. Roep and J.W.A. Smit, published in The Netherlands Journal of Medicine 2002;60(7):269-5, an error was made in the first author's name. It should be P. Martín Martorell instead of P.M. Martorell. Our apologies for any inconvenience caused.

ANSWER TO PHOTO QUIZ (FROM PAGE 362)

A PATIENT WITH SKIN AND BONE LESIONS - G. VERVOORT

Because of the inflammatory lesions in both the bone (especially claviculae) and skin, the diagnosis of SAPHO syndrome (synovitis, acne, pustulosis, hyperostosis and osteitis) was made. Three subtypes of SAPHO are distinguished with the following common features: sternoclavicular hyperostosis and sterile inflammatory bone lesions, and skin eruptions. SAPHO syndrome groups together sternocostoclavicular hyperostosis, chronic recurring multifocal osteomyelitis, and pustular arthro-osteitis. Aetiology and pathogenesis of SAPHO are unknown. The syndrome often runs a protracted course, with relapses and remissions but without resulting in serious disability. Treatment is symptomatic using non-steroidal anti-inflammatory drugs.
The patient was treated with topical corticosteriods and the skin lesions disappeared in just a few weeks. Bone scintigraphy four months after admission revealed no progression of the lesions; X-ray of the left clavicula no longer showed any radiographic abnormalities.

A year later she volunteered as a blood donor in our hospital. Unexpectedly, routine screening showed positive syphilis serology: Venereal Disease Research Laboratory (VDRL) test 1:128; *T. pallidum* haemagglutination assay (TPHA) 1:40960; fluorescent treponemal antibody absorption (FTA-abs) positive. HIV serology was repeatedly negative. There was no history of drug abuse or sexual promiscuity. However, her husband was treated with antibiotics by the general practitioner because of an ulcerative lesion on the penis.

Histopathological revision showed spirochetes in both clavicula and the uterus (*figure 3* and *4*). The patient was now diagnosed as having late syphilis with skin lesions and gummas of the bone. She was treated with penicillin G 2 million units six times daily intravenously for three weeks. After treatment the VDRL titre decreased. One year after treatment, bone scintigraphy revealed no abnormalities.

Syphilis is a chronic infection caused by *Treponema pallidum*. Late syphilis is defined as the stages of syphilis that occur after early or latent syphilis, typically involving the central nervous system, cardiovascular system or the skin, viscera and bones. The hallmark of late syphilis of the skin or viscera is gummas, granulomatous lesions that can easily be mistaken for sarcoidosis and tuberculosis. Skeletal gummas most frequently involve the long bones of the legs. Presenting symptoms usually include tenderness and pain and less frequently swelling; trauma may predispose a specific side to involvement. Radiographic abnormalities include periostitis and destructing or sclerosing osteitis.

Treatment of late syphilis requires prolonged courses of penicillin G. For late syphilis without central nervous system involvement a two-week course of intramuscular penicillin G. would be adequate. In our patient lumbar puncture failed, so the patient was treated as having neurosyphilis with a three-week course of intravenous penicillin G.

The response to treatment is monitored by the VDRL titre. In our patient, the VDRL titre dropped rapidly to 1:16 and has remained so for >6 years, but never became negative. She remained asymptomatic.

