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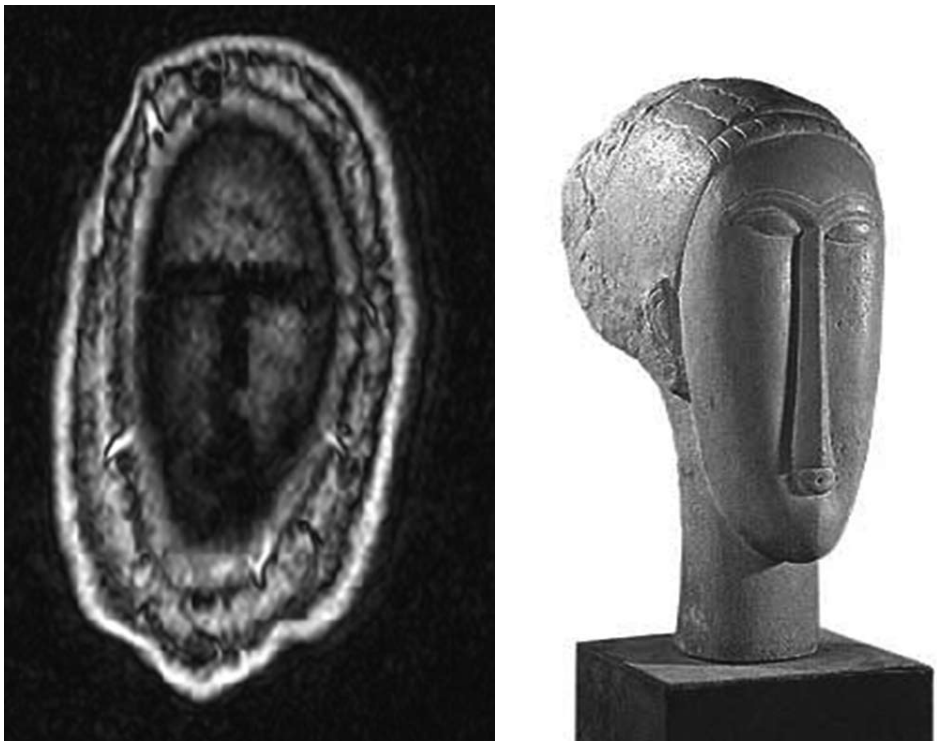

PHOTO QUIZ: The Modigliani head, see page 270

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EDITORIAL

Autoantibodies against multiple tissues in type 1 diabetes

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Diabetes mellitus type 1 (DM1) results from a T-cell mediated destruction of the insulin-producing β-cells in the pancreatic islets of Langerhans. The role of autoantibodies in the pathogenesis of this disease is unclear.[1] Whether islet autoantibodies count as 'smoke or fire' in β-cell destruction remains a question. Approximately 20% of DM1 patients are seronegative for islet autoantibodies, indicating that autoantibodies are not required for the development of DM1, whereas the vast majority of subjects with islet autoantibodies remain nondiabetic. The targets of islet autoantibodies including insulin, glutamic acid decarboxylase-65 (GAD65) and the tyrosine phosphatase insulinoma antigen-2 (IA-2) are not tissue specific, while autoantibodies against these are not disease specific. Yet islet autoantibodies are the most apt predictors of clinical manifestation of DM1 in first-degree relatives of patients. In particular, high titres of multiple autoantibodies are considered synonymous with preclinical diabetes.

In their contribution in this issue of the journal, De Graaff, Smit and Radder studied the literature on autoantibodies with specificity for other target tissues than pancreatic islets, with possible clinical implications.[2] Indeed, DM1 is frequently associated with multiple autoimmune features that are not limited to pancreatic islets.[3,4] Autoantibodies against thyroid tissue (thyroglobulin (Tg) and thyroid peroxidase (TPO)), parietal cells in the stomach (PCA), adrenal cortex (adrenocortical antibodies, ACA) or gut (endomysium, EMA) occur in widely diverse frequencies in different cohorts of DM1 patients, even though this is not accompanied with associated clinical symptoms in the majority of cases.

