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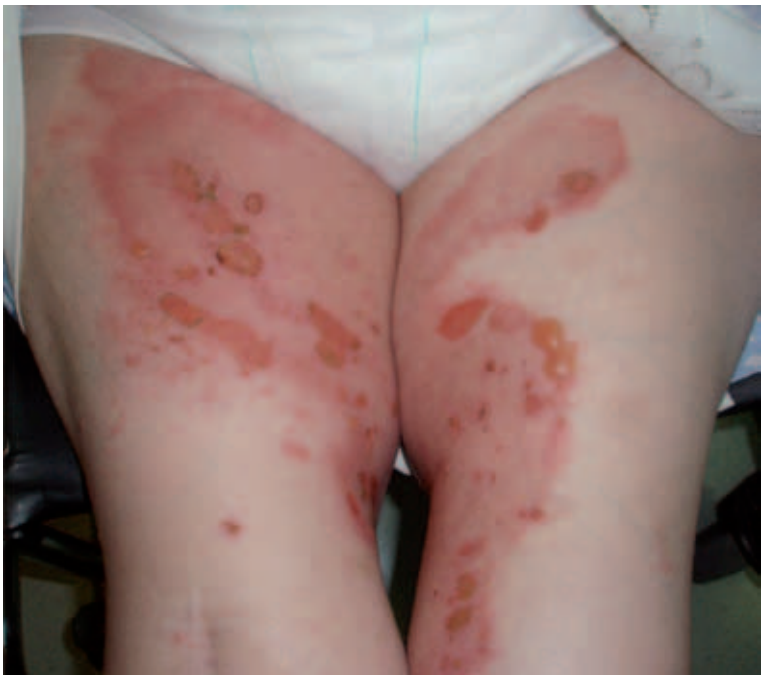

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REVIEW

Symptom experience, nonadherence and quality of life in adult liver transplant recipients

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ABSTRACT

Survival outcomes after liver transplantation in adult patients have gradually improved with a five-year survival of about 70% and a ten-year survival of about 60%. The present review focuses on relevant patient-reported outcomes such as self-perceived side effects of immunosuppressive drugs, medication nonadherence and long-term health-related quality of life after liver transplantation. These entities are interrelated but have often been studied separately.

Self-perceived symptom experience in liver transplant recipients has not been studied extensively. Symptoms that cause distress differ between men and women, e.g. symptoms related to cosmetic side effects of drugs.

Medication nonadherence seems to be infrequent, but if present may have serious consequences. Important risk factors were found to be the costs of drugs, age <40 years, psychiatric disorders, side effects of drugs, beliefs that drugs were harmful, and large influence of the liver transplant on the patient's life.

Health-related quality of life is satisfactory, but below the level of the general population. Results, however, must be interpreted with caution as quality-of-life improvements may have been overstated due to variables such as selection bias (e.g. exclusion of severely ill and deceased patients), too many short-term studies, and suboptimal methodology. Presently we lack data on the influence of recurrence of disease, *'de novo'* diseases and gender differences on health-related quality of life in liver transplanted patients.

KEYWORDS

Adherence, compliance, concordance, quality of life, side effects, solid organ transplantation

INTRODUCTION

For several decades liver transplantation (LT) has been the accepted treatment for a gradually expanding variety of indications.[1,2] Life expectancy improved over time, due to better surgical techniques and preoperative and postoperative care,[1,3] with a five-year survival of about 70%, and a ten-year survival of about 60%.[2,4] An update on liver transplantation by Verdonk *et al.* was recently published in this journal.[1]

Formerly, the results of solid organ transplantation were mostly evaluated from the perspective of clinicians in terms of objective clinical outcomes, such as postoperative complications, renal impairment, hypertension, malignancies, osteoporosis, diabetes, and patient and graft survival. Nowadays, it is increasingly recognised that an evaluation of outcomes should incorporate the subjective experiences of the patients.[5]

The Food and Drug Administration in the USA strongly recommends that patient-reported outcomes (PRO) should be incorporated to evaluate the impact of treatment on patients' daily life and well-being. A patient-reported outcome can be defined as *'any outcome based on data provided by patients or by patient proxy as opposed to data provided from other sources'*.[5] Patient-reported outcomes may help to improve the quality of health care, and need to be taken into account when developing new drugs. PROs that are of importance to liver transplant patients are symptom experience, medication adherence and health-related quality of life.

The effectiveness of the treatment after solid organ transplantation depends both on the skills of the healthcare team and on the life-long, active cooperation of the patient.[6,7] Side effects as a consequence of taking of immunosuppressive medications may occur. Assessment of side effects as perceived by the patients provides the

transplant field with valuable information regarding the benefit and burden of immunosuppressive regimens.[8] A relationship has been found between symptom experience and nonadherence and health-related quality of life in heart, renal and lung transplant recipients.[9-23] The current review explores whether evidence supports these relationships in liver transplant patients.

Adherence (also called compliance or concordance) is defined as: 'the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes – corresponds with the *agreed* recommendations from a healthcare provider'.[24]

Nonadherence with the immunosuppressive regimen in solid organ transplant recipients is recognised as a major long-term problem with a negative impact on clinical outcome [25-30] and worse economic outcome.[31-34] The majority of research on adherence in transplantation, however, has been done in renal and heart transplantation. No reviews have been published on medication adherence in liver transplant patients specifically.

Health-related quality of life (HRQOL) is also recognised as an important patient-reported outcome. Solid organ transplantation remains a chronic condition which can have a high impact on the daily life and well-being of the patient.[35,36] For liver transplant patients specifically, health-related quality of life may well be influenced by long-term side effects of drugs, and by the status of the liver as *'de novo'* disease or recurrent disease may develop.[37-39]

METHODS

The present review focuses on experience of symptoms related to side effects of immunosuppressive drugs, medication nonadherence and long-term health-related quality of life in adult liver transplant patients. The databases PubMed, PsychInfo, Cinahl, preCinahl, and the Cochrane Library, from 1966 to October 2008, were searched with the help of a medical librarian. A combination of following search terms was used: liver transplantation, liver transplant*, compliance, non(-) compliance, non(-)adherence, adherence, concordance, symptom experience, symptom frequency, symptom distress, subjective side effects, subjective adverse effects, quality of life, general health status, long-term. Further selection criteria were English language publications and focusing on adult patients. Excluded were articles that focused on living donation. The articles found on all of the topics were screened and in addition to this search strategy the references of the publications were searched for additional publications. In total we found 41 publications on the three subjects: six studies on symptom experience, 14 studies with the main focus on medication nonadherence and 21 studies on long-term HRQOL. From the 21 studies on long-term HRQOL five studies that were published after the latest meta-analysis were selected for this review.

SYMPTOM EXPERIENCE

Symptom experience is a critical post-transplant outcome and it provides the transplant field with valuable information regarding the benefit and burden of immunosuppressive regimens as perceived by the patients.[8] Symptom experience refers to the patient's subjective experience of side effects related to immunosuppressive drugs and it can be divided in perceived symptom occurrence (cognitive part of symptom experience) and perceived symptom distress (emotional part of symptom experience).[8-12] Symptom occurrence is described along the dimensions of frequency, duration and severity of perceived side effects of immunosuppressive medications. Symptom distress, expressing the emotional burden related to side effects, demonstrates how patients are affected in daily life by these symptoms.[5] Many clinical symptoms may not be perceived by the patients as very distressing, and also the level of distress may differ in patients.[20] For instance, the patients may worry more about skin alterations, sexual disorders, depressive symptoms and stomach complaints than about hypertension or renal impairment. The amount of perceived distress was found to be related to health-related quality of life and to nonadherence in heart, renal and lung transplant recipients.[9-23] Kidney transplant recipients with a higher level of symptom occurrence and symptom distress for instance had a higher rate of nonadherence.[19] More 'drug holidays' as a measure of nonadherence and a higher level of symptom occurrence and distress was related to a worse quality of life in lung transplant recipients.[23] It is important to find evidence about symptom experience in liver transplant patients for three reasons: (1) healthcare workers must be informed that symptom occurrence and/or a higher level of perceived distress may worsen the patient's well-being and that it may lead to nonadherence, (2) it can be used to educate the patient and his/her relatives about the side effects of immunosuppressive medications and (3) it can be used in developing new drugs and in prescribing existing immunosuppressive medications based on patients' preferences.[20,40] Only a few studies were retrieved that report on symptom experience after liver transplantation.[40-45] Foley *et al.*[41] report a low score on occurrence of symptom frequency and perceived distress in 26 liver transplant recipients. Most frequently reported symptoms were fatigue, bodily appearance, excessive hair growth and overeating. However, the sample was rather small and the response rate was only 59%. Karam *et al.*

assessed 'Measures of Disease', more specifically physical symptoms and severity of symptom distress, in 126 liver, 229 renal and 113 heart transplant recipients as part of long-term quality-of life-assessment.[44] The 'Measures of Disease' reported by the transplant patients were significantly worse than in the general population, with the worst score for renal transplant patients. The symptom distress score for psychological symptoms revealed that renal transplant recipients had a worse HRQOL than liver transplant recipients. However, limited specifications about the symptoms were provided.

We assessed symptom experience in 108 adult liver transplant patients[40] with the 29-item Modified Transplant Symptom Occurrence and Symptom Distress Scale [9,10,46] and found that increased hair growth was the most frequent symptom in male and female recipients.

The most distressing symptom in women was excessive and/or painful periods, while in men this was impotence. Male and females did not differ with respect to symptom frequency, but overall symptom distress was more serious in women than in men. It was also shown that the most frequently reported symptoms do not necessarily cause the most perceived distress. Stomach, back and muscle complaints were listed in the Top Ten of most distressing symptoms for both sexes. Dividing the sample in a cohort with a short-term (1-4 years) and a long-term (5-18 years) follow-up, clear differences over time and between genders were noted. Women in the long-term cohort reported more cosmetic side effects. A decrease in symptom frequency and symptom distress was not seen in the long-term cohort. This might be explained by the fact that comorbidity from long-term immunosuppression increases through the years and by the effect of ageing, but this needs further investigation in future studies. No relationship was found between symptom experience and prednisolone nonadherence as measured with electronic monitoring.[47]

Drawing firm conclusions from these few studies on symptom experience, however, is difficult, because the studies used different measurement instruments, and the symptoms assessed were not always described in detail. Symptoms that cause distress may differ between men and women. Furthermore, no conclusions can be drawn about the relationship with different immunosuppressive regimens, nor about the relationship with medication adherence and HRQOL.

NONADHERENCE WITH IMMUNOSUPPRESSIVE MEDICATION

Patients' adherence to immunosuppressive medications plays a key role in obtaining and maintaining a good clinical outcome. Fourteen studies on nonadherence in adult patients after liver transplantation were retrieved.[14,15,25,28,47-56] Most of the studies that were published before 2000 included only small numbers of patients.

Measurement of nonadherence

Measurement of medication nonadherence can be divided into direct and indirect methods.[57] Direct methods are: direct observation or measurement of a drug (metabolite) in blood or urine. Indirect methods are patient self-report, collateral report, pill counts, rates of prescription refills, assessment of clinical outcome, electronic medication monitors (EM), and measurement of physiological markers (i.e. heart rate of patients taking β-blockers).[57-60]

Adherence measurement methods in adult liver transplant adherence studies have been: monitoring blood levels of calcineurin inhibitors,[15,20,25,48-51] self-report,[15,51-54] collateral report,[25] retrospective chart review,[14,51,55] clinical outcome,[25,28,49,51,55,56] electronic monitoring (EM)[47] and appointment nonadherence.[51] The diagnostic accuracy of each method has been discussed extensively by several authors.[57,59-62] Recent research findings using cross validation and diagnostic research suggest that a combination of several measurement methods has higher sensitivity compared with using a single method.[57,62]

Establishing nonadherence in clinical practice

In clinical practice a simple measure to establish suspected nonadherence, e.g. a patient is not responding to therapy, is by just asking the patient at a scheduled follow-up how often he/she could not take the medication as prescribed in the last four weeks and what caused this omission.[57] Another useful method is to contact the patient's pharmacy about refilling prescriptions[57] or to ask the patient to bring the medication along to a scheduled appointment with the physician or clinical nurse specialist.

Prevalence of nonadherence

As the retrieved studies use different methodology it is not easy to derive a general nonadherence prevalence rate. Schweizer *et al.*[15] reported the first prospective adherence study (n=13) among adult liver transplant recipients. Nonadherence was suspected when unexplained decreases in cyclosporin blood levels were observed. Three of 13 liver transplant recipients were found to be nonadherent. In a retrospective study among 118 patients who had undergone liver transplantation for alcoholic liver cirrhosis, Berlakovich *et al.*[28] reported that 19 recipients (16%) were not within the target range of whole blood trough levels of the calcineurin inhibitor. This, however, in itself does not prove nonadherence. We studied prednisolone nonadherence with the use of electronic monitoring and found an overall high level of dosing adherence for prednisolone (median of 99%), except that

timing adherence, which describes 'the percentage of days that opening of the bottle was within three hours of the subject's chosen time of day to routinely take their prednisolone dose', was low in about one-third of the patients.[47] Dew *et al.*[63] analysed adherence after solid organ transplantation, and included seven liver transplant studies on medication nonadherence in her meta-analysis. Liver transplant recipients had a medication nonadherence rate of 6.7 cases per 100 patients per year (PPY) *vs* 15 cases per 100 PPY in heart transplant recipients and 36 cases per 100 PPY in renal transplant recipients.

The limited available evidence suggests that adherence for medication intake after liver transplantation seems to be good, and more favourable than in other transplant recipients. Nonadherence should of course also be evaluated in view of the possible clinical consequences of medication nonadherence.

Clinical consequences of nonadherence

Medication nonadherence must have a measurable effect on the clinical outcome for it to be clinically relevant.[64] The ultimate goal is to develop a clinically relevant definition of nonadherence indicating the level of nonadherence that is connected with increased risk for poor clinical outcome. Review of nonadherence studies in renal transplant recipients revealed that nonadherence was associated with poor clinical outcome, e.g. rejection episodes and graft loss.[29,65] Research in heart transplant populations[27] with electronic monitoring showed that minor deviations from the dosing schedule were associated with increased risks of late acute rejection, graft loss, and mortality.

In a retrospective review by Mor *et al.*[25] in 375 liver transplant patients it was found that nonadherence accounted for 34.6% of late acute rejection episodes. In a retrospective study among 118 patients who had undergone liver transplantation for alcoholic liver cirrhosis, Berlakovich *et al.*[28] reported that late acute rejection differed significantly between the adherent patients (5% with acute rejection) and the nonadherent patients (22% with acute rejection). In our study concerning prednisolone nonadherence, we looked for a relationship between nonadherence and clinical outcomes during a two-year follow-up including liver tests, acute rejection episodes, changes in dosages of immunosuppression, hospital re-admissions, and patient and graft survival.[56] Except for a somewhat higher alkaline phosphatase in patients who were less adherent, no relationship between prednisolone nonadherence and clinical outcome parameters was found.[56] It is possible, however, that the level of nonadherence in our patient population was too low to be of clinical significance. O'Carroll *et al.*[51] conducted a retrospective audit in 435 Scottish patients who were beyond one year after LT. Approximately one out of 100 patients died from poor adherence and nonadherence may have played a role in the development of chronic rejection.

These studies show on the one hand that medication nonadherence may have serious consequences for graft and patient survival. On the other hand the level of nonadherence must be substantial with abstinence of medication probably for many weeks.