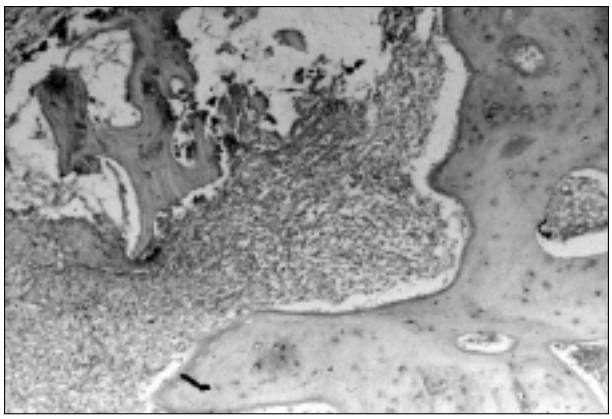

Figure 3

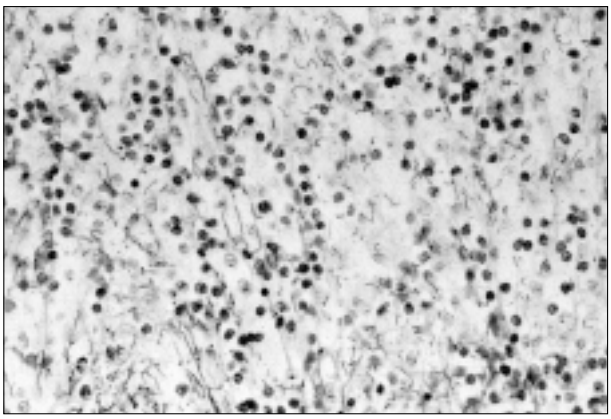

Figure 4

ABOUT THE COVER

Colour etching 'Grachten bloeien' (Canals bloom)

Els Maasson

This month's cover, entitled 'Grachten bloeien' (Canals bloom), shows a colour etching by Els Maasson.

Els Maasson (1967) has her studio in the old city centre of Delft, the Netherlands. She attended the Royal Academy of Art in The Hague from 1987 to 1992. She exhibits her work at many individual and group exhibitions in the Netherlands, for example the South Holland Graphic Art Manifestation in The Hague in 1993, 1995, 1997, 1999, and 2001, Landgoed Voorlinden in Wassenaar, Galerie Zuid Holland Provinciehuis in The Hague, Galerie Inkt in Delft, Stadsgalerie Woudrichem and Galerie Expoline in Scherpenzeel. Her exhibitions abroad include Galerie La Dame Bastet in Halle (Belgium), Exhibition of the Union Feminine Artistique et Culturelle in the European Parliament in Brussels, and Galerie Comunica in Tokyo.

For this particular etching she was inspired by the water lilies in the old canals in Delft. Before starting to design the picture, she wrote a poem about the flowering canals. The poem is cited on the etching. From the upper right corner, clockwise it reads: Canals bloom...they let me sense... that my heart is too small...for beauty and for pain.

To achieve an etching like this one, she cuts the relevant forms from zinc plate. By piling plates she obtains a profound relief in the paper. She loves the colour gold, as it is used in ancient religious art, icons and Jugendstil. The present etching, which was printed with two plates and two colours, has been hand-coloured in gold.

Major themes in her work are animals, nature and text.

A limited edition of original prints (size 27 x 30 cm) of this month's cover is available at a price of € 225. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

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The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the Editor are welcomed.

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Language
The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process number the pages; also we would appreciate seeing the line numbers in the margin (Word: page set-up - margins - layout - line numbers). Divide the manuscript into the following sections: Title page, Abstract, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

A *Covering letter* should accompany the manuscript, identifying the person (with the address, telephone and telex numbers, and e-mail address) responsible for negotiations concerning the manuscript: the letter should make it clear that the final manuscript has been seen and approved by all authors. Conflicts of interest, any commercial affiliations, consultations, stock or equity interests should be specified. In the letter 1-3 sentences should be dedicated to what this study adds. All authors should sign the letter.

The *Title page* should include authors' names, degrees, academic addresses, address for correspondence including telephone, fax and e-mail, and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for proprietary names of substances and materials. At first mention of a chemical substance, use the correct chemical designation as well as the generic name.

The *Abstract,* not exceeding 200 words, should be written in a structured manner and with particular care, since this will be the only part of the article studied by some readers. In original articles, the abstract should consist of four paragraphs, labelled Background, Methods, Results, and Conclusions. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

The *Introduction* should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

Acknowledgement: All finding sources should be credited here. Also a statement of conflicts of interest should be put here.

References should be numbered consecutively (in square brackets) as they appear in the text. Type the reference list with double spacing on a separate sheet. References should accord with the system used in Uniform requirements for manuscripts submitted to biomedical journals (N Engl J Med 1991;324:424-8).

Examples:

[1.] Smilde TJ, Wissen S van, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.

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Please note that the first six authors should be listed; when seven or more, list only the first three and add *et al*. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised.

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Brief reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Articles published in this section should be no longer than 1000 words, and be supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references.

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After external and editorial review of the manuscript, the authors will be informed about acceptance, rejections or revision. Unless stated otherwise in our letter, we require revision within three months.

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