What is the cause of the occurrence of non-islet autoantibodies and comorbidity of autoimmune diseases in DM1? First, the genetic predisposition to DM1 is shared with other autoimmune diseases. Polymorphisms of the human leucocyte antigen (HLA) are perhaps most important in this regard. Indeed, HLA-DR3 predisposes to a variety of autoimmune diseases including DM1, thyroiditis and coeliac disease. Recently, it was shown that development of DM1 is associated with impaired function of the regulatory T cells, in spite of equivalent frequencies of these immune suppressors in the blood of patients compared with nondiabetic control subjects.[5] It is conceivable that an impaired capacity of the immune system to keep immune abnormalities in check may lead to loss of immune tolerance to other tissues.

A prominent observation by De Graaff and colleagues involves the considerable degree of inconsistency between the reports in their literature study. An important confounder is HLA predisposing to many autoimmune diseases. A caveat is therefore that the probability of developing any type of autoantibody or autoimmune disease may be associated with genetic predisposition, rather than the development of DM1. Since the frequency of HLA-DR3 differs considerably across Europe (coincident with the local incidence of DM1), it may not be unexpected that the frequencies of autoantibodies in the control populations differed between the reports. Some of the supposed increased frequencies of autoantibodies in DM1 patients compared with nondiabetic control subjects will be confounded by inappropriate matching for HLA: the frequency of EMA autoantibodies often appears to be quite similar in DM1 patients to that of HLA-DR3 positive nondiabetic control subjects. As the authors noticed, the prevalence of several tissue-specific autoantibodies increases with age, and with the disparities in age of the cohorts compared here, the frequencies of autoantibodies will vary. Consistency between the various reports will further suffer from the small sizes of the study cohorts and ascertainment bias.

There are some striking differences between islet autoantibodies and autoantibodies against other tissues. With age, islet autoantibodies become less frequent, whereas those against the thyroid and possibly parietal cells increase. Furthermore, while a gender bias in DM1 is negligible, there is a female preponderance for

autoantibodies against the thyroid and possibly the adrenals. Disparities between reports may again be attributed to insufficient matching between patient and control populations for age, gender and HLA.

What are the clinical consequences of autoantibody positivity? As indicated above, the presence of islet autoantibodies has not yet been shown to be pathogenic. There is no evidence that islet autoantibodies impair β-cell function. Intriguingly, changes in autoantibody titres during experimental immunotherapy are discordant with clinical benefit, while immunointervention strategies targeting B-cells or antibodies (e.g., plasmapheresis, intravenous immunologlobulin therapy) have failed thus far.[1] Remarkably, transplacental transfer of maternal islet autoantibodies from diabetic mothers appears to prevent rather than precipitate DM1 in the offspring. This is in contrast to the situation in preclinical animal models of the disease. Indeed, the nonobese diabetic mouse spontaneously develops an immune mediated β-cell destruction that is clearly B-cell dependent. This may represent yet another case where animal models may be misleading. As every model represents an inbred population, this underscores the appropriateness of De Graaff and colleagues to exclude case reports in their literature study.[6] A clinical trial in progress assessing the effect of anti-B-cell therapy (rituximab) may shed light on the role of B cells in the pathogenesis of DM1 (see www.diabetestrialnet.org for details).

For several other tissue-specific autoantibodies, the evidence supporting a role in the disease process is more compelling. Antibodies against thyroid, gastric parietal cells and adrenal cortex have unequivocally been shown to affect target tissue function. EMA autoantibodies are very strongly associated with villous atrophy in biopsies of the small intestine, even in cases where clinical symptoms of coeliac disease are lacking. Interestingly, an important target of EMA autoantibodies is the enzyme tissue transglutaminase (tTG). This enzyme is critically important in the pathogenesis of coeliac disease, as it modifies gluten components by deamidation, introducing epitopes of pathogenic T cells in coeliac disease. Despite the striking discovery that tTG is the main target of autoantibodies in coeliac disease, it remains to be elucidated whether, and if so, how they contribute to disease. Given the exceptionally strong similarities between coeliac disease and DM1 that include comorbidity, epidemiology, rise in incidence, genetic predisposition and gluten as environmental risk factor, it is conceivable that autoimmune components contribute to dietary triggering of the symptoms of coeliac disease.