Economic consequences of nonadherence

Nonadherence with the immunosuppressive regimen has found to be associated with poor economic outcome, but has not been studied in adult liver transplant patients thus far.[31-33] Economic consequences, using data from the renal transplant literature, include higher healthcare costs among nonadherent patients in comparison with adherent patients in terms of hospital care, retransplantation, ambulatory care, nursing homes, productivity loss and 'out-of-pocket' expenses of patients and relatives.[31-33] On the other hand, when lifetime costs of adherent *vs* nonadherent renal transplant patients were compared, Cleemput *et al.*[33] found lower costs in nonadherent patients over lifetime, due to a shorter life span in nonadherent patients (i.e. a median survival of 12 *vs* 16 years). Yet quality adjusted life years (QALYs) were higher in adherent patients.[33]

Risk factors for nonadherence

Knowing that nonadherence can have a negative impact on outcomes after transplantation, clinicians should be aware of possible risk factors for nonadherence so that adequate interventions can be undertaken. Reported risk factors in liver transplant patients are higher costs of medications,[15] age <40 years,[47] psychiatric disorders,[14,15] side effects of medications,[14,15,51] beliefs that medications were harmful,[51] and experiencing a large effect of the transplant on the patients' daily life.[51] More studies are needed to judge the influence of higher level factors related to the healthcare centre and healthcare providers. For example, in a multicentre study of renal transplant patients using electronic monitoring, associations were found between the transplant centre and adherence.[66] Another study showed that nonadherence rates were higher in the United States compared with Europe, and highlight that healthcare system factors, such as insurance coverage, are possibly an influencing factor of higher nonadherence rates in the USA.[7,63,67-69]

Interventions

No intervention studies to enhance medication adherence in adult liver transplant patients have been published, to our knowledge. As nonadherence is a complex behaviour, usually not predictable and individual to every patient, it is difficult to develop effective strategies to enhance adherence.[7,70] Several reviews about interventions in other chronic illness patient populations have been published.[71-74]

One conclusion they have in common is that no 'magic bullet' was found and that very few effective interventions significantly affected clinical outcomes in the long term. Patient education is important and may include discharge teaching and introducing a self-medication programme.[57,70] Once-daily medication dosing and simplifying dosing so that it fits into the lifestyle of the patient may improve adherence.[34,57,71,74] Of further importance is investment in a good relationship between the healthcare professionals and the patient, with more frequent interactions with attention to adherence.[57,71,74] Additionally, this includes means of easy communication by phone or e-mail and broadening opening hours of the outpatient clinic to shorten waiting times.[57] Interventions need to be tailored to the individual patient. A combination of educational, behavioural and affective interventions seems to be most effective, but they are complex and labour-intensive.[71,74]

LONG-TERM HEALTH-RELATED QUALITY OF LIFE

The World Health Organisation defined Health as *'a state of complete physical, mental and social well-being and not merely the absence of disease'*.[75] General HRQOL improves significantly from pre- to post-LT but most findings refer to a relatively short duration of follow-up.[37-39]

Long-term results indicate that HRQOL, after the initial improvement from pre- to post-LT, remains rather stable through the years and is not always negatively influenced by comorbidity and clinical side effects of medications.[37-39] Results show, however, that LT patients have significant deficiencies in most QOL areas when they are compared with healthy controls.[40] The impact of aetiology of liver diseases on HRQOL, such as alcoholic liver disease, HCV infection, acute liver failure, remains inconclusive with contradicting findings of HRQOL gains. The assumption was made by Tome *et al.*[39] that recurrence of disease, e.g. hepatitis C, and development of *'de novo'* diseases, e.g. diabetes mellitus, after LT might be of higher influence on a worsened HRQOL than the original aetiology of the disease.[39] In view of sexual functioning and employment more recent studies show that females tend to have a lower HRQOL compared with males. Sexual health was found to be unchanged after LT compared with the period before LT when data of longitudinal studies were combined. Employment rates varied considerably after transplantation.[39] Unemployment was predicted by age, longer duration of disability before LT, unskilled workers, lower income, and unemployment status.[39] Five new publications, one qualitative and four quantitative studies, assessing long-term HRQOL have been published since the most recent meta-analysis by Tome *et al.*[36,39,76-79] Median follow-up ranged from 4.4 years to more than 15 years after LT. The main findings of these studies are that varying levels of physical and psychosocial disability may persist for many years after LT, although patients describe having productive and meaningful lives with a positive outlook despite remaining uncertainty about the future.[36] Physical impairment led to significantly lower employment[76] but did not have an impact on satisfaction and self-care.[77] Long-term HRQOL did not seem to be related to the level of clinically observed comorbidity[77] or to the use of calcineurin inhibiting drugs.[78] Job rehabilitation in the first year after LT had a positive influence on long-term HRQOL.[78] Age above 60 years, female gender and post-transplant complications as recurrent disease and osteoporosis were associated with poorer physical functioning.[79]

These new studies also report a lower HRQOL than in the general population. In three of the four quantitative studies the SF-36[80] was used as a generic instrument to measure HRQOL[76,78,79] and one study also used a disease specific questionnaire.[79]

In summary, so far recent HRQOL studies add evidence in that QOL remains satisfactory in the long term after LT, but lower compared with that of the general population. Although some of the recent studies on long-term HRQOL contribute to earlier assumptions that overall long-term HRQOL does not seem to be affected by the level of comorbidity and that female patients experience a worse HRQOL compared with men, more studies on long-term HRQOL after LT are needed to gain more understanding.

CONCLUSIONS

The present review focused on three important patient-reported outcomes in adult liver transplant patients, i.e. perceived subjective side effects of immunosuppressive drugs (i.e. symptom experience), medication nonadherence and long-term health-related quality of life. Clearly, these entities are interrelated but have often been studied separately.

We found that self-reported symptom experience in liver transplant recipients has not been studied extensively. Differences between different immunosuppressive regimens have not been explored so far in this respect. Also the relationship between symptom experience and medication adherence and HRQOL needs further study. Special attention should be paid to the level of perceived symptom distress and its impact on the daily life of the patient as a high level of distress might lead to nonadherence and worse HRQOL, as experienced in kidney, heart and lung transplant patients.

Medication nonadherence as measured to date seems to be infrequent, but if present may have serious consequences. Important risk factors included age <40 years, and side effects of medications.[15,47,51] More studies are needed to gain more insight into clinically relevant nonadherence and to judge the influence of the healthcare centre and healthcare providers. More studies into the prevalence of medication nonadherence and corresponding risk factors are needed before appropriate intervention studies can be developed. Evidence from studies in chronically ill patients and other organ transplant patients show us that there is not one single effective intervention available and that a combination of multidimensional and multi-level interventions may be effective for long-term results to enhance adherence.[71,74] This is an important area for future research, yet the clinical consequences of nonadherence in liver transplant patients should also determine if this is a priority.

Results show that long-term HRQOL is satisfactory, but it is below the level of the general population. These results must be interpreted with caution as HRQOL benefits after liver transplantation may have been overstated due to variables such as selection bias (e.g. exclusion of severely ill and deceased patients), too many short-term studies, and suboptimal methodology.[39] In addition, HRQOL will also be affected by cultural, economic and social factors which are difficult to incorporate in research.[38,39,81] In studying HRQOL from the perspective of patient-reported outcomes it is recommended to use both a disease-specific questionnaire and a generic questionnaire. The former detects disease-specific changes and the latter allows comparison of results with other groups of patients with chronic diseases. Presently we lack data on the influence of recurrence of disease and of *'de novo'* diseases in adult liver transplant patients. Also gender differences should be given more attention.

RECOMMENDATIONS

Two main recommendations can be made. Firstly it is important that assessment of adherence is an integrated part of the treatment plan of the patient, and poor adherence should always be considered when a patient is not responding to therapy.[57] Secondly, physicians should be aware of the possible influence of subjective side effects of immunosuppressive drugs on medication adherence and of the impact of corresponding distress on the daily life of the patient. In the future, medication regimens should not only be based on clinical data alone, but, when possible, also on subjective patient-reported outcomes.

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REVIEW

Hypomagnesaemia due to use of proton pump inhibitors – a review

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ABSTRACT

Magnesium homeostasis is essential for many intracellular processes and depends on the balance of intestinal absorption and renal excretion. Hypomagnesaemia may arise from various disorders.

We review the literature on hypomagnesaemia due to the use of proton pump inhibitors, as illustrated by a case of a 76-year-old woman with muscle cramps and lethargy caused by hypomagnesaemia and hypocalcaemia with a low parathyroid hormone level while using esomeprazole, a proton pump inhibitor (PPI). After oral magnesium repletion both abnormalities resolved. Fractional magnesium excretion was low, excluding excessive renal loss. A causal relation with PPI use was supported by the recurrence of hypomagnesaemia after rechallenge. In the past decade our understanding of transcellular magnesium transport was enhanced by the discovery of several gene mutations i.e. transient receptor potential melastin (TRPM) 6 and 7. In this light we discuss the possible aetiology of proton pump inhibitor related hypomagnesaemia.

KEYWORDS

Hypocalcaemia, hypomagnesaemia, proton pump inhibitors, TRPM6, TRPM7

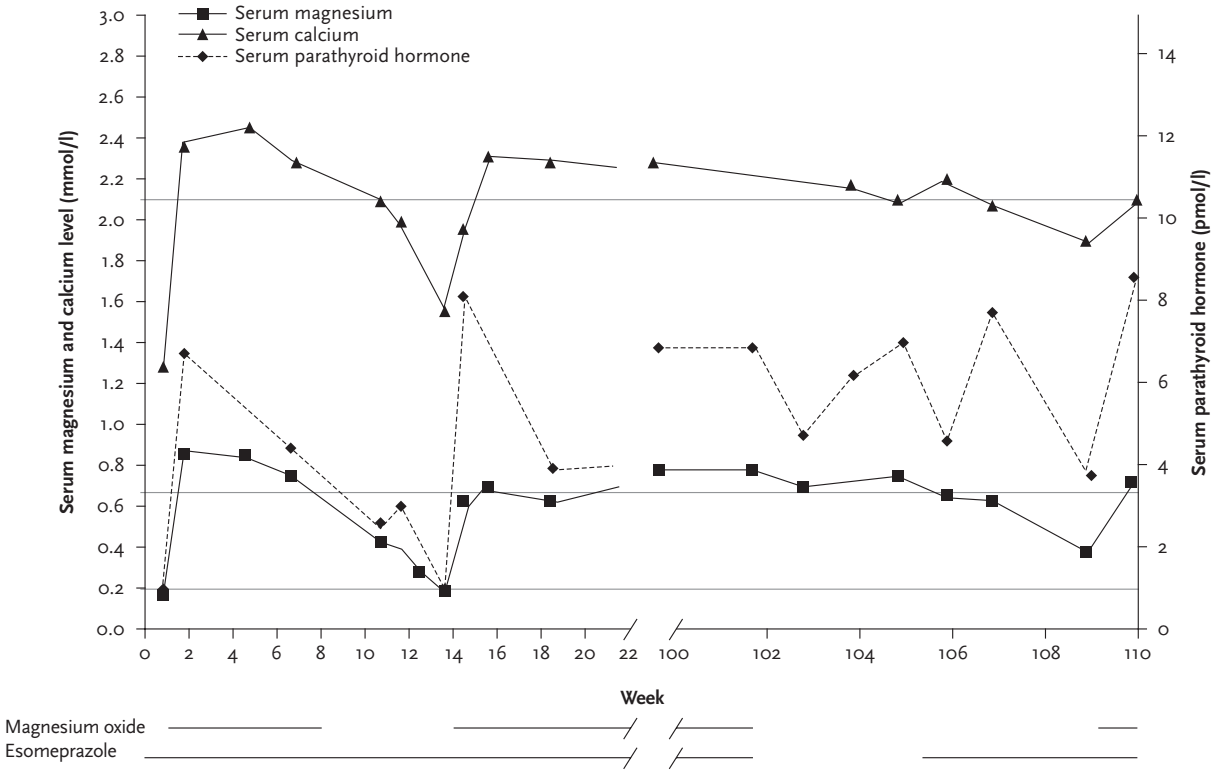
INTRODUCTION

Magnesium is implicated in many biochemical and physiological processes in our body as an essential cation. Although homeostasis of magnesium is tightly regulated by a dynamic interplay of intestinal absorption, exchange with bone and renal excretion, hypermagnesaemia and hypomagnesaemia occur. Hypomagnesaemia may arise from various disorders and can cause tetany, convulsions, cardiac arrhythmias, hypoparathyroidism and hypocalcaemia. Recently three case reports described six adults with hypomagnesaemia and hypocalcaemia which resolved after withdrawal of the proton pump inhibitor.[1-3] In this case we present another patient with severe hypomagnesaemia due to extra renal magnesium loss associated with the use of esomeprazole.

CASE REPORT

A 76-year-old woman presented at our emergency department with lethargy and muscle cramps in the abdomen and extremities. Her medical history included an appendectomy, diverticulosis, gastro-oesophageal reflux symptoms, sliding diaphragmatic hernia, benign paroxysmal positional vertigo and ischaemic heart disease. There was no history of other gastrointestinal operations, chronic alcoholism or use of laxatives. She did not complain of nausea, vomiting or diarrhoea. For one year she had been taking esomeprazole (40 mg/day) because of the indication for aspirin and the history of reflux symptoms. Her other medications were aspirin, diltiazem, oxazepam, psyllium fibres and simvastatin. Physical examination showed no abnormalities, Trousseau's and Chovstek's signs were negative. On admission the laboratory findings showed hypocalcaemia (1.26 mmol/l), hypomagnesaemia (0.18 mmol/l), hypokalaemia (3.2 mmol/l) and a low parathyroid hormone (0.9 pmol/l). Her serum vitamin D3 level was normal. There was neither alkalosis nor acidosis. We started intravenous calcium and magnesium suppletion. In three days the calcium, potassium, magnesium and parathyroid hormone (PTH) level normalised and her symptoms slowly resolved (*figure 1*). We interpreted these results as severe hypomagnesaemia with secondary hypoparathyroidism. After 11 days she was discharged

Figure 1. *Course of laboratory parameters over time*



with oral magnesium supplements (magnesium oxide 500 mg three times daily). Two months later the magnesium was discontinued. Within four weeks a dramatic drop in the serum magnesium and calcium followed. The fractional magnesium excretion was 0.69% (*table 1*). This suggests a total body magnesium deficiency due to extra renal magnesium wasting. About two years later we challenged the notion that proton pump inhibitors can cause hypomagnesaemia by impairing the intestinal magnesium uptake. We discontinued the magnesium supplements and the esomeprazole. The serum magnesium and calcium level did not change in four weeks. Because of increasing symptoms of gastro-oesophageal reflux we let her resume the esomeprazole. Within four weeks the serum magnesium level dropped to 0.4 mmol/l. The pattern we observed in our patient fits nicely with the theory of proton pump inhibitor induced intestinal magnesium loss that was suggested in three recent publications on similar case histories.[1-3]

DISCUSSION

We describe an adult patient presenting with severe hypomagnesaemia and hypocalcaemia while using a proton pump inhibitor. Discontinuation of her treatment with magnesium supplements resulted in hypomagnesaemia and hypocalcaemia. We also documented that during withdrawal of magnesium supplements and esomeprazole the electrolyte balance did not change. There are several disorders which may cause hypomagnesaemia. In recent years several genes were shown to be involved in renal magnesium and to some extent intestinal magnesium transport. FXYD2 gene mutation causes autosomal dominant renal wasting with hypocalciuria due to mutations in the γ subunit of Na,K-ATPase at the basolateral membrane of the distal convoluted tubule (DCT). SLC12A3 gene mutation causes Gitelman's syndrome due to a problem in the Na,K-2Cl$^-$ co-transporter. Mutations in genes encoding claudin 16 and claudin 19, structural proteins found as part of the tight junction between cells of the thick ascending limb of Henle's loop, cause familial hypomagnesaemia syndrome with hypercalciuria and nephrocalcinosis. Groenestege *et al.* showed that EGF plays an important role in renal magnesium regulation.[4] Pro-EGF is a membrane protein expressed at high levels in the luminal and low levels in the basolateral membranes of the DCT. P1070L mutation in the EGF gene prevents EGF secretion, especially in the basolateral space. This reduces TRPM6 activity, the key

channel that allows magnesium reabsorption. Therefore EGF antagonists i.e. gefitinib, cetuimab, erlotinib and panitumumab induce hypomagnesaemia.

In this case there was no SLC12A3 gene mutation. Renal conservation of magnesium and calcium during esomeprazole use was normal (*table 1*), which was also described earlier by Epstein *et al.* and Shabajee *et al.*[2,3] This suggests that the problem did not arise in the kidney.