Even though clinical symptoms are often lacking, the recommendation by De Graaff and colleagues to monitor for subclinical autoimmune disease associated with autoantibodies against tissues other than islets is warranted and worthy of implementation in diabetes care.

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REVIEW

Overview of research on health-related quality of life in patients with chronic liver disease

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ABSTRACT

Health-related quality of life (HRQoL) has become an important outcome measure in patients with chronic liver disease (CLD). In this article, an overview is given of the most common measurement instruments of HRQoL, determinants of HRQoL in patients with CLD, and current developments in the implementation of routine measurement of HRQoL in daily clinical practice. Well-developed generic instruments of HRQoL are the Short Form-36 (SF-36), the Nottingham Health Profile (NHP) and the Sickness Impact Profile (SIP). Well-developed liver disease-specific HRQoL instruments are the Hepatitis Quality of Life Questionnaire (HQLQ), the Chronic Liver Disease Questionnaire (CLDQ), the Liver Disease Quality Of Life Questionnaire (LDQOL), and the Liver Disease Symptom Index 2.0 (LDSI 2.0). Commonly used HRQoL measures in cost-effectiveness studies are the Health Utilities Index (HUI), Short Form-6D (SF-6D) and the EuroQol-5D (EQ-5D). HRQoL of patients with chronic liver disease has been shown to be impaired, with patients with hepatitis C showing the worst HRQoL. Disease severity, pruritis, joint pain, abdominal pain, muscle cramps, fatigue, depression and anxiety have been associated with HRQoL in patients with CLD. Recently, studies assessing the feasibility and effectiveness of measuring HRQoL in daily clinical practice have been performed, generally showing positive results regarding the discussion of HRQoL-related topics, but mixed results regarding the added value of actual improvement in HRQoL. Furthermore, logistic and attitudinal barriers seem to impede successful implementation. Nevertheless, given the importance of HRQoL in liver patients, we should persist in measuring and subsequently improving HRQoL in clinical practice.

KEYWORDS

Hepatitis, liver, quality of life

INTRODUCTION

Due to continuously improving medical treatment, many formerly lethal diseases have nowadays become chronic. It has been calculated that one quarter to one third of the adult population in the Netherlands has a chronic disease (van den Berg & van den Bos 1989, Monthly Indicators, Statistics Netherlands (CBS) 3, 4-21). The increasing prevalence of chronic disease in developed countries has led to an increased focus on the emotional and social well-being of patients as well as their physical well-being, referred to as health-related quality of life (HRQoL). To illustrate the increasing interest in HRQoL in medical treatment, a count of hits in PubMed when entering the search term 'quality of life' in title and/or abstract shows an increase of over 31-fold in the past 20 years (from 2266 articles in 1986 to 70,796 articles in 2006). Despite this increase in research, the impact on clinical practice has been limited: to date, HRQoL assessment has largely been restricted to patients in a research environment. However, the importance of using HRQoL information for the improvement of physician consultations is increasingly being acknowledged. In 1992, a large conference was dedicated to the topic of 'Applications of health status assessment measures in clinical practice',[1] and in June of 2007, another conference on this topic took place (www.isoqol.org). Furthermore, several high-impact articles have been published on this topic since 2001.[2-4] This article will discuss HRQoL specifically for patients with chronic liver disease (CLD), its measurement, and current developments in the implementation of routine measurement of HRQoL in clinical practice.