Gastrointestinal causes are diarrhoea, short bowel syndrome, fistula, a magnesium free diet and very rarely a malabsorption syndrome. The average daily diet contains approximately 360 mg (=15 mmol) magnesium.[5] About 24 to 75% of this ingested magnesium is absorbed, depending on dietary contents and body stores. In a normal secreting stomach the high H+ concentration is competing with the metal ions for the ligand binding sites in food and so liberating the metals.[6] Gastric acid suppression therapy with proton pump inhibitors causes low gastric hydrochloric acid secretion. Theoretically we can assume that the absorption of magnesium falls in hypochlorhydric subjects but evidence for magnesium malabsorption in proton pump inhibitor users is lacking. Limited studies show conflicting conclusions.[6] The intestinal magnesium absorption occurs in two ways: in a passive paracellular manner and in an active saturable transcellular manner. About 90% of the absorption happens passively and linear with the dietary magnesium content.[7] Free magnesium follows its concentration gradient and flows down the paracellular pathway. The fact that treatment with oral magnesium supplements is effective suggests a normal passive paracellular pathway and a problem in the active transcellular route. The active transcellular pathway has become clearer since the identification of TRPM6 and TRPM7. These channels conduct divalent cations (magnesium and calcium) into the cell following the transmembrane electrochemical gradient.[7] TRPM6 is expressed along the entire gastrointestinal tract, in the kidney (predominantly in the DCT), in testis and lung tissue, TRPM7 is omnipresent in tissues. A mutation in TRPM6 was found responsible for primary hypomagnesaemia with secondary hypocalcaemia (HSH). This rare autosomal recessive disorder was first described by Paunier and colleagues in 1968.[8] Patients present in early infancy with diarrhoea, tetany and/or convulsions. Premature stop mutations, exon deletions, frame shifts and inserted splice sites are the identified mutations and lead to loss of function of the TRPM6 channel. This disease, together with the expression pattern of TRPM6, showed us that TRPM6 is responsible for the absorption of magnesium renally and in the intestines.[9] There are several hormonal and other factors that have an influence on TRPM6/7 expressions and their magnesium transport. Li *et al.* showed that the TRPM6/7 channel activity is potentiated by external protons.[10] In another *in vitro* study they demonstrated that glutamates in the pore-forming region of TRPM6/7 are responsible for the divalent selectivity and pH sensitivity. Mutations in these negatively charged amino acids produced dramatic changes in the channel function with a decreased calcium and magnesium permeation. Proton pump inhibitor users lack intestinal protons, i.e. they have a more alkalic intraluminal pH. To our knowledge clinical manifestations have not been described in heterozygotic mutations in TRPM6/7. Could it be that variants in TRPM6/7 may predispose to the side effect of proton pump inhibitors as described in this article? The effect of proton pump

Table 1. *Electrolyte balance and other biochemical data at admittance, during and after magnesium supplements and esomeprazole*

	Normal value	Day 1	3 months*	6 months	2 years and 1 month**	2 years and 2 months***
Serum magnesium	0.65-1.05 mmol/l	0.18	**0.39**	0.67	0.74	**0.4**
Serum calcium	2.10-2.60 mmol/l	1.26	1.99	2.18	2.09	1.85
Serum potassium	3.5-5.0 mmol/l	3.3	4.1	4.6	4.1	3.7
Serum albumin	35-55 g/l	40.9	44.3	n.d.	n.d.	n.d.
Serum creatinine	50-100 umol/l	91	108	n.d.	112	99
Serum vitamin D3	30-130 nmol/l	39	n.d.	58	n.d.	n.d.
Serum parathyroid hormone	1.0-7.5 pmol/l	0.9	3	3.9	7.0	4.6
Urinary magnesium	3.0-5.0 mmol/24 h	n.d.	0.11	n.d.	n.d.	n.d.
Urinary calcium	1.25-10 mmol/24 h	n.d.	0.08	n.d.	n.d.	n.d.
Urinary creatinine	10-12 mmol/24 h	n.d.	6.4	n.d.	n.d.	n.d.
Urinary volume	ml/24 h	n.d.	1600	n.d.	n.d.	n.d.

n.d. = not determined. *Three weeks after stopping magnesium supplements with continuing esomeprazole. **Plasma magnesium still remains normal four weeks after stopping magnesium supplements and esomeprazole. ***Plasma magnesium drops dramatically four weeks after resuming esomeprazole without magnesium supplements.

inhibitors on TRPM6/7 has not yet been determined, so this field still remains an active area of research.

While awaiting additional studies, we suggest that magnesium loss in proton-pump inhibitor users should be considered in the differential diagnosis of hypomagnesaemia of any age. This newly recognised side effect may not even be extremely rare once prescribers become aware of its existence.

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ORIGINAL ARTICLE

Amoxicillin/clavulanate (Augmentin®) resistant *Escherichia coli* in bacterial peritonitis after abdominal surgery – clinical outcome in ICU patients

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ABSTRACT

Bacterial resistance to antimicrobial agents is of great concern to clinicians. Patient outcome after infection is mainly dependent on the sensitivity of the bacterium to the agent used.

We retrospectively studied 89 postoperative intensive care unit (ICU) patients with proven *Escherichia coli* peritonitis and investigated the clinical consequences of the *E. coli* resistance to amoxicillin/clavulanate.

Significantly increased mortality, days of ventilation and ICU stay were noted in the co-amoxicillin/clavulanate resistant group. Furthermore, our results demonstrate that the sensitivity of *E. coli* to amoxicillin/clavulanate in the postoperative ICU setting has decreased in recent years. We can conclude that the current antibiotic regimen for the empirical treatment of ICU patients with peritonitis, as used in our hospital, needs to be changed. A switch, for instance, to ceftriaxone (Rocephin®) in combination with metronidazole and gentamicin, instead of the present regimen of amoxicillin/clavulanate in combination with gentamicin, seems preferable.

KEYWORDS

Abdominal surgery, amoxicillin/clavulanate, augmentin, clinical outcome, *Escherichia coli,* peritonitis, resistance

INTRODUCTION

In surgical practice, significant morbidity and mortality is caused by infection from resistant pathogens.[1,2] Hospitals are considered to be very important for the containment of antimicrobial resistance.[3,4] Resistant pathogens may cause infections that are associated with higher morbidity and mortality rates and increase medical costs. The prevalence of resistance to nearly all important micro-organism/antibiotic combinations is generally higher among isolates from patients hospitalised in intensive care units (ICU). *Enterobacteriaceae* have emerged as one of the major causes of infections and account for approximately 30% of all bloodstream infections. Antibiotic resistance surveillance programmes have demonstrated an increase in resistance among these gram-negative pathogens.[5,6]

MATERIAL AND METHODS

We retrospectively studied postoperative ICU patients and looked at the clinical consequences of *Escherichia coli* resistance to amoxicillin/clavulanate. The outcomes of ICU patients with severe bacterial peritonitis, empirically treated with a combination of gentamicin and amoxicillin/clavulanate, were collected. The patient charts of 89 consecutive postoperative ICU patients with peritonitis from whom *E. coli* was cultured between 2000 and 2005 were studied. The outcomes of ICU patients with severe bacterial peritonitis, empirically treated with a combination of gentamicin 5 mg/kg once daily and amoxicillin/clavulanate 1.2 g 4 times/day for five days, were collected. In the case of renal impairment dosage schedules were adjusted. The patients were divided into two groups: an *E. coli* amoxicillin/clavulanate resistant and a sensitive population according to the microbiologic cultures. The

number of days of admission in the ICU department, the number of ventilation days, the use of inotropic agents and the mortality rate of the patients were scored, together with APACHE scores. In order to determine the susceptibility of the aerobic gram-negative bacilli to the antimicrobial agent, the VITEK 2 Gram Negative Identification and Susceptibility Cards were used.[5,6] The breakpoint for sensitivity of *E. coli* was determined at a minimum inhibiting concentration (MIC) of ≤8 mg/ml in accordance with published standards.[7-11] A MIC value of 16 mg/l was regarded as being of intermediate sensitivity and a value of ≥32 mg/l as being resistant. Rates of resistance in the hospital and the ICU were compared over the studied years.

For statistical analyses, a χ^2 test with a Yates correction was performed.

RESULTS

Table 1 shows the characteristics of the two populations studied. There are significant differences (χ^2 Yates correction; p<0.0.5) between the two population groups with regard to stay in the ICU, mortality and ventilation days. Male patients showed a significantly higher prevalence of *E. coli* resistance to the amoxicillin/clavulanate combination compared with female patients. The scored use of inotropic agents did not show a significant difference between the sensitive and the resistant group.

The APACHE scores of the two patient groups were comparable. This means that the difference in mortality between sensitive and resistant patients cannot be explained by their initial overall disease severity as estimated by the APACHE score.

The percentages of amoxicillin/clavulanate sensitive *E. coli* strains isolated from patients in different settings are presented in *table 2*. The difference in *E. coli* resistance between 2000 and 2005 was not significant in *E. coli* strains cultured elsewhere in the hospital for either amoxicillin/clavulanate or ceftriaxone. In contrast, there was a significant difference (χ^2 with Yates correction p<0.05) between amoxicillin/clavulanate sensitive *E. coli* strains on the ICU between 2000 and 2005. Moreover, in 2005, this was also true for *E. coli* strains isolated in the hospital and the ICU, indicated with a cross in *table 2*.

In the years 2000 to 2005, the *E. coli* strains were for 96% sensitive to the other administered antibiotic, gentamicin, in both treatment schedules. Resistance to both antibiotics occurred in none of the treated patients. Combined resistance to both antimicrobial agents of all *E. coli* strains was altogether less than 1%.

Table 1. *Study population and differences in patient outcome on the ICU with severe bacterial peritonitis, divided in amoxicillin/clavulanate resistant and sensitive Escherichia coli groups*[*]

	Resistant	Sensitive
Male	20 (71%)	27 (44%)
Female	8 (29%)	34 (56%)
Total number of patients	28	61
Days in the ICU[*]	14	8.3
Mortality[*]	18 (64%)	18 (30%)
Ventilation days[*]	9.9	4.9
APACHE scores	17.4	18.4

[*] χ^2 with Yates correction p<0.05.

DISCUSSION

For many years, methods have been sought to measure and define the absolute value of susceptibility of micro-organisms to antimicrobial agents. The value most often used is the minimal inhibitory concentration (MIC), although the minimum bactericidal concentration (MBC) has also been employed.[9] Because MBC values are more time consuming to obtain, they are currently

Table 2. *Numbers (N) and percentages (%) of amoxicillin/clavulanate (AC) and ceftriaxone (CO) sensitive Escherichia coli cultured in Atrium Medical Centre in all hospital departments and the ICU, showing significant decrease of sensitivity (*) between 2000 and 2005 of AC sensitive Escherichia coli on the ICU (horizontally) and in 2005 between the hospital and ICU E. coli strains (*†) (vertically)*

Setting	2000		2001		2002		2003		2004		2005	
	AC	CO	AC	CO	AC	CO	AC	CO	AC	CO	AC	CO
Hospital	N=674		N=798		N=604		N=608		N=613		N=689	
%	91	100	91	100	93	99	94	98	93	98	88*†	97
ICU	N=473		N=386		N=314		N=333		N=289		N=415	
%	82*	99	86	100	85	97	86	97	83	99	69*†	92

[*] χ^2 with Yates correction p<0.05.

considered to be the reference value for the susceptibility of a micro-organism to a drug. The MIC is used as an instrument to predict outcome of therapy. Words such as susceptible (S) to indicate that the chance of success following antimicrobial therapy is good and resistant (R) that failure is imminent, are increasingly being used. However, in providing interpretations of MIC values measured in the lab and only providing that interpretation, valuable information is lost to the clinician. It stands to reason that reporting values found together with their interpretation is superior. The MIC could be given with a probability of treatment being successful according to the *a priori* available information.[10] This, instead of a relatively crude interpretation of drug effectiveness being expressed as S (susceptible), I (intermediate) or R (resistant). These terms are open to different interpretations depending on the criteria used by different institutions.

It has been suggested that the level of a β-lactam antibiotic should be above the MIC of the targeted bacteria (around 50% of the dosing interval). The pharmacodynamic profile of amoxicillin is such that with the MICs of most bacterial strains (including *E. coli* higher than 8 mg/l) mean this target cannot be reached.[9,10] Thus most intermediately sensitive *E. coli* strains with a 'Vitek MIC' of 16 mg/l can better be considered resistant.

Boyed *et al*. have previously reported the higher prevalence of *E. coli* resistance to amoxicillin/clavulanate in men.[10] There has not been a good explanation for this phenomenon other than the fact that urinary tract infections in men are often complicated and treated by antibiotics for a longer period.

Bruinsma *et al*. (2002) studied antibiotic use and antibiotic resistance of *E. coli* and stated that the differences in antibiotic consumption observed might lead to changes in antibiotic resistance in the near future. They strongly recommended surveillance of antibiotic use and antibiotic resistance to control the development of antibiotic resistance as surveillance provides epidemiological data on the basis of which antibiotic guidelines can be amended.[12,13] In order to prevent resistance, it is advised to limit the use of antimicrobial agents to certain clear indications.[14]

Amoxicillin/clavulanate resistant *E. coli* is an underestimated problem in ICUs. In the protocol of many hospitals, amoxicillin/clavulanate is initiated empirically in patients with peritonitis. This combination is often used as its broad spectrum covers the anaerobe flora, including the prominently cultured *Bacteroides fragilis*. In our population, the mortality in the group of patients with amoxicillin/clavulanate resistant *E. coli* was found to be significantly higher than in the amoxicillin/clavulanate sensitive group. Days of stay in the ICU and ventilation days were also significantly longer. These outcomes may indicate that we have to alter the empirical antimicrobial therapy in postoperative peritonitis patients admitted to the ICU contrary to the SWAB (Stichting Werkgroep Antibiotica Beleid; in English: Working Party on Antibiotic Policy) guideline which lists amoxicillin/clavulanate as an option in the treatment of secondary bacterial peritonitis in the Netherlands (http:customid.duhs.duke.edu/NL/Main/Diagnosis.asp?DiagnosisID=340). When choosing ceftriaxone, the sensitivity percentages remain higher than 95% for the principal pathogen (*E. coli*) (*table 2*). Coverage for anaerobes can be secured by the addition of metronidazole.

It seems that the sensitivity percentage of *E. coli* has decreased in the last five years. This is particularly true for the ICU population in the hospital in our study. This may be explained by the increased amoxicillin/clavulanate prescription by family physicians.[14]

When resistance rates of a species approach the 10% threshold, the efficacy of that particular antimicrobial agent decreases.[14,15] This was also the case for our specific group of ICU patients.

Considering the results of our study, we can conclude that the current antibiotic regimen for the empirical treatment of ICU patients with peritonitis, as used in our hospital, should be changed: a combination of, for instance, ceftriaxone, metronidazole and gentamicin instead of amoxicillin/clavulanate in combination with gentamicin seems preferable. We believe that this change of protocol could result in reducing mortality and possibly the number of ventilation days of ICU patients. Follow-up studies are needed to evaluate the outcome of such antibiotic regimen changes.

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Netherlands
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ORIGINAL ARTICLE

Current clinical care compared with new Dutch guidelines for hepatitis C treatment

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ABSTRACT

Background: Recently, the Dutch Association of Gastroenterology and Hepatology issued new guidelines for the treatment of chronic hepatitis C virus (HCV). These guidelines reflect the current standard of care. Before these guidelines were published and implemented we (1) studied the current clinical care of HCV patients among Dutch physicians, and (2) identified areas for future refinement in the current treatment.

Methods: We conducted a non-targeted survey among Dutch medical specialists in Gastroenterology, Hepatology and Internal Medicine who actively treat HCV patients. The questionnaire contained items about facility, duration and dosing of treatment, and side effect management using clinical vignettes followed by short questions.

Results: We received 49 questionnaires from treating HCV specialists. The majority (65%) of respondents treat HCV patients during regular outpatient clinics, while 35% treat these patients in a separate setting dedicated to the care of HCV patients. The majority of physicians follow the stipulated dosage regimens of pegylated interferon (88%) and ribavirin (83%). A minority (13%) exceed the advised dosage of ribavirin. Side effects such as neutropenia are mostly managed by decreasing the interferon dosage (42%). Some 35% of physicians reduce ribavirin if haemoglobin levels drop below 5.4 mmol/l, and 41% initiate erythropoietin treatment.

Conclusion: Dutch clinical practice reflects the recently issued HCV guidelines. An important area of refinement in treatment of HCV is the management of side effects.

KEYWORDS

Current practice, guidelines, hepatitis C, survey

INTRODUCTION

Chronic hepatitis C virus (HCV) is one of the most common chronic viral infections throughout the world and is a primary cause of cirrhosis and hepatocellulair carcinoma.[1] Treatment of HCV has improved steadily during the last decade, and with the current standard of care, that is treatment with pegylated interferon and ribavirin, sustained virological response (SVR) rates of 50% among genotypes 1 and 4, and 80% among genotypes 2 and 3 have become possible.[2,3]

The selection of patients who benefit most from therapy and close monitoring of hepatitis C treatment are crucial components of hepatitis C therapy. These aspects, and the wealth of clinical data that stem from a large number of clinical trials, have led to the formulation of international guidelines.[4,5] Given the intensive treatment schedule with side effects, long treatment duration and high costs of therapy, it is important that physicians follow evidence-based guidelines in order to improve patient outcome and give the best care available.[4,5] Recently, the first Dutch national guideline 'Treatment of chronic hepatitis C virus infection' was formulated by the Dutch Association of Gastroenterologists and Hepatologists.[6] This guideline for HCV mono-infection provides recommendations on diagnostic evaluation, choice of the (initial) antiviral treatment and the follow-up during and after antiviral therapy (*table 1, figure 1*).[6]

Although clinical trials have demonstrated the benefit of combination therapy in patients with chronic HCV, how this translates into actual benefit in practice that can be attributed to knowledge of and adherence to guidelines is unclear. The primary objective of this study was to evaluate current clinical practice among Dutch physicians who actively see and treat chronic HCV patients. A secondary objective was to identify dimensions of the guideline that potentially benefit from refinement or adjustment.