CHRONIC LIVER DISEASE

CLD is one of the most prevalent diseases in the world. The most common causes of CLD, hepatitis B virus (HBV) and hepatitis C virus (HCV), have been estimated to affect 360 million and 200 million people worldwide respectively (www.epidemic.org, 4-12-2006). In addition, alcohol is another main cause of end-stage liver disease worldwide, and alcoholic liver disease is the second most common reason for liver transplantation in the United States.[5] In the Netherlands, CLD affects approximately one in 400 people (www.statline.cbs.nl, 4-12-2006). CLD is a serious disease that is associated with significant morbidity and mortality. Patients may suffer from specific complications of cirrhosis such as hepatic encephalopathy, ascites and variceal bleedings. Furthermore, fatigue, joint pain, pruritis, loss of appetite, depression, abdominal pain, worries about complications of the disease, decreased sexual interest/activity, loneliness, hopelessness, problems with social interaction and problems with memory/concentration have been associated with CLD.[6-12] Given the many effects that CLD may have on patients, HRQoL should be considered an important outcome measure in the treatment of CLD patients.

DEFINITION OF HEALTH-RELATED QUALITY OF LIFE

HRQoL was adapted from the more general and wide-ranging concept 'quality of life' (QoL). Because this is such a broad concept, there is no universally accepted definition for QoL. In this study we have adopted the WHO definition of the multidimensional concept of QoL: 'individuals' perceptions of their position in life in the context of the culture and value system in which they live and in relation to their goals, standards, and concerns'.[13] Due to the multidimensionality of the concept, it is not practical (or perhaps not possible) to assess all that is meant by QoL simultaneously. Therefore, a more limited and focused assessment should be undertaken. With regard to chronic illness, QoL should be determined by health parameters, and not by more general parameters such as economic status or environment since these are often distant from health or medical concerns.[14] This has led to the concept of HRQoL. HRQoL ranges from negatively valued aspects of life, including death, to the more positively valued aspects such as role function or happiness. The general consensus is that it consists of three core domains: psychological functioning (well-being and emotional status), social functioning, and physical functioning.[15] It should be noted that this definition of HRQoL is from a patient or clinical perspective, which is the main focus of this article. HRQoL can also be looked at from a cost-effectiveness perspective. This will be described more elaborately in the paragraph on utility measures.

USE OF HRQOL ASSESSMENTS IN HEALTH CARE

In general, there are four main uses of HRQoL assessments in health care: 1) treatment comparisons in clinical trials, 2) patient population studies to evaluate the burden of the disease in terms of HRQoL, 3) health economics evaluations to determine the best use of health care resources, and 4) treatment choices in individual patient care.[14] This article will focus on elements mentioned in point two, i.e. levels of HRQoL in patient populations with various forms of liver disease, and elements mentioned in point four, i.e. HRQoL assessment at individual patient level.

Measurement of HRQoL

HRQoL includes a physical, a social, and a mental component, each of which consist of multiple subcomponents. For example, the mental component can consist of depression, but also of anxiety. Typically, these components can not be readily observed. Indeed, one of the arguments for asking patients to judge their own HRQoL with the use of questionnaires is that it has been shown that physicians are generally unable to adequately judge their patients' HRQoL.[16] Judgements of physicians do not only deviate from those of patients, they also differ between physicians.[16] Especially this last variability makes it difficult to obtain 'objective' judgements of HRQoL. Measurement of HRQoL is therefore done by means of standardised, self-administered questionnaires. Note that the patients' judgements about their own HRQoL are still subjective: patients with the same physical state might give us different views about their HRQoL, but this outcome no longer depends on the observer. There are two basic types of HRQoL questionnaires that measure HRQoL from this patient perspective: generic questionnaires and disease-specific questionnaires. A third type of HRQoL questionnaires exists that measures HRQoL from a cost-effectiveness perspective. These are called utility measures.

Generic questionnaires

Generic HRQoL questionnaires include a spectrum of domains of HRQoL that apply equally to various patient populations. Generic questionnaires have the advantage that the scores of the patients can be compared with the scores of other patient populations and/or a healthy control population. A disadvantage is that generic instruments are not designed to identify disease-specific domains that may be important to establish clinical changes.[17] The most

generic form is just one question 'how is your quality of life today', with for instance a visual analogue scale (VAS) as answering mode. The three most commonly used generic HRQoL instruments, according to a recent review,[18] are the Nottingham Health Profile (NHP), the Medical Outcomes Study Short Form-36 (SF-36) and the Sickness Impact Profile (SIP) (*table 1*). The SIP has a broad coverage of topics, but is therefore very long.[19] The NHP focuses on more severe levels of disability and has thus been known to be less sensitive to changes in conditions where effects are relatively mild.[20,21] The SF-36 is sensitive to a wider range of disability levels, from the general population to patients with severe levels of disability.[22] All three instruments have sufficient psychometric properties, as shown in *table 1*. For health care workers interested in a broad range of HRQoL topics, we recommend using the SIP if it is feasible for the patients to complete this lengthy instrument. Shorter instruments are the NHP and the SF-36. Since the NHP is less sensitive in patients with relatively mild conditions, we recommend the use of the SF-36, which is applicable to a broader range of conditions. Furthermore, the SF-36 is currently the most used HRQoL instrument in studies worldwide, and shorter versions are available.

Disease-specific questionnaires

Disease-specific questionnaires are designed to be valid only for a specified condition and have the advantage of providing greater specificity and sensitivity.[23] Four liver disease-specific HRQoL questionnaires have been developed and used extensively (*table 2*). The first liver disease-specific HRQoL questionnaire to be systematically developed and employed was the Hepatitis Quality of Life Questionnaire (HQLQ),[9] followed by the Chronic Liver Disease Questionnaire (CLDQ),[10] the Liver Disease Quality Of Life questionnaire (LDQOL),[11] and lastly, the Liver Disease Symptom Index 2.0 (LDSI 2.0).[12] All four instruments have strengths and weaknesses. The HQLQ consists of the widely validated generic SF-36 with five added disease-specific subscales, but it excludes patients with other chronic liver disease than HCV. The CLDQ is a short and therefore feasible questionnaire, but is unable to discriminate between more advanced stages of liver disease. The LDQOL addresses a variety of domains, but is therefore very long (101 items). This may be a problem when completion time is limited, or multiple questionnaires are being administered. The LDSI 2.0 is a short questionnaire that measures nine possible liver disease-specific symptoms, as well as the *hindrance* that

Table 1. *Specifications of the three most commonly used generic HRQoL instruments*

	Nottingham Health Profile (NHP)	Medical Outcomes Study Short Form-36 (SF-36)	Sickness Impact Profile (SIP)
Authors	Hunt *et al.* 1980, 1985[20,21]	Ware *et al.* 1992[88] (Validation study Brazier *et al.* 1992[22])	Bergner *et al.* 1981[19]
No. of items	38	36	136
No. of subscales	7	8	12
Total score	No	Yes	Yes
Reliability	IC: Cronbach's α = 0.70 - 0.85 (Dutch population (86)) TRT: r = 0.75 - 0.88	IC: Cronbach's α > 0.84 (social functioning, α = 0.73) TRT: r = 0.60 - 0.81	IC: Cronbach's α = 0.94 TRT: r = 0.87 - 0.97
Validity*	CV: Ill *versus* healthy people DV: Between groups with various health statuses in a Dutch population[85]	Conv. V: Correlations between four comparable dimensions of SF-36 and NHP were high (r = -0.55 to -0.93) DV: Correlations between non-comparable dimensions of SF-36 and NHP were low (r = -.018 to -0.35)	Conv. V: E.g. Activity of Daily Living Index: r = 0.55- 0.61 DV: E.g. explained variance of Speech Pathology Ratings: R^2 = 0.30) Clinical validity Descriptive validity
Subscales	Energy Pain Emotional reactions Sleep Social isolation Physical mobility	Physical functioning Role limitations due to physical problems Role limitations due to emotional problems Mental health Vitality Bodily pain General health perception Social functioning	Ambulation Body care/movement Mobility Social interaction Alertness behavior Emotional behavior Communication Sleep and rest Eating Work Home management Recreation/pastimes