Table 1. *Main recommendations for treatment and management of chronic HCV patients*[6]

	Dutch guideline[6]	% followed guideline*
Dosage		
HCV genotype 1 and 4	Peg-IFN α-2a 180 µg/week + weight-based RBV/day or	83%
	Peg-IFN α-2b 1.5 µg/kg/week + weight-based RBV/day	
HCV genotype 2 and 3	Peg-IFN α-2a 180 µg/week + 800 mg RBV/day or	88%
	Peg-IFN α-2b 1.5 µg/kg/week + weight-based RBV/day	
Duration		
HCV genotype 1 and 4	48 weeks of treatment	67%
	Exception: <600,000 viral load (IU/ml) at baseline with RVR: 24 weeks of treatment	55%
HCV genotype 2 and 3	24 weeks of treatment	70%
	Exception: with RVR: 12-16 weeks of treatment	-
Side effects		
Neutropenia	ANC <0.75 x 10^9/l: PEG-IFN dose to 75%	35%
	ANC <0.375 x 10^9/l: PEG-IFN dose to 50%	-
Anaemia	Hb <5.0 mmol/l: give erythropoietin	25%
	Hb <4.0 mmol/l: decrease dose RBV to 800 mg/day and give erythropoietin and blood transfusion	-

HCV = hepatitis C virus; Peg-IFN = peginterferon; RBV = ribavirin; RVR = rapid viral response; ANC = absolute neutrophil count; Hb = haemoglobin. *% physicians who treat according to the Dutch guideline.

METHODS

We conducted a non-targeted survey among Dutch medical specialists in Gastroenterology, Hepatology and Internal Medicine. These physicians were selected from membership directories of the Dutch Society of Hepatology and the Infectious Disease Society of the Netherlands and were sent a postal questionnaire; physicians who actively treated HCV patients were asked to return the questionnaire.

The postal questionnaire included four important issues: the first part discussed the setting of the treatment: a regular outpatient clinic or a separate setting dedicated to the care of HCV patients. The second and third part of the survey focused on peginterferon and ribavirin treatment for HCV patients: detailed questions using clinical vignettes were included about the treatment duration and dosage of the drugs, respectively. The last part focused on side effects, their consequences, and management in terms of dose reduction or treatment of possible side effects.

The questionnaire consisted of clinical vignettes followed by a mix of short multiple-choice and open-ended questions. For example: 'A male HCV genotype 1 patient starts the combination therapy (peginterferon 180 µg/week and weight-based ribavirin daily); at the start of the treatment the haemoglobin (Hb) level is 8.0 mmol/l. After eight weeks the patient's Hb has decreased to 5.4 mmol/l. What to do? 1) Continue combination therapy. 2) Stop ribavirin (whether or not temporarily). 3) Reduce ribavirin to 50%. 4) Give erythropoietin. 5) Give blood transfusion.' Data from returned questionnaires were entered into a Microsoft Access database, and frequency tables were provided by SPSS 14.0 for Windows.

RESULTS

A total of 64 questionnaires were received. Although explicitly indicated, 15 responding specialists were not actively treating HCV patients and were excluded from further analysis. Forty-nine questionnaires were suitable for analysis.

Setting

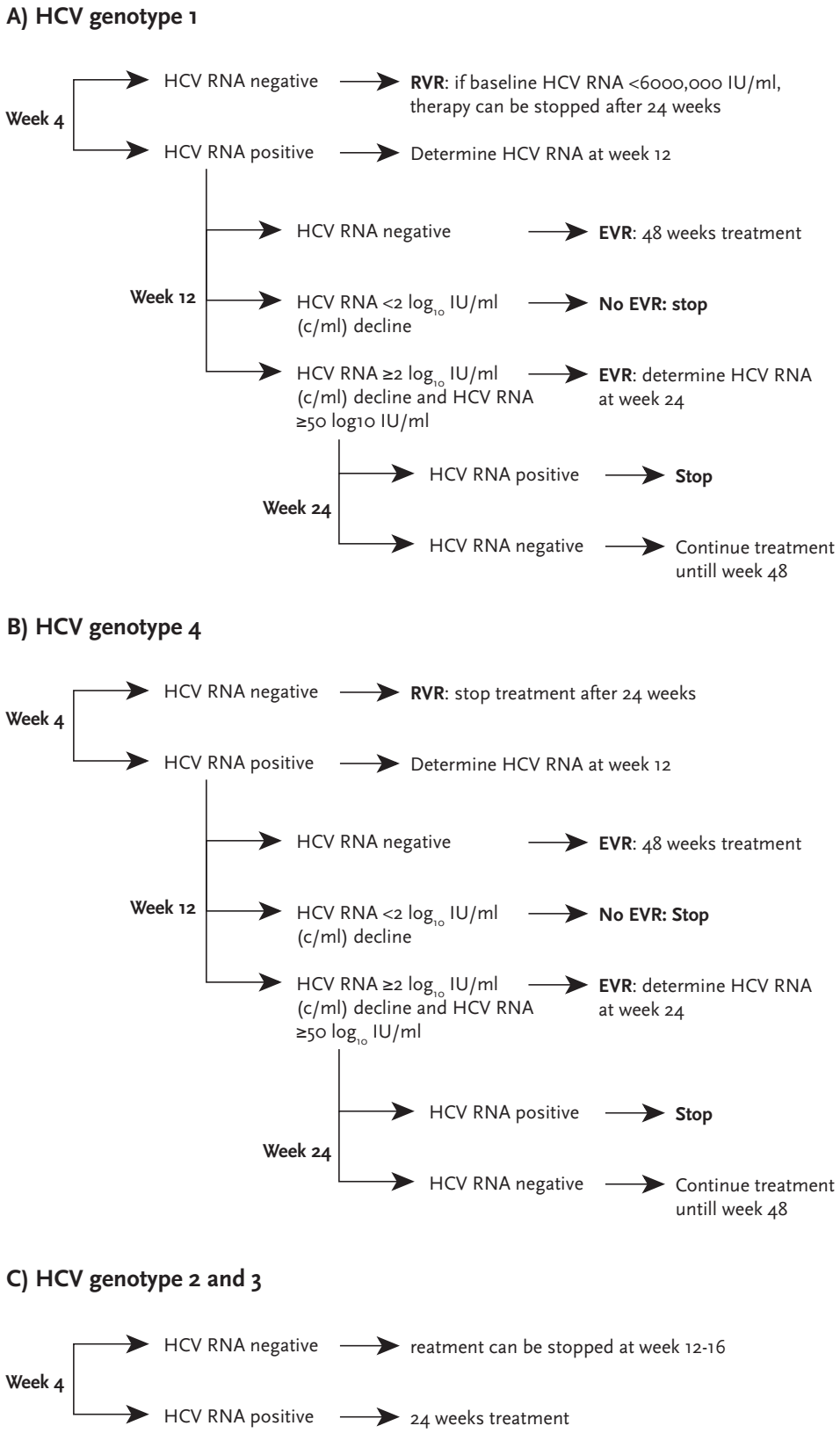
The majority (65%) of respondents treat HCV patients during regular outpatient clinics, while 35% treat patients in a separate setting dedicated to the care of HCV patients.

Viral load, genotype and length of treatment

Week 12 and 24 measurements of viral load are important to judge treatment outcome. When asked, 67% of physicians would treat HCV genotype 1 patients with a detectable HCV viral load at week 12 and an undetectable HCV viral load at 24 weeks for a total of 48 weeks (*table 1*), while 13 of the 49 respondents (27%) choose longer treatment (72 weeks). This is an area of controversy since the guideline indicates 48 weeks, but recent evidence suggests that prolonged treatment up to 72 weeks is beneficial in these cases.[7,8]

The guideline recommends treating HCV genotype 3 patients for 24 weeks. Some 70% of respondents treat HCV genotype 3 patients with significant cirrhosis for 24 weeks, while a minority (22%) will treat for 48 weeks. For HCV genotype 3 patients who had a negative viral load at week 4, the guideline recommends treating for 12 to 16 weeks. Thirty-seven percent of the respondents would treat for this period. The remainder choose a longer treatment duration than currently advised in the guideline.

Figure 1. *Flowchart for the treatment of patients with chronic hepatitis C mono-infection according to the recently issued Dutch guideline*[6]



Respondents were also divided on treatment duration of genotype 1 patients with low viraemia (<600,000 IU/ml) at the start of treatment, and a negative viral load at week 4. While following the guideline, some 55% would treat for 24 weeks, and 45% would treat for 48 weeks.

Ribavirin

The recommended dosage of ribavirin for HCV genotype 1 patients is weight-based. Indeed, the majority of physicians treat an HCV genotype 1 patient with a weight of 104 kg with ribavirin on a weight-based schedule (83%). A minority (13%) consistently exceeds this dosage.

Peginterferon

The vignette on the peginterferon dosage in an HCV genotype 1 patient weighing 150 kg shows that 88% of the physicians administer the dosage exactly as stated in the guideline. Thus, peginterferon-α-2a, 180 µg/week, or weight-based peginterferon-alfa-2b, 1.5 µg/week. The remainder choose a higher dosage than currently advised in the guideline.

Side effect management

Neutropenia is a common event in HCV combination therapy and is usually attributed to the peginterferon component. Upon managing a patient with profound neutropenia (0.8 x 10^9/l), 35% would continue current treatment, 42% would reduce (halve) the prescribed dosage of peginterferon, and 15% stop peginterferon (*figure 2*).

Anaemia is caused by ribavirin-induced haemolysis, and if asked to decide for a patient with haemoglobin <5.4 mmol, the majority of physicians (41%) would initiate erythropoietin treatment, while 35% of physicians would reduce the ribavirin. Twenty-five percent continue both pegylated interferon and ribavirin unchanged (*figure 3*).

Figure 2. *Management choices in treatment of neutropenia 0.8 x 10^9/l*

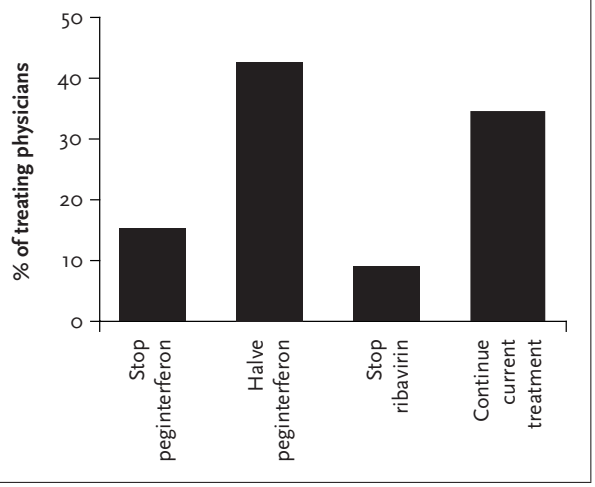
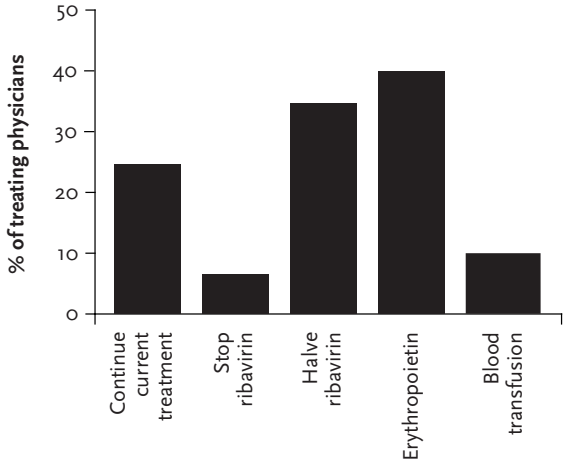


Figure 3. *Management choices in treatment of haemoglobin levels below 5.4 mmol/l*



DISCUSSION

Our survey shows that most physicians treat patients with HCV in clinical practice according to this recently issued HCV guideline.

Still there are some areas of controversy. Almost half of the physicians treat HCV genotype 1 and 3 patients with RVR longer than currently needed. In terms of financial aspects, occurrence of severe side effects and convenience for patients, shorter treatment duration with retained efficacy is desirable.

Some 13% of the physicians use higher ribavirin dosages than currently advised. Some studies show that a higher starting dose of ribavirin is associated with a lower relapse rate and higher rate of SVR.[9-11] High-dose ribavirin is also associated with more frequent and serious side effects such as anaemia, which require erythropoietin in many cases.[9-11] More studies are necessary to judge whether the benefits of a higher ribavirin dosage needed to achieve SVR outweigh the disadvantages of the side effects.[12]

Management of side effects has low evidential value and the current literature is mostly based on expert opinion. When a side effect, e.g. neutropenia or anaemia, occurs peginterferon and ribavirin are often reduced or stopped too soon,[13] and this may hamper the achievement of SVR. On the other hand, if treatment of anaemia by erythropoietin is initiated too early, it leads to unnecessary expenses.[9,14] Supporting evidence in order to issue guidelines is clearly needed. Along the same line, neutropenia due to peginterferon can be controlled by granulocyte colony stimulating factor, but it is unclear which cut-off point should be used for initiating therapy.

This study has some limitations. We did not ask for respondents' demographics, professional background and/or hospital setting. It is unclear whether our findings are generalisable to all Dutch medical specialists in Gastroenterology, Hepatology and Internal Medicine.

Altogether, data from our survey indicate that Dutch physicians treat their HCV patients as described in the recently issued HCV treatment guideline. There is a paucity of data that enables evidence-based management of side effects during HCV therapy.

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ORIGINAL ARTICLE

Earlier stages of colorectal cancer detected with immunochemical faecal occult blood tests

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ABSTRACT

Background: The aim of colorectal cancer screening is to improve prognosis by the detection of early cancer and precursor stages. We compared the stage distribution of asymptomatic colorectal cancer patients detected by a positive immunochemical or guaiac-based faecal occult blood test (FOBT) with symptomatic colorectal cancer patients.

Methods: In a longitudinal cohort study tumour stages were assessed in 144 symptomatic (mean age 69.3 years, 56% male) and 41 asymptomatic colorectal cancer patients (mean age 64.9 years, 56% male) of which 11 were detected with guaiac FOBTs (G-FOBT, Hemoccult-II®) and 30 with immunochemical FOBTs (I-FOBT, OCSensor®). Stage distributions were used to calculate average stage specific predicted five-year survival rates and to analyse group differences with Wilcoxon log-rank test.

Results: Colorectal cancer was detected in significantly earlier stages in symptomatic compared with asymptomatic patients (p<0.0001). Average stage specific predicted five-year survival was 59.1% in symptomatic and 76.6% in asymptomatic patients. Compared with the symptomatic patients the stage distribution for colorectal cancer patients detected with Hemoccult-II was not significantly different (p=0.29), whereas colorectal cancer was detected at significantly earlier stages with the OCSensor (p<0.0001). Treatment could be confined to colonoscopy in 27% of the asymptomatic patients compared with 3% of the symptomatic patients (p<0.0001). Cancer distribution over the colon was comparable between symptomatic and asymptomatic patients (p=0.3).

Conclusions: Compared with symptomatic patients, patients detected by FOBT and especially immunochemical FOBT, presented significantly more often at earlier stages suggesting increased survival. Additionally treatment could more often be confined to colonoscopy.

KEYWORDS

Colorectal cancer, faecal occult blood test, mass screening, survival

INTRODUCTION

Colorectal cancer is, after breast cancer, the second most frequent cancer in women, and after lung and prostate cancer, the third most frequent cancer in men in the Netherlands. The lifetime risk of developing colorectal cancer is approximately 6% (www.IKCnet.nl). In 2003, colorectal cancer was responsible for more than 56,000 life-years lost and for 70,000 disability adjusted life-years in the Netherlands. Total costs for management of colorectal cancer patients amounted to 230 million euros.[1] The ageing population will have an enormous impact on the incidence of colorectal cancer and costs in the near future. Until 2025, the incidence of colorectal cancer will gradually increase by 40% in the Netherlands and medical costs for colorectal cancer patients will considerably increase due to growing application of biologicals.[2] When colorectal cancer is detected as a result of symptoms, the prognosis is still rather poor with an average five-year survival between 50 to 60% (www.IKCnet.nl).[3] In order to improve the prognosis of colorectal cancer, colorectal

cancer should be detected in earlier stages or even precursor stages. Colorectal cancer is a heterogeneous disease arising from a complex series of molecular events. The successive evolution of normal colonic mucosa to a benign adenoma, then to an adenomatous polyp containing cancer, and then to a potentially life-threatening invasive cancer is associated with a series of genetic events occurring over a period of approximately ten years.[4] So, many years pass before colorectal cancer becomes clinically manifest and therefore there is an opportunity to improve prognosis by early detection of colorectal cancer with screening.