IC = internal consistency; TRT = test-retest reliability; CV = construct validity; Conv. V = convergent validity; DV = discriminant validity.
***All reported validities have been established.**

patients experience from having these symptoms. The LDQOL, HQLQ and CLDQ fail to address this hindrance, even though having a certain symptom does not always automatically mean that HRQoL is impaired. Psychometric properties of the four instruments are sufficient, as shown in *table 2*. The LDQOL can be used when administration of a lengthy questionnaire is not an issue, and the aim is to obtain information on a wide range of liver disease-specific HRQoL domains. When a short questionnaire is preferred, the LDSI 2.0 is recommended over the CLDQ since it takes symptoms *and* hindrance of these symptoms into account. The HQLQ may be an efficient instrument for health care professionals interested in the HRQoL of patients with HCV, since it comprises generic and disease-specific items simultaneously.

UTILITY MEASURES

Utility measures originated in health economics, and form an important subgroup of generic measures that are used in cost-effectiveness studies[24] and medical decision-making analyses.[25] With utility measures, quality adjusted life years (QALYs) can be computed, which can provide an indication of the benefits gained from a variety of medical procedures in terms of quality of life and survival of the patient. Utility 'values' of health states are typically determined by asking healthy people to rate HRQoL of hypothetical health states, for instance characteristic health states of liver patients, instead of the patients themselves. Consequently, coping is not included. Sophisticated techniques such as Standard Gamble and Time Trade-Off are used to estimate the utility values between 0.00 (a poor state of health) and 1.00 (normal health).[24,25] Besides using these sophisticated but labour-intensive methods, there are generic 'off the shelf' quality of life instruments that provide the utility value as additional outcome. The three most used utility measures are the Health Utilities Index (HUI),[26] the SF-6D[27] and the EuroQoL EQ-5D[28] (*table 3*). We prefer the EQ-5D and HUI over the SF-6D, as the SF-6D has shown a floor effect, especially in liver patients.[29]

HRQOL IN PATIENTS WITH CHRONIC LIVER DISEASE

The vast majority of studies assessing HRQoL in patients with CLD have focused on patients with chronic HCV infection. This interest of the research community in HCV may be explained by the severity of this form of CLD as well as by the debilitating side effects of interferon, which is used to treat some of these patients.

Table 2. *Specifications of the four liver disease-specific HRQoL measures*

	Hepatitis quality of life questionnaire (HQLQ)	**Chronic liver disease questionnaire (CLDQ)**	**Liver disease quality of life questionnaire (LDQOL)**	**Liver disease symptom index 2.0 (LDSI 2.0)**
Authors	Bayliss *et al.* 1998[9]	Younossi *et al.* 1999[10]	Gralnek *et al.* 2000[11]	Unal *et al.* 2001[7]
No. of items	69	29	101	18
No. of subscales	13	6	20	9
Total score	No	Yes	No	Yes
Reliability	IC: Cronbach's $\alpha > 0.80$	TRT: ICC = 0.59	IC: Cronbach's $\alpha > 0.70$ (1 subscale $\alpha = 0.62$)	IC: Cronbach's $\alpha > 0.79$
Validity*	CV: E.g. correlations between limitations and physical factor of the SF-36 (r = 0.69) DV	CV: Worse CLDQ scores with increased disease severity	CV: Worse LDQOL scores with increased disease severity for all subscales	CV: Correlations between symptom severity items and their accompanying hindrance items: r = 0.52 - 0.80)
Subscales	8 subscales of the SF-36 (see *table 1*) + Limitations due to chronic hepatitis C Health distress due to chronic hepatitis C Positive well-being Sleep somnolence Health distress	Fatigue Activity Emotional function Abdominal symptoms Systemic symptoms Worry	8 subscales of the SF-36 (see *table 1*) + CLD-related symptoms CLD-related effects on activities of daily living Concentration Memory Sexual functioning Sexual problems Sleep Loneliness Hopelessness Qual. of social interaction Health distress Self-perceived stigma of CLD	Itch Joint pain Pain in the right upper abdomen Sleepiness during the day Worry about family situation Decreased appetite Depression Fear of complications Jaundice