In 2006, colorectal cancer screening studies were started in the Netherlands randomly employing two different types of faecal occult blood tests (FOBTs): guaiac-based FOBTs and immunochemical FOBTs. In order to verify if prognosis of colorectal cancer is improved by FOBT screening and if improvement depends on the type of FOBT, we compared the stage distribution of colorectal cancer patients detected as a result of FOBT screening with colorectal cancer patients detected because of symptoms.

Table 1. *Stage specific average five-year survival rates for colorectal cancer*

T	N	M	Dukes	Stage	5-year survival*
Tis	No	Mo	-	0	98%
T1	No	Mo	A	I	93%
T2	No	Mo	A	I	93%
T3	No	Mo	B1	IIA	85%
T4	No	Mo	B2	IIB	72%
T1,2	N1	Mo	C1	IIIA	83%
T3,4	N1	Mo	-	IIIB	64%
Each T	N2	Mo	C2	IIIC	44%
Each T	Each N	M1	D	IV	8%

***According to data from the Surveillance, Epidemiology, and End Results (SEER) programme.**

MATERIALS AND METHODS

Between July 2006 and March 2007, asymptomatic subjects with a positive FOBT were invited for colonoscopy. These subjects were recruited from two colorectal cancer screening studies in the Netherlands with a comparable design, registered at Current Controlled Trials (ISRCTN57917442). All invited participants were asymptomatic, between 50 and 75 years of age and without apparent family history of colorectal cancer. Data from the largest of the two studies comparing the performance of two types of FOBTs, supported by the Netherlands Organisation for Health Research and Development (ZonMW: number 50-50115-98-060, project 63000004), were published previously.[5] From the patients with colorectal cancer detected in these studies, the following data were collected: age, gender, location of the tumour, surgical or endoscopic treatment and TNM classification. These data were also collected from 144 consecutive symptomatic colorectal cancer patients without a family history of colorectal cancer in the same region in the Netherlands and in the same period, from July 2006 to March 2007. Tumour staging was performed according to the American Joint Committee on Cancer (AJCC) system, also called the TNM system, which describes stages using Roman numerals I through IV. Three experienced pathologists staged all the detected colorectal cancers of both the symptomatic and the asymptomatic patients. According to data from the Surveillance, Epidemiology, and End Results (SEER) programme, each stage has an average stage specific five-year survival (*table 1*).[6] The follow-up after our study was too short to measure actual five-year survival and furthermore actual five-year survival would have to be corrected for over-diagnosis and lead-time bias, the extent of which is largely unknown. Therefore the stage specific average five-year survival rates were used to predict the five-year survival of the patients in our study group. For each colorectal cancer stage we assumed that the five-year survival would eventually prove to be identical for symptomatic or asymptomatic patients and identical to the SEER data. Of course this approximation method has certain drawbacks, but also advantages, which we will discuss in the discussion.

In the asymptomatic patients participating in the colorectal cancer screening study, the type of FOBT was also registered.[5] Two types of FOBT were used, a guaiac-based FOBT (Hemoccult-II®) and an immunochemical based FOBT (OCSensor®). A specific advantage of the OCSensor® is that the test is quantitative allowing the cut-off value for positivity to be changed. As threshold for positivity, the manufacturer recommends a cut-off value of 100 ng/ml, applied in several studies.[7-12] The literature as well as data provided by the manufacturer show that the test results of the OCSensor® are reliable in the range from 50 ng/ml to 2000 ng/ml.[13] In the previous publication in Gastroenterology we compared the guaiac FOBT Hemoccult-II® with the I-FOBT OCSensor®. In that publication, for generalisability with the previous studies, we presented data for the I-FOBT with a cut-off value of 100 ng/ml. However, we invited all patients for colonoscopy with an I-FOBT result of ≥50 ng/ml, corresponding to about 10 µg haemoglobin per gram faeces.[14] In the current study we use the data of all invited patients with a cut-off value of 50 ng/ml.

With immunochemical faecal occult blood tests, as little as 0.3 ml of daily blood loss in the stool can be detected.[15] In contrast, the Hemoccult-II® test is a qualitative test in which the minimal amount of blood that can be

detected in faeces is probably about ten times higher than immunochemical FOBTs.[15,16] Therefore, we speculated that the immunochemical FOBT OCSensor® detects colorectal cancer at earlier stages.

We calculated descriptive statistics of the symptomatic and asymptomatic study populations. We used nonparametric analysis with the Wilcoxon log-rank test to compare the stage distribution between the two groups. Statistical significance was accepted if the level of probability of a type I error was ≤5% ($p<0.05$). Statistical analyses were performed with SAS system for windows, software version 8.02 (SAS Institute Inc., Cary, NC, USA).

Table 3. *Staging of colorectal cancer in symptomatic and asymptomatic patients*

Colorectal cancer stage	Symptomatic patients N=144 (100%)	Asymptomatic patients N=41 (100%)
I	6 (4%)	20 (49%)
IIA	23 (16%)	6 (15%)
IIB	43 (30%)	0 (0%)
IIIA	15 (10%)	3 (7%)
IIIB	12 (8%)	5 (12%)
IIIC	13 (9%)	4 (10%)
IV	32 (22%)	3 (7%)

RESULTS

Colonoscopies in the asymptomatic group with a positive FOBT revealed in total 41 patients with colorectal cancer (23 male, 18 female; mean age 64.9 years, range 53 to 75 years). Nine of the tumours were located in the rectum, 19 in the sigmoid, three in the descending colon, one in the splenic flexure, two in the transverse colon, one in the hepatic flexure, four in the ascending colon, and two in the caecum (*table 2*). Twenty of the tumours were classified as stage I, six as stage IIA, three as stage IIIA, five as stage IIIB, four as stage IIIC, and three as stage IV (*table 3*). The average predicted five-year survival deduced from the TNM staging system and SEER programme (*table 1*) in this group of 41 patients was 76.6 (SD 25%). In 11 (27%) of these 41 patients tumour treatment could be confined to endoscopy. Tumours were found in 11 patients with a positive Hemoccult-II® and in 30 patients with a positive OCSensor®. The predicted five-year survival for the patients with a positive Hemoccult-II® test was 60.5 (SD 37%), and for the patients with a positive OCSensor® 82.4 (SD 16%).

Table 2. *Location of colorectal cancer in symptomatic and asymptomatic patients*

Colorectal cancer location	Symptomatic patients N=144* (%)	Asymptomatic patients N=41 (%)
Rectum	61 (42%)	9 (22%)
Sigmoid colon	33 (23%)	19 (46%)
Descending colon	3 (2%)	3 (7%)
Splenic flexure	3 (2%)	1 (2%)
Transverse colon	5 (3%)	2 (5%)
Hepatic flexure	5 (3%)	1 (2%)
Ascending colon	17 (12%)	4 (10%)
Caecum	15 (10%)	2 (5%)
Unknown	4 (3%)	- (0%)

***Two patients had two concurrent colorectal tumours.**

Colonoscopies performed in subjects referred with symptoms, such as visible blood loss and changed bowel habits, revealed 144 patients with colorectal cancer (81 male, 63 female; mean age 69.3 years, range 32 to 93 years). Sixty-one of the tumours were located in the rectum, 33 in the sigmoid, three in the descending colon, three in the splenic flexure, five in the transverse colon, five in the hepatic flexure, 17 in the ascending colon, 15 in the caecum and for four tumours the specific location was uncertain (*table 2*). Two patients had a double tumour and these patients were staged according to the most advanced carcinoma. Six of the patients were classified as stage I, 23 as stage IIA, 43 as stage IIB, 15 as stage IIIA, 12 as stage IIIB, 13 as stage IIIC, and 32 as stage IV (*table 3*). The predicted five-year survival for this group of 144 patients was 59.1 (SD 29%). In four (3%) of these 144 patients tumour removal could be confined to endoscopy.

Colorectal cancer was detected significantly earlier in symptomatic patients compared with asymptomatic patients ($p<0.0001$). Average stage specific predicted five-year survival was 59.1% in symptomatic and 76.6% in asymptomatic patients. Additionally, treatment for colorectal cancer could be confined to colonoscopy in 27% of the asymptomatic patients compared with 3% of the symptomatic patients ($p<0.0001$). Ten (24%) of the colorectal cancer tumours in asymptomatic patients and 45 (31%) of the colorectal cancer tumours in symptomatic patients were located proximal of the descending colon ($p=0.3$).

Compared with the symptomatic patients the stage distribution for colorectal cancer patients detected with Hemoccult-II was not significantly different ($p=0.29$), whereas colorectal cancer was detected significantly earlier in patients detected with OCSensor compared with the symptomatic patients ($p<0.0001$). The average predicted five-year survival was 82.4% for the asymptomatic patients detected with the OCSensor® and 60.5% for the patients detected with the Hemoccult-II®.

DISCUSSION

In this study we demonstrated that colorectal cancer in asymptomatic persons aged between 50 and 75 years with a positive FOBT is on average detected at a significantly earlier stage compared with symptomatic subjects with colorectal cancer diagnosed in the same time period and in the same geographical region. In addition, treatment of colorectal cancer could significantly more often be confined to colonoscopy in the asymptomatic screening group than in the symptomatic group. The most important finding in this study was that, compared with symptomatic patients, colorectal cancer was detected in significantly earlier stages for the patients detected with the I-FOBT, but not for patients detected with the G-FOBT.

We have compared the data from our study with data from a German study by Hüppe *et al*. For easy comparison with our study we calculated the gain in predicted five-year colorectal cancer survival according to stage distribution from the data in their study in 5066 asymptomatic patients (participating in colonoscopy screening) and in 4099 symptomatic patients.[17] The 18% gain in survival from their data compares well with the 22% gain in average predicted survival we observed with FOBT screening. In addition Hüppe *et al*. had followed up their population for up to five years (on average 33 months). Up to the time of their publication no subjects in the screening group had died compared with 20% of the symptomatic colorectal cancer patients, confirming the predicted gain in five-year survival according to stage distribution.[17] We propose this indicates that the stage distribution of colorectal cancer detected by primary colonoscopy screening is comparable with the stage distribution of colorectal cancer detected by faecal occult blood testing. Additionally the follow-up data by Hüppe *et al*. indicate that colorectal cancer survival predicted by stage distribution might on average correlate well with actual survival. We think this is interesting for policy makers who are considering implementing colorectal cancer screening with either primary colonoscopy or FOBT.

Another important finding of our study was the difference that we observed in the stage distribution between subjects with colorectal cancer detected after a positive Hemoccult-II® test, compared with a positive OCSensor® test. Both the Hemoccult-II® test and the OCSensor® test are developed to detect haemoglobin in faeces. In contrast to the Hemoccult-II® test, the OCSensor® test is specific for human haemoglobin and the test is quantitative, allowing the cut-off values for positivity to be shifted. The Hemoccult-II® test is probably at least ten times less sensitive to haemoglobin compared with the OCSensor® test.[15,16] The stage distribution of colorectal cancer patients detected with the OCSensor® in this study was significantly better than the stage distribution in the symptomatic patients. In contrast, the stage distribution of colorectal cancer patients detected with the Hemoccult-II® was not significantly different from the symptomatic patients. Therefore we propose that colorectal cancer patients detected by a positive OCSensor® can probably on average expect to have an increased five-year survival compared with those with a positive Hemoccult-II®.

Although slightly more proximal colorectal cancers were detected in the symptomatic patients compared with the asymptomatic patients, this difference was not statistically significant. Therefore, tumours in the proximal colon are also detected by FOBT. For the OCSensor® this was confirmed in a recent study, where it was shown that the amount of blood in the stool detected in patients with proximal colorectal cancer is lower than in distal colorectal cancer, but never below the cut-off value of 50 ng/ml that we used in our study population.[14] In another study with a different immunochemical FOBT (Magstream 1000®), a lower sensitivity for proximal compared with distal colorectal cancer was reported, but this difference was not statistically significant.[18] Wexner *et al*. demonstrated there was no difference in colorectal cancer location for Hemoccult-II® negative and Hemoccult-II® positive colorectal cancer patients.[19] Fujita *et al*. also failed to detect any differences in colorectal cancer distribution over the colon in asymptomatic patients screened with guaiac slides compared with symptomatic patients.[20]

CONCLUSION

We demonstrated that colorectal cancer is detected at significantly earlier stages in asymptomatic patients by a positive FOBT compared with symptomatic patients. In addition, treatment of colorectal cancer could be confined to colonoscopy in significantly more asymptomatic patients with a positive FOBT than in the symptomatic group. Furthermore, there was no difference in distribution of colorectal cancer over the colon between the asymptomatic and symptomatic colorectal cancer patients, indicating that tumours in the proximal colon are also detected by FOBT. Colorectal cancer was not detected at significantly earlier stages in patients detected with the Hemoccult-II compared with symptomatic patients. However, colorectal cancer was detected in significantly earlier stages in patients detected with the OCSensor compared with symptomatic patients.

NOTE

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CASE REPORT

Food-dependent Cushing's syndrome

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ABSTRACT

It has recently been proposed that other hormones than ACTH can control cortisol production in Cushing's syndrome with bilateral adrenal hyperplasia. We present a case of food-dependent Cushing's syndrome. After a positive response of cortisol production during mixed meals, several tests identified glucose-dependent insulinotropic polypeptide (GIP) as the driving hormone responsible for the cortisol overproduction. Identification of aberrant hormone receptor expression is of importance because it may create a possibility for pharmacological treatment.

KEYWORDS

ACTH-independent macronodular adrenal hyperplasia (AIMAH), food-dependent Cushing's syndrome, glucose-dependent insulinotropic polypeptide (GIP)

INTRODUCTION

Cushing's syndrome is usually caused by excessive ACTH secretion, such as in pituitary adenomas (as in Cushing's disease) and ectopic ACTH secretion (as in small cell lung carcinomas and carcinoids); less frequently it is ACTH-independent, frequently caused by cortisol-producing adrenal adenomas or carcinomas. However, ACTH-independent bilateral adrenal hyperplasia has also been described. Adrenocortical cells then have the capacity to secrete cortisol in the absence of detectable ACTH levels. In recent years, the presence of aberrant hormone receptors on adrenal cells of patients with ACTH-independent Cushing's syndrome has been demonstrated.[1] Hormones or peptides other than ACTH control cortisol secretion in these cases, for example glucose-dependent insulinotropic polypeptide (GIP) via binding to ectopic receptors on adrenal cells.[2]

We report a new case of bilateral adrenal enlargement based on food-dependent Cushing's syndrome, a less known variant of Cushing's syndrome.

CASE REPORT

A 57-year-old woman was referred to our outpatient clinic because of accidentally discovered bilateral adrenal enlargement. She complained of weight gain, abdominal discomfort, easy bruising, trembling and palpitations. No muscle weakness or psychological problems, such as depression, were reported. Physical examination revealed hypertension (160/100 mmHg), centripetal fat distribution without signs of muscle atrophy and ankle oedema. No moon face or buffalo hump were noticed. The early morning fasting plasma cortisol level could not be suppressed (323 nmol/l; reference value <50 nmol/l) by 1 mg dexamethasone administered the previous evening. The 24-hour urinary free cortisol excretion was also elevated (539 nmol/24 hours; reference values 30 to 150 nmol/24 hours). ACTH levels were below the detection limit (<10 ng/l) at all times. Further investigations showed that the patient's 24-hour urinary free cortisol excretion appeared to be lower (444 nmol/l) during a fasting day as compared with values on non-fasting days (539 nmol/l). Plasma cortisol levels increased after mixed meals and decreased during fasting. Prolonged fasting was associated with a progressive decrease in serum cortisol levels (*figure 1*). Food-dependent Cushing's syndrome was suspected.

On intravenous infusion of GIP (0.6 µg/kg, while fasting) the plasma cortisol level more than doubled already 15 minutes after infusion (*figure 2*). The response of cortisol to ACTH administration (250 µg) was similar and

Figure 1. *Plasma cortisol levels while fasting (■) and during normal food intake (▼)*

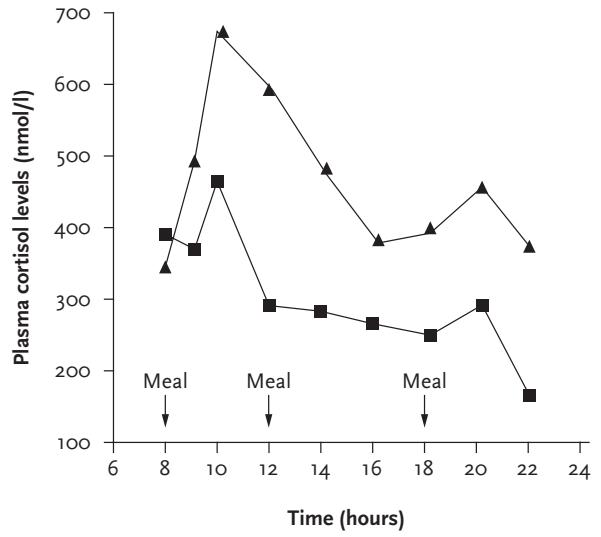


Figure 3. *Plasma cortisol levels during food intake (■) and during treatment with octreotide pre-meals (▼)*

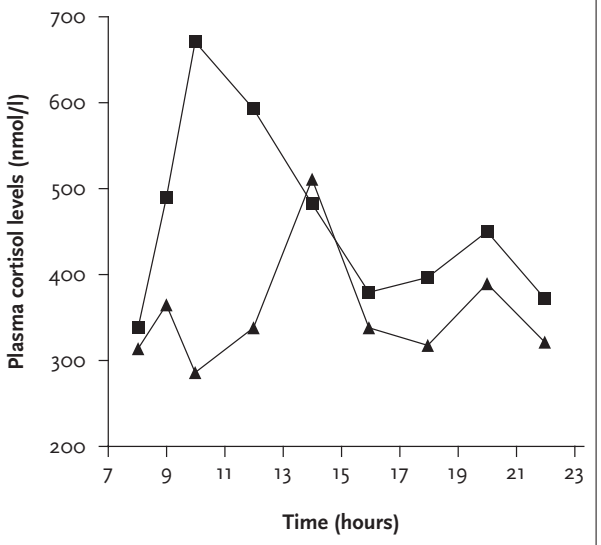
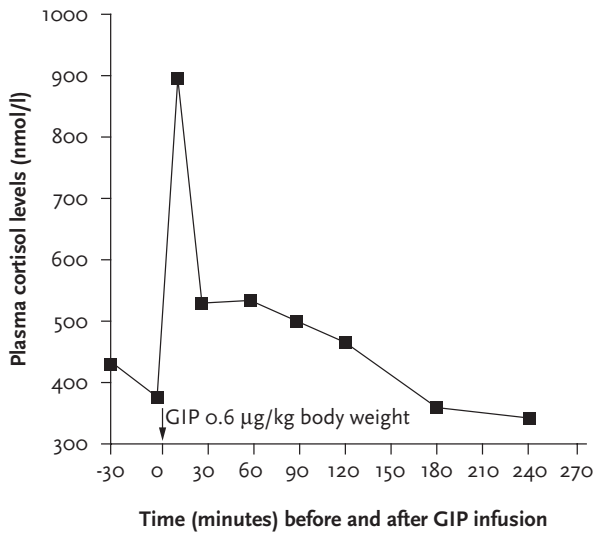


Figure 2. *Effect of a single infusion of GIP (0.6 µg/kg body weight) on plasma cortisol levels*



served as a reference test (data not shown). This proved food-dependent Cushing's syndrome in this patient.

A posture test was performed to screen for receptors to angiotensin II, vasopressin and catecholamines and the tests were negative.

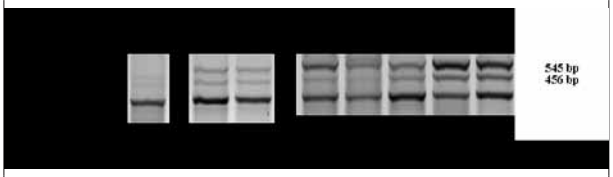
Octreotide treatment before meals (0.1 mg subcutaneously 3 times/day) reduced plasma cortisol levels (*figure 3*). Urinary cortisol excretion was 414 nmol/l, lower than the other values (in the fasting or the non-fasting state). According to our experience and that of others reported in the literature,[3,4] octreotide therapy only results in short-term suppression of the hypercortisolism. It was, therefore, decided not to treat the patient with octreotide. She subsequently underwent laparoscopic bilateral adrenalectomy. The left adrenal tissue weighed 11 grams and the right adrenal lesion weighed 120 grams. Both lesions were categorised as benign adrenocortical adenomas.

Total RNA was extracted from adrenals using Trizol (Invitrogen). Multiplex RT-PCR using QuantumRNA 18S rRNA as an endogenous control (Spencer & Christensen 1999) was performed according to recommendations by the manufacturer, as follows: the RT-PCR reaction was terminated when all samples were in the linear range of amplification. For each gene, seven RT-PCR reactions with a pool of cDNA from normal adrenals were performed using the specific primers, submitted to PCR and one tube was removed every four cycles, starting from the 12th cycle; then, all the PCR products were resolved in ethidium bromide stained 1.5% agarose gel, detected by Typhoon and quantified by ImageQuant. Cycle number was plotted against the log of the signal and a straight line was obtained for samples in linear range of amplification; the medium point was chosen. Multiplex PCR using two sets of primers, one to amplify the cDNA of interest and a second to amplify an endogenous control (18S rRNA - QuantumRNA 18S Internal Standards, Ambion Inc. Austin-Texas, USA), was performed. The product level of the gene of interest was normalised against the product from the control reaction. The PCR reactions contained 10 mM Tris-HCl (pH 8.3), 1.5 mM

MgCl2, 50 mM KCl, 0.2 mM each of deoxy-dNTP, 20 pmol each of sense and anti-sense primers specific for the gene of interest, 2 µl of RT reaction, 2.5 U Taq DNA polymerase and 2 µl of 18S PCR Primer Pair.[5] GIP receptor was found to be over-expressed on the adrenal tumour cells as compared with a normal adrenal or normal human pancreas (*figure 4)*.

She gained weight in the first year postoperatively, and then lost a total of 3 kg. The hypertension resolved. The patient developed type 2 diabetes five years postoperatively, which is now well controlled with oral glucose-lowering drugs.

Figure 4. *GIP receptor expression in control pancreas, control adrenal and in 5 cases of GIP-dependent adrenal AIMAH with Cushing's syndrome*



Relative quantitative RT-PCR products in agarose gel, showing the two GIPR isoforms (545 and 456 bp) and the internal control r18s (324bp). Sample in lane 3 with the asterisk is the patient reported in this study.

DISCUSSION

In recent years, new aetiologies of ACTH-independent unilateral and bilateral adrenal hyperplasia have been added to the spectrum of ACTH-independent Cushing's syndrome.

It has been established that the aberrant stimulation of steroidogenesis in ACTH-independent macronodular adrenal hyperplasia (AIMAH) and in some unilateral adenomas can be driven by ectopic receptors such as those for glucose-dependent insulinotropic peptide or gastric inhibitory polypeptide (GIPR), β-adrenergic receptors, vasopressin (V_2-V_3 vasopressin receptor), serotonin (5-HT_7 receptor) and probably angiotensin II receptor (AT1R); it can also result from increased expression or altered activity of eutopic receptors such as those for vasopressin (V_1 vasopressin receptor), luteinising hormone/human chorionic gonadotropin (LH/hCGR) and serotonin (5-HT_4 receptor).[1] The molecular mechanisms leading to the abnormal expression of eutopic and ectopic receptors in the adrenal glands of patients with AIMAH and less commonly in adrenal adenomas remains not completely understood.

In our case, the driving force behind the increased adrenal cortisol production appeared to be meals, i.e. a peptide or hormone released after meals, instead of ACTH. All healthy persons produce GIP from endocrine K cells in the small intestine (duodenum and proximal jejunum) after oral food intake.[6] This release increases with higher fat and carbohydrate intake and decreases with protein intake. GIP has a positive effect on the release and synthesis of insulin, thus its name: glucose-dependent insulinotropic polypeptide.

In patients with food-dependent Cushing's syndrome, GIP is released in physiological concentrations in the small intestine; however, because of the binding to the ectopic or overexpressed adrenal GIP receptors this results in a postprandial increase in plasma cortisol. Cortisol exerts its negative feedback on ACTH and CRH synthesis and leads to suppressed levels of plasma ACTH and thereby to low fasting cortisol levels. In some patients with GIP-dependent Cushing's syndrome, plasma cortisol levels are not as low, possibly as the result of the expression of more than one aberrant receptor. In this case report it was remarkable that in the fasting state cortisol levels also increased around the meal times (morning and evening). This could be a result of the presence of other aberrant receptors, but this has not been tested. In addition, no cortisol increase was found after lunch during feeding and fasting; this could be due to a bigger contribution of physiological morning and evening increases in cortisol than the result of the influence of GIP. The investigators did not screen for all possible receptors such as the glucagon receptor and the serotonin (5-HT4) receptor; a posture test was performed to screen for receptors to angiotensin II, vasopressin and catecholamines; these tests were negative. The presence of other aberrant receptors could, however, have influenced the cortisol pattern in this patient.

Until recently GIP receptors (GIPR) had not been found on either normal human adrenal cells[2] or on adrenal cells of a non-food-dependent Cushing's syndrome patient.[7] Baldacchino *et al.* found GIPR to be expressed in a large number of human adult and foetal tissues. Data obtained by gene array and semi-quantitative RT-PCR showed an increase in the expression of several genes implicated in GIPR expression in the adrenal adenomas or bilateral macronodular hyperplasia of patients with GIP-dependent Cushing's syndrome. They were, however, also increased in some patients with non-GIP-dependent cortisol-secreting adenomas or with ACTH-dependent Cushing's disease.[7] Further studies are necessary to clarify the molecular mechanisms responsible for the over expression of GIPR in zona fasciculata cells.

Octreotide administration before each meal prevented meal-induced increase of plasma cortisol and GIP levels.[3]

However, pretreatment with octreotide only decreases the cortisol response to meals for a few weeks or months, in most other cases.[4,8] This is possibly due to downregulation of somatostatin receptors on GIP-secreting intestinal cells. Adrenalectomy seems to be the best treatment for GIP-dependent Cushing's syndrome. As an alternative to surgery, new pharmacological therapy will require development of molecules which can block the GIP receptor efficiently.

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CASE REPORT

Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour

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ABSTRACT

Background: In up to 20% of patients with renal cell cancer (RCC) an inflammatory response consisting of low-grade fever, weight loss and an elevated ESR and CRP may occur with modest granulocytosis and thrombocytosis. Clinical and experimental data suggest a pathogenic role for tumour-derived cytokine production, especially interleukin-6.

Case report: A 79-year-old female with RCC presented with low-grade fever, weight loss and overt granulocytosis and thrombocytosis. Radiological examination revealed a right-sided renal tumour. During nephrectomy a gradient between the IL-6 levels in the renal artery and vein was demonstrated, providing direct evidence for *in vivo* production of IL-6 by the tumour affected kidney, which was confirmed by the demonstration of IL-6 in the tumour cells by immunohistochemical staining and in the supernatant of the homogenised tumour. Cytogenetic examination revealed complex abnormalities including a gain of chromosome 7. In addition we demonstrated production of IL-1α, IL-1β, IL-8 and ICAM-1 in the tumour with systemic elevated levels of IL-6 and IL-8 with secondary increased serum G-CSF and TPO levels.

Conclusion: We have provided direct evidence for the production of pro-inflammatory cytokines by renal cancer cells in a patient with RCC and a profound inflammatory response, with a central role of IL-6, probably due to a gain of chromosome 7. The extreme granulocytosis and thrombocytosis may have resulted from the secondary systemic production of G-CSF and TPO.

KEYWORDS

Cytokines, inflammation, interleukin-1, interleukin-6, renal cell carcinoma, trombocytosis

INTRODUCTION

Ten to forty percent of patients with renal cell cancer (RCC) experience paraneoplastic phenomena during the course of the disease.[1] The symptoms may result from the production of humoral factors by the tumour or by adjacent tissue in response to the tumour or via immune modulation. Erythrocytosis is the most well-known paraneoplastic haematological event. Although two-thirds of the renal tumours produce erythropoietin, erythrocytosis is reported in 1 to 8% of the patients.[1] Overt granulocytosis (>50/nl) has occasionally been reported in patients with G-CSF or GM-CSF producing RCC.[2-4] Modest granulocytosis and thrombocytosis occurs in up to 20% of the patients and is thought to be part of a systemic inflammatory response since it is usually associated with anaemia, fever, weight loss, and increased serum levels of CRP and interleukin-6.[5] Here we report a patient with RCC, who presented with fever, weight loss and marked granulocytosis and thrombocytosis. We performed extensive cytokine profiling of the tumour and normal renal tissue as well as in blood samples obtained from peripheral blood, renal vein and renal artery. We demonstrated production of IL-1α, IL-1β, IL-6, IL-8 and ICAM-1 in the tumour with systemic elevated levels of IL-6 and IL-8 with secondary increased serum levels of granulocyte-colony stimulating factor (G-CSF) and thrombopoietin (TPO).

CASE REPORT

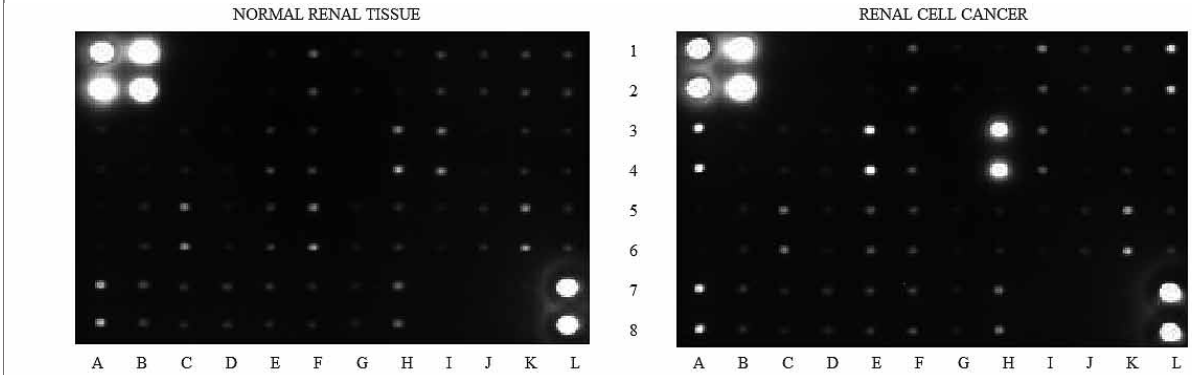
A 79-year-old female presented with low back pain, low-grade fever and 6 kg weight loss in three months' time. Physical examination was unremarkable. Laboratory analysis showed an ESR of 123 mm (RR <30) and serum CRP of >200 mg/l (RR <8). Haemoglobin level was 6.1 mmol/l (RR 7.4 to 10.2), granulocytes were 40/nl (RR 1.5 to 7.0) with 665/nl thrombocytes (RR 150 to 400). An extensive biochemical profile showed a serum albumin level of 31 g/l (RR 34 to 48) but was otherwise normal. Computed tomography showed a right-sided renal tumour with extension into the pyelum without distant metastases. During radical nephrectomy samples of renal artery and vein were collected and GM-CSF, G-CSF, IL-1α, IL-6, IL-8 and TPO were determined by conventional ELISA (BioSource, CA, USA) or by human inflammation Th1/Th2 cytometic bead array (CBA; BD Biosciences, San Jose, USA) according to the manufacturer's guidelines. As shown in *table 1*, a 1.5 gradient was detected for IL-6 between the renal vein and artery, indicating production of IL-6 by the tumour infiltrated kidney. The systemic level, as reflected by the concentration in the renal artery, of IL-6 was increased to 6.2 pg/ml (RR 0.2 to 4.6), and of IL-8 was increased to at least 80 pg/ml (RR <10) while the levels of GM-CSF, G-CSF, IL-1α and TPO were within the normal range. Pathological examination showed a renal cell cancer with extensive sarcomatoid differentiation (Fuhrman grade 4) with a diameter of 8 cm with regional lymph node metastases. In the tumour neutrophilic infiltration was present. Cytogenetic examination showed complex abnormalities: 47, XX, + 18 (two cells), 48, XX, +9, +10 (one cell), 92, XXXX (three cells), 77-93, XXXX, +18, +7, -9mar[cp4] (four cells) and 47, XX, +10 (one cell). Sample suspensions of the tumour and the normal adjacent renal tissue were made by homogenising 4 mm^3 tissue in 2 ml PBS using a Potter's homogeniser. Cytokine

Table 1. *Results of cytokine profiling*

Cytokine	Tumour/normal tissue	Renal vein/artery
IL-1α	7.5#*	1.0#
IL-1β	76.3*$	n.d.
IL-6	5.9#*$	1.5#$
IL-6sR	1.8*	n.d.
IL-8	11.5#*$	0.9#$
G-CSF	n.d. (due to inconsistent results)	1.2#$
GM-CSF	0.5#*	0.9#
TPO	1.3#	1.1#
ICAM-1	1.6*	n.d.

The ratio is expressed by means of combining the results of the conventional ELISA (#), the Quantibody Human Inflammation Array 3 (*) and/or the human inflammation TH1/Th2 cytometic bead array ($). n.d. = not determined.

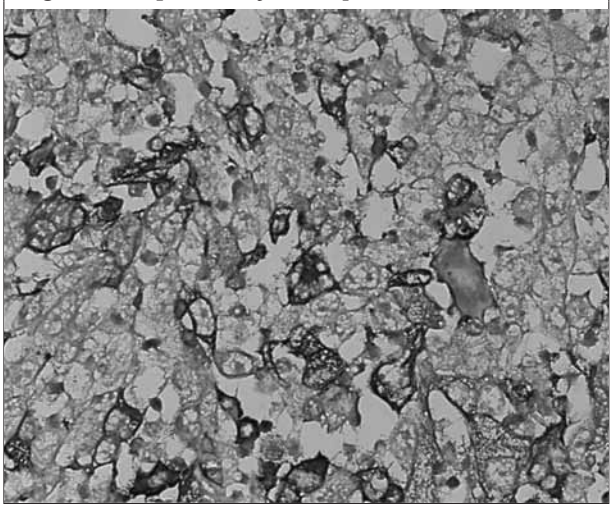
Figure 1. *RayBio human inflammation antibody array 3 in comparison with the normal renal tissue the renal cell cancer shows increased expression of IL-1α, IL-1β, IL-6, IL-8, ICAM-1 and CCL-5*



	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	Eotaxin	Eotaxin-2	G-CSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
2	POS	POS	NEG	NEG	Eotaxin	Eotaxin-2	G-CSF	GM-CSF	ICAM-1	IFN-γ	I-309	IL-1α
3	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12p40	IL-12p70
4	IL-1β	IL-2	IL-3	IL-4	IL-6	IL-6sR	IL-7	IL-8	IL-10	IL-11	IL-12p40	IL-12p70
5	IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
6	IL-13	IL-15	IL-16	IL-17	IP-10	MCP-1	MCP-2	M-CSF	MIG	MIP-1α	MIP-1β	MIP-1δ
7	CCL-5	TGF-β1	TNF-α	TNF-β	sTNF RI	sTNF RII	PDGF-BB	TIMP-2	Blank	Blank	Neg	Pos
8	CCL-5	TGF-β1	TNF-α	TNF-β	sTNF RI	sTNF RII	PDGF-BB	TIMP-2	Blank	Blank	Neg	Pos

profiling of the supernatant of these samples was done by the above-mentioned ELISA and bead array as well as by Quantibody Human Inflammation Array 3 (RayBiotech, Norcross, GA, USA). As indicated in *table 1* and *figure 1*, concentrations of IL-1α, IL-1β, IL-6, IL-8 and ICAM-1 in the supernatant of the tumour tissue were higher compared with normal renal tissue. There was no evidence for production of GM-CSF, G-CSF, TPO and a variety of other cytokines (*figure 1* and *table 1*). Immunohistochemical staining showed the presence of IL-6 in the cytoplasma of the renal tumour cells (*figure 2*) while the staining for IL-8 was more prominent in the tumour infiltrating granulocytes. After nephrectomy the granulocyte and platelet counts nearly normalised. However, the patient's condition deteriorated due to rapidly progressive metastatic disease and she died 54 days after nephrectomy. At that time the granulocytes were 72.2/nl and thrombocyte count 810/nl. There were increased serum levels of G-CSF (182 pg/ml [RR 7.8 to 38.9]), IL-6 (23.9 pg/ml), IL-8 (80 pg/ml) and TPO (105 U/ml [RR 4-32]), while the serum concentrations of IL-1α and GM-CSF were within the normal range.

Figure 2. *Expression of IL-6 by the renal tumour*



DISCUSSION

This patient with RCC presented with systemic inflammatory response and profound granulocytosis and thrombocytosis. We have provided evidence for production of IL-6 by the renal cell tumour by the presence of an IL-6 level gradient of 1.5 between the renal vein and artery, a high concentration of IL-6 in the supernatant of the tumour compared with that of normal adjacent renal tissue and the demonstration of IL-6 in the cytoplasm of the tumour cells by immunohistochemical staining. In addition, cytokine profiling of the supernatant of the tumour revealed an indication for production of IL-1α, IL1-β, IL-8, and ICAM-1 by the tumour affected kidney. This resulted in elevated systemic levels of IL-6 and IL-8.

IL-6, encoded by a gene located on chromosome 7p21-p14, is a pleiotropic cytokine which promotes the growth and action of cytotoxic T cells, acts synergistically with IL-3 in haematopoiesis and induces differentiation of various cells including megakaryocytes. IL-6 has a central role in the acute-phase response by stimulating hepatocytes to produce acute-phase proteins such as CRP and fibrinogen and suppress albumin production. It is produced primarily by lymphocytes and monocytes, but also by numerous other tissues, including a variety of tumours.[6] Normal renal cells may express low amounts IL-6 which may be upregulated by exogenous stimuli.[7] In the vast majority of renal cancer cell lines as well as in freshly isolated renal cell cancer specimens IL-6 is the predominantly produced cytokine.[8,9] Furthermore, serum IL-6 levels are elevated in many patients with RCC. In most studies there is a relation between the serum IL-6 level and the systemic inflammatory response reflected by fever, weight loss and elevated CRP or ESR.[5,10-11] In approximately 15 to 25% of these patients mild granulocytosis and/or thrombocytosis may occur.[12] Thrombocytosis, granulocytosis and elevated serum IL-6 levels are usually associated with a poor prognosis.[12,13] Since IL-6 is not expressed in all cases of RCC, additional factors to induce IL-6 production must be present. Increased IL-6 production has been demonstrated in renal cell tumours with mutated p53[14] and in renal cell cancer with gain of an additional chromosome 7 as in our patient.[6] A variety of cytogenetic abnormalities, such as gain of chromosome 7, may occur in the different RCC histological subtypes, including those with sarcomatoid features, with diagnostic and prognostic implications.[15,16] Once expressed, IL-6 acts as an autocrine factor.[6]

Since we performed the cytokine profile in the supernatant of the homogenised tumour it is questionable whether production of IL-1α, IL-1β, IL-8 and ICAM-1 originated from the tumour or from infiltrating neutrophils. It may be hypothesised that tumour-derived IL-1α, IL-1β, IL-8 and ICAM-1 induce migration and attraction of the neutrophils. Furthermore, the produced IL-6 and IL-8 prohibit the infiltration of (tumour-specific) cytotoxic T-cells, which may explain the lack of tumour infiltrating lymphocytes and may contribute to the aggressive course of the disease in our patient.[17] Otherwise, it may be assumed that the invading neurophils are responsible for additional cytokine production, such as IL-8, as indicated by the immunohistochemical staining of IL-8 in both the tumour cells and neutrophils. Neutrophils are able to produce IL-8; however, they cannot produce IL-1 and IL-6, neither constitutively nor post-stimulation.[18] So both the tumour cells and the

invading neutrophils may be responsible for different parts of the systemic inflammatory response.

Maturating neutrophils and platelets bind G-CSF and TPO respectively which may result in a reciprocal relationship between the cytokines and the circulating cells.[19] The fact that serum G-CSF and TPO is increased during extreme granulocytosis and thrombocytosis indicates excessive production of these cytokines. We found no evidence for production of G-CSF or TPO within the tumour.

It is very likely that the increased systemic level of G-CSF may be the result of the tumour-derived production of IL-1, while the increased TPO level is secondary to the production of IL-6 by the tumour.

CONCLUSION

We report a patient with RCC with low-grade fever, weight loss and elevated ESR and CRP resulting from the release of pro-inflammatory cytokines within the tumour affected kidney with a central role for tumour-derived IL-6, probably due to a gain of chromosome 7. The extreme granulocytosis and thrombocytosis may have resulted from the secondary systemic production of G-CSF and TPO.

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LETTER TO THE EDITOR

Diagnosis of Wilson's disease

I read the paper of Kok *et al.*[1] in this journal with some surprise. The authors try to make the case that the patient they describe was difficult to diagnose with Wilson's disease and it was only molecular analysis of the ATP7B gene for mutations that saved the day. In fact, the patient should have been quite easy to diagnose if they had done quantitative copper assay on the liver biopsy instead of only a copper stain. The authors acknowledge that copper stains are unreliable for diagnosis, but for some reason never obtained a quantitative liver copper. They seem to justify not doing a quantitative copper assay by referring to a paper that tries to make the case that liver copper assays vary wildly in Wilson's disease,[2] but that is not the experience of most Wilson's disease workers.[3,4]

When this patient first appeared with a urine copper of 2.31 µmol/24 hours (150 µg/24h) and a mildly reduced ceruloplasmin, Wilson's disease was a highly likely diagnosis – normal urine copper is not <1.5 µmol/24 hours (97.5 µg/24 hours) as the authors state, but is about 0.8 µmol (52 µg/24 hours). Assaying copper on the liver biopsy would have confirmed the diagnosis, and the patient would not have had to suffer damage for an additional 15 years without treatment until molecular analysis was done.

Molecular analysis of the gene has its problems. Because of the large array of causative mutations, it is difficult to assess for all the mutations. And about 25% of the time, complete sequencing of the coding regions fails to reveal two causative mutations. Assay of copper in liver biopsies is still the 'gold standard.'

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RESPONSE TO LETTER TO THE EDITOR

Diagnosis of Wilson's disease

We appreciate the comments made by Dr Brewer.[1,2] He argues that the diagnosis of Wilson's disease (WD) should have been made using quantitative copper assay of liver specimen, and suggests that this is the 'gold standard'. Unfortunately, contrary to his statement, there is no gold standard for diagnosis in WD. If this were to be true, there would be no need for diagnostic criteria as have been developed for this disease. Indeed, as we have experienced, the diagnosis of WD in an individual with chronic hepatic disease is not straightforward. We concur that liver copper measurement is a valuable diagnostic test, but the diagnostic yield has been subject to debate and as Dr Brewer agrees, (a)typical WD cases with normal copper quantification have been reported.[3]

Indeed, in hindsight with all the diagnostic clues available as laid out in the paper, it is fairly straightforward to make the diagnosis in this case. Unfortunately, as we have learned, clinical reality is sometimes different and diagnosing an atypical case of WD may be a challenging task. Then why publish such a case? The prime reason for publication of our case was to post the readers of this journal on the diagnostic pitfalls in WD. As duly noted there are multiple confounders in WD such as atypical age at presentation, non-characteristic laboratory parameters and unusual clinical features.[4] In addition, we hope to draw attention to the possibility of mutational analysis of ATP7B to make the right diagnosis. Dissemination of this information helps other clinicians to make better decisions for their patients in the future, and that is central to this case report.

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LETTER TO THE EDITOR

Severe and symptomatic hyponatraemia after moxifloxacin intake

Hyponatraemia, i.e., serum sodium levels below 134 mmol/l, is one of the most common electrolyte disorders, affecting up to 1% of all hospitalised patients and as many as 18% of nursing home patients. Despite the relatively large number of potential causes of hyponatraemia, antidiuretic hormone (ADH) dysregulation appears to be the most common. In the syndrome of inappropriate antidiuretic hormone secretion (SIADH), which consists of hyponatraemia, inappropriately elevated urine osmolality, excessive urine sodium excretion, and decreased serum osmolality, hyponatraemia is a result of excess water accumulation in the body.[1] Causes of SIADH can be divided into central nervous system diseases, pulmonary disorders, malignant diseases, drugs, surgery, and idiopathic. SIADH is a well-established side effect of certain psychoactive drugs, comprising antidepressants as well as antipsychotic and antiepileptic drugs, and anticancer agents.[2] In contrast, antibiotics are rare causes of drug-induced SIADH;[3] however, they require clinical attention. Therefore, we wish to add a case of moxifloxacin-associated SIADH.

A 73-year-old woman was started on antibiotic treatment with moxifloxacin 400 mg daily for acute febrile upper airway infection. The patient has been on steady antihypertensive medication, comprising hydrochlorothiazide 12.5 mg, amlodipine 5 mg, ramipril 10 mg, bisoprolol 5 mg, and olmesartan 20 mg once daily, for several years. In the following days, nausea, tiredness, agitation/anxiety, disorientation, dysarthria, and muscle cramps occurred. After an incidental fall on day 3, the patient was referred to our emergency room. Laboratory studies showed marked hyponatraemia (108 mmol/l, normal: 136 to 148) with corresponding serum hypo-osmolality (230 mOsm/kg, normal: 275 to 300) and urine osmolality (525 mOsm/kg, normal: 50 to 1400) exceeding serum osmolality, indicating SIADH in the absence of renal, adrenal, and thyroid failure. Moxifloxacin was discontinued and intravenous saline treatment was initiated. Cranial computed-tomography and chest X-ray proved negative. Serum sodium concentration normalised within eight days with the antihypertensive medication continued and the patient recovered fully.

A score of 7 out of a possible 12 on the Naranjo Adverse Drug Reaction Probability Scale indicated a probable relationship between hyponatraemia and moxifloxacin. However, it is worth noting that some of the antihypertensive drugs taken by the patient may have contributed to or aggravated the moxifloxacin-associated hyponatraemia. While diuretics, including thiazides, are common causes of drug-induced hyponatraemia, angiotensin-converting enzyme inhibitors and amlodipine are rare causes.[2]

Clinicians should be alert to hyponatraemia as a rare potential adverse effect of moxifloxacin, or possibly quinolones as a class, in addition to their well-known central nervous system adverse effects. Concomitant use of diuretics, advanced age, and female gender are risk factors for drug-induced hyponatraemia.

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Netherlands
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PHOTO QUIZ

A well-circumscribed density along the right heart border

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CASE REPORT

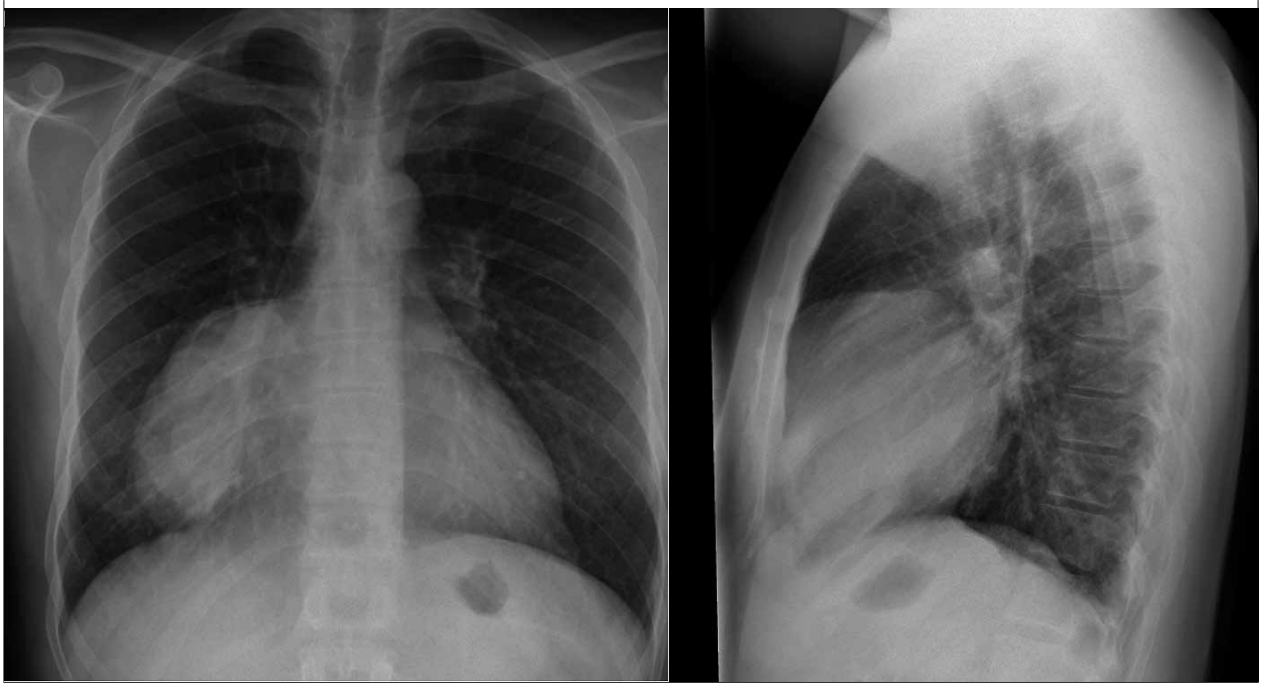
A 25-year-old male was evaluated because of a density along the right heart border, which was accidentally diagnosed during a tuberculosis screening. He was asymptomatic and his medical history was unremarkable. He denied shortness of breath, cough, fever or history of trauma *and his weight was stable*. He had smoked 15 cigarettes daily for 10 years and he had worked in the metal industry for four years. The physical examination was unremarkable and his laboratory tests were normal.

The chest radiograph showed a density along the right heart border (*figure 1*) and further analysis was performed.

WHAT IS YOUR DIAGNOSIS?

See page 199 for the answer to this photo quiz.

Figure 1. *Chest radiograph (posteroanterior and lateral) showing a well-circumscribed density along the right heart border*



ANSWER TO PHOTO QUIZ (ON PAGE 198)

A WELL-CIRCUMSCRIBED DENSITY ALONG THE RIGHT HEART BORDER

DIAGNOSIS

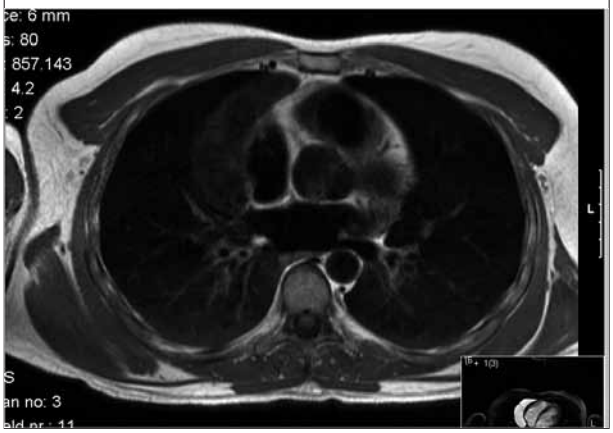
Pericardial cysts are benign and rare intrathoracic findings with an estimated prevalence of 1:100,000 persons.[1,2] Pericardial cysts are most frequently located at the right cardiophrenic angle (51 to 70%), less frequently at the left cardiophrenic angle (28 to 38%) and rarely in other mediastinal locations (8 to 11%).[1,2]

They may be either congenital or acquired.[3] The size of these cysts varies from 2 to 28 cm as reported by Braude *et al.* (10 cm in our patient).[2]

Pericardial cysts are often discovered as an incidental finding on imaging studies obtained in an asymptomatic patient.[1,2] They are not usually associated with symptoms and follow a benign course but rare complications of pericardial cysts have also been reported and include haemorrhage into cysts with tamponade, cysts rupture, torsion or erosion of the cysts into adjacent structures, such as the right ventricular wall. Obstruction of the right mainstem bronchus has also been reported.[2,3]

Patel *et al.* believe that echocardiography is a superior noninvasive modality to delineate the exact position of a pericardial cyst and to differentiate a cyst from other potential diagnoses such as a prominent fat pad, ventricular aneurysm, prominent atrial appendage, aortic aneurysm, and solid tumours.[1] The differential diagnosis of a cardiophrenic angle mass also includes lipoma, and Morgagni hernia.[4] Doppler is particularly helpful in differentiating a pericardial cyst from other vascular structures such as an aneurysm.[1] Echocardiography and a heart MRI confirmed the diagnosis in our patient (*figure 2*). After three years of follow-up, the pericardial cyst in our patient was slightly enlarged and the patient still had no symptoms. The cyst enlargement was the reason why our patient underwent surgical removal of the pericardial cyst. His postoperative recovery was unremarkable.

Figure 2. *The heart MRI showed pericardial cysts along the right heart border*



Surgical excision of pericardial cysts or percutaneous aspiration is, in general, only recommended in symptomatic patients[2] although if cysts are large as in our case, cyst removal should be considered because of the possibility of complications such as haemorrhage into the cyst with tamponade.[1,3]

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PHOTO QUIZ

Bullous dermatosis

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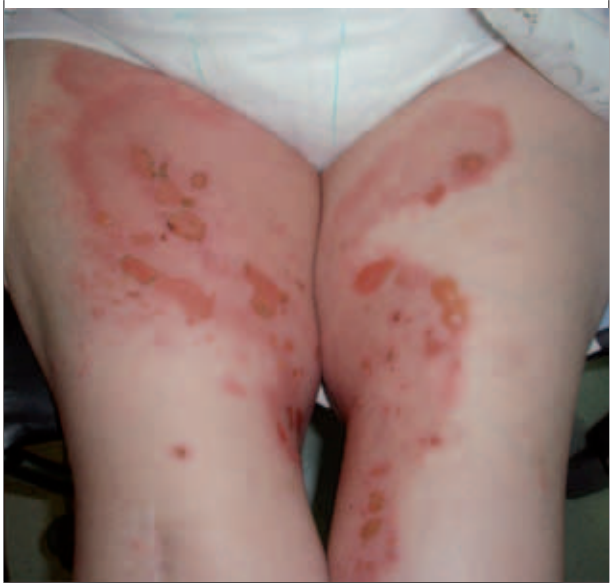
CASE REPORT

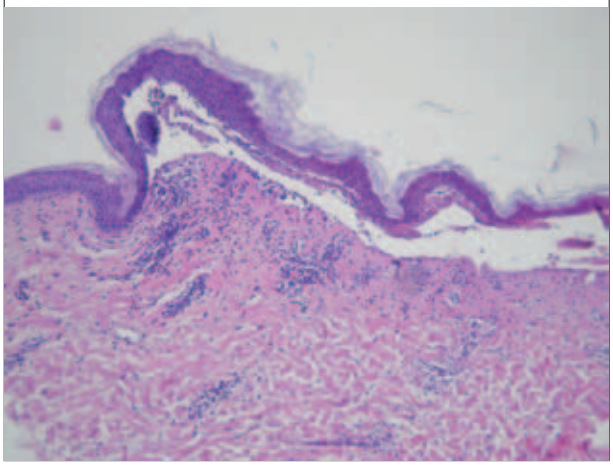
An 83-year-old woman presented with a six-month history of intensely itching cutaneous bullous lesions affecting the trunk and limbs. Her medical history included type 2 insulin-dependent diabetes mellitus and autoimmune hypothyroidism in treatment with levothyroxine. Initially, she had been misdiagnosed as bullosis diabeticorum and treated with topical antiseptics with no improvement.
Physical examination revealed multiple erosions and tense bullae on an erythematous base, filled with clear fluid, located predominantly on trunk and extremities (*figure 1*).

Figure 1. *Clinical image showing tense blisters situated on inflamed skin along the thighs (severe pruritus was reported by the patient)*



No blister spreading was detected after the application of tangential pressure to the skin (negative Nikolsky's sign). Mucous were not involved.
Laboratory evaluation revealed a high absolute eosinophil count (1456 x 10^9/l) and a slightly elevated sedimentation rate without other abnormalities. Skin biopsy showed a subepidermal blister with a dermal leucocyte infiltrate rich in eosinophils (*figure 2*).

Figure 2. *Histological image of a skin biopsy from the edge of a blister reveals a subepidermal blister with a dermal leucocyte infiltrate rich in eosinophils*



WHAT IS YOUR DIAGNOSIS?

See page 201 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 200)

BULLOUS DERMATOSIS

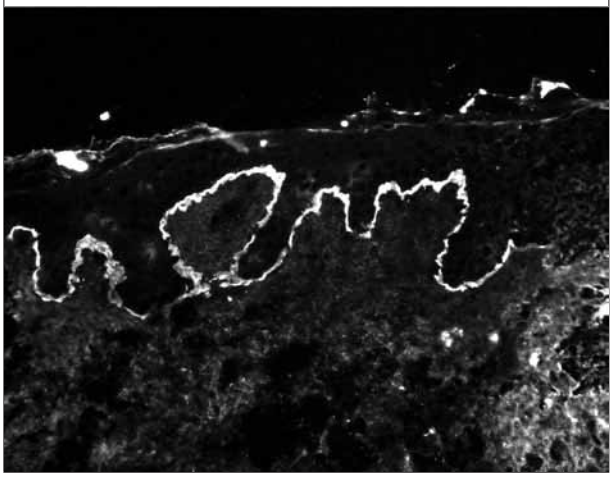
DIAGNOSIS

On direct immunofluorescence, linear reactivity for C3c and IgG was found at the dermal-epidermal junction (*figure 3*), confirming the diagnosis of bullous pemphigoid. Oral prednisone (0.5 mg/kg/day), oral dexchlorpheniramine and topical steroids were administered, achieving complete resolution of the lesions without scarring within two months. During the next 12 months, the patient only presented intermittent outbreaks of localised lesions that were successfully treated with the application of topical corticosteroids in short cycles of one to two weeks.

Bullous pemphigoid (BP) is a chronic, autoimmune blistering disease that primarily affects the skin. BP is characterised by the presence of immunoglobulin G (IgG) autoantibodies specific for the hemidesmosomal BP antigens BP230 (BPAg1) and BP180 (BPAg2). IgG autoantibody and C3 deposition seem to be T cell mediated, HLA restricted and possibly related to dysregulation of auto-reactive regulatory T cells.[1]

It occurs mainly in the elderly and rarely in children. Onset is typically between 60 and 80 years of age.[2] Patients with BP present with tense blisters with cutaneous involvement. Oral mucosal lesions are present in approximately one third of patients. The onset of BP may be either subacute or acute, with widespread, tense blisters. Significant pruritus is frequently present. In some patients, the blisters arise after persistent urticarial lesions. These blisters are usually distributed in the upper and lower extremities, groin, axilla, and abdomen.[3]

Figure 3. *Direct immunofluorescence microscopy of perilesional skin from a patient with bullous pemphigoid demonstrates in situ continuous linear deposits of C3c and IgG along the dermoepidermal junction*



The diagnosis of BP is confirmed with histological and immunopathologic studies. The histology of a BP lesion is characterised by a subepidermal vesicle or bullae, with an inflammatory infiltrate consisting predominantly of eosinophils and polymorphonuclear cells. Direct immunofluorescence studies of perilesional skin demonstrate the presence of immunoglobulins, most frequently IgG and C3, along the epidermal basement membrane zone.

The differential diagnoses for BP are cicatricial pemphigoid, pemphigus vulgaris, drug-induced bullous disorders, epidermolysis bullosa acquisita, herpes gestationis, dermatitis herpetiformis, linear IgA dermatosis and other bullous eruptions such as bullous erythema multiforme, bullous lupus erythematosus or porphyria cutanea tarda. Autoantibody formation in BP may also have a paraneoplastic aetiology, especially in lymphoproliferative neoplasms.

The treatment of BP is determined by the extent of involvement and rate of disease progression. As in other autoimmune bullous diseases, the goal of therapy is to decrease blister formation, to promote healing of blisters and erosions, and to determine the minimal dose of medication necessary to control the disease process. Localised lesions can be controlled with topical corticosteroids. In patients with progressive disease with involvement of multiple sites, systemic corticosteroids are often necessary.[2] Prednisone provides good control of the disease and the accompanying symptoms, including pruritus. In some patients, antihistamines can be used to control the pruritus. Recently, Rituximab has been described to be successfully applied in corticosteroid-refractory BP patients.

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Netherlands
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PHOTO QUIZ

A young man with progressive dysphagia

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CASE REPORT

In 2008, a 27-year-old Caucasian male presented with dysphagia which had been present for over two years. His symptoms had been progressive over the last few months with solid food impaction, which resolved spontaneously, several times a week. His weight had remained stable and he did not complain of pyrosis, acid reflux or chest pain. An upper gastro-intestinal (GI) tract endoscopy had already been performed in 2006, but a clear aetiology had not been established. Proton pump inhibitor (PPI) treatment had not relieved these symptoms. Medical history revealed an allergic reaction of unknown origin at the age of six with angio-oedema of buccal and genital mucosa. In addition he reported a strong family history of allergic rhinitis, from which he also suffers. An upper GI tract endoscopy was performed and revealed a characteristic finding *(figure 1A* and *1B)*.

WHAT IS YOUR DIAGNOSIS?

See page 203 for the answer to this photo quiz.

Figure 1A. *Endoscopic picture of upper oesophagus showing concentric fixed ring-like mucosal deformation or 'trachea-like' aspect*

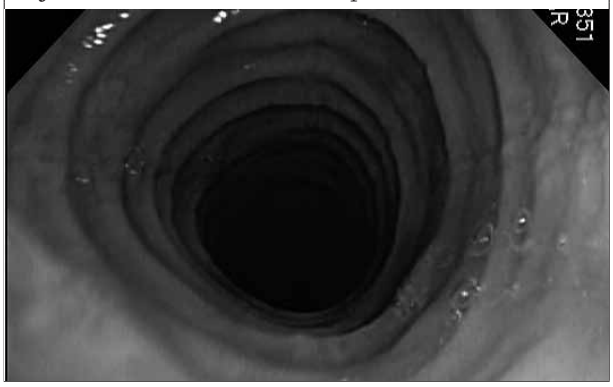
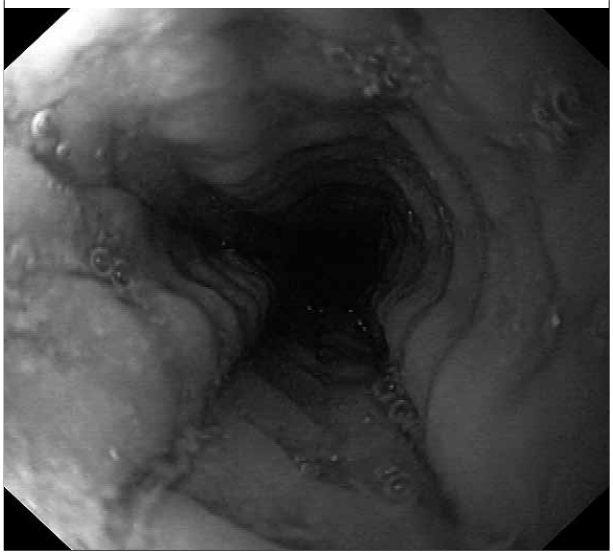


Figure 1B. *Endoscopic picture of mid oesophagus showing oesophageal furrowing with longitudinal lines and nodular aspect of mucosa, indicative of mucosal oedema and thickening*



ANSWER TO PHOTO QUIZ (ON PAGE 202)

A YOUNG MAN WITH PROGRESSIVE DYSPHAGIA

DIAGNOSIS

At endoscopy fixed rings and longitudinal furrowing were seen in the upper and mid oesophagus *(figure 1)*. Biopsy of upper oesophageal tissue revealed dense eosinophilic infiltration (240 eosinophils per high-power field (HPF) of 0.2 mm^2) *(figure 2)*.

Laboratory examination showed marked elevation of serum IgE (802 IU/ml), without eosinophilia. Serum allergy testing was positive for several food allergens (milk, wheat and peanuts), aerogenic allergens (grass and tree pollen) and dust mites.

These macroscopic findings, the eosinophilic infiltrates, the lack of response after two months of PPI treatment and the diminution of symptoms following initiation of topical steroid therapy (swallowing fluticasone propionate 250 µg/puff, two puffs twice daily) confirmed the diagnosis.

EO has been described for over three decades, but has become an entity of increasing interest because of its apparent rising prevalence with an incidence of around 1:10,000 persons/year. EO predominantly affects children and young adults. Men are more commonly affected than women.[1,2]

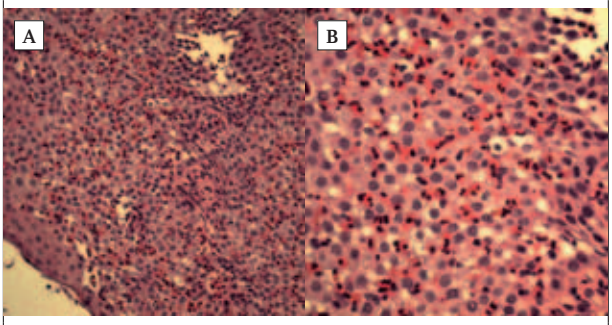
Common presenting symptoms in adults are dysphagia, food impaction and gastro-oesophageal reflux disease (GERD) symptoms. Its natural history has not been clarified, but no effect on life expectancy or development of (pre)malignant oesophageal disease has been reported.[1,2]

A diagnosis can be made on endoscopic and microscopic findings, after exclusion of other illnesses associated with oesophageal eosinophilia, such as eosinophilic gastroenteritis, GERD and Crohn's disease. At endoscopy longitudinal furrowing, whitish exudates, fixed rings and strictures can be seen in the proximal, mid or distal oesophagus. The lack of macroscopic abnormalities, however, does not exclude the disease. Therefore, in patients with typical symptoms but without endoscopic abnormalities, biopsies of normal mucosa should be considered.[2] Microscopic features associated with EO are >15 intraepithelial eosinophils per HPF, eosinophilic microabscess formation, superficial epithelial layering of eosinophils and basal zone hyperplasia.[1] Peripheral eosinophilia, high total IgE and food specific IgE have been described. An association with allergic rhinitis, eczema and asthma has also been recognised.[1]

Recommended treatment strategies for EO include diet therapy with specific food restrictions and topical or systemic steroids.[2,3] Oesophageal strictures, a complication of EO, might be safely dilated, although oesophageal perforation has been described.[4]

This case illustrates the significant burden of this disease and emphasises the importance of improved awareness.

Figure 2. *Light microscopic picture of upper oesophageal tissue*



Marked infiltration of eosinophils, layered from submucosa to superficial epithelium, and microabscess formation (clustering of 4 or more eosinophils) (haematoxylin and eosin stain; original magnification A) x 100 and B) x 200).

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

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