IC = internal consistency; ICC = intra class correlation; TRT = test-retest reliability; CV = construct validity; DV = discriminant validity.
***All reported validities have been established.**

Table 3. *Specifications of the mostly used utility measures*

	EuroQol-5D (EQ-5D)	Health utilities index (HUI 3)	Short form-6D (SF-6D)
Authors	EuroQol Group 1990, Brooks 1996[28]	Feeney *et al.* 1995[26]	Brazier *et al.* 2002[27]
No. of items	5	31	10
No. of dimensions	5	8	6
Nr. of unique health states	243	972,000	18,000
Total score	Yes	Yes	Yes
Reliability	TRT: ICC = 0.81	TRT: ICC = 0.87	TRT: ICC = 0.83
Validity*	CV: Spearman correlation with HUI 3 = 0.80 Spearman correlation with SF-6D = 0.70 DV: Able to discriminate between mildly, moderately, severely and very severely disabled patients	CV: Spearman correlation with EQ-5D = 0.80 Spearman correlation with SF-6D = 0.69 DV: Able to discriminate between mildly, moderately, severely and very severely disabled patients	CV: Spearman correlation with EQ-5D = 0.70 Spearman correlation with HUI 3 = 0.69 DV: Able to discriminate between mildly, moderately, severely and very severely disabled patients
Dimensions	Mobility Self-care Usual activity Pain/discomfort Anxiety/depression	Vision Hearing Speech Ambulation Dexterity Emotion Cognition Pain	Physical functioning Role limitations Social functioning Pain Mental health Vitality

TRT = test-retest; ICC = intraclass correlation; CV = construct validity; DV = discriminant validity. All tests of reliability and validity were performed in a sample of patients with multiple sclerosis.[87] *All reported validities have been established.

Side effects of interferon may include fever, aching muscles, fatigue, depression, aggression, impotence, hair loss and eczema. These side effects often have consequences for family life, work, and other aspects of daily living. Indeed, studies assessing HRQoL in HCV patients with and without interferon treatment have shown the HRQoL of these patients to be impaired.[30-34] Studies including CLD patients with other disease aetiologies than HCV also show impaired HRQoL.[35-39] Of all patients with CLD, those with HCV seem to have the worst HRQoL.[35]

Determinants of HRQoL in patients with chronic liver disease

Despite the many studies that have shown a reduced HRQoL in hepatology, relatively few studies have investigated which factors influence liver patients' HRQoL. That is a problem when we want to move from just measuring HRQoL towards treatments that improve HRQoL. Disease severity, as indicated by stage of fibrosis (absent, early or advanced) or Child Pugh scores, seems to determine HRQoL.[8,37,39,40] Such a relationship between disease severity and HRQOL seems fairly self-evident as we are dealing with 'health related' quality of life. Nevertheless some studies did not find this relationship.[32,41,42] This may have been due to the relatively small number of patients with CLD in a more advanced stage that were included in these studies: Foster *et al.* (1998) did not include patients with cirrhosis, Kramer *et al.* (2005) excluded patients with decompensated cirrhosis and most patients in the study had mild chronic hepatitis (Child Pugh stage A without ascites). Over 70% of the patients in the study performed by Hauser *et al.* (2004) did not have cirrhosis. Besides disease severity, physical symptoms of CLD such as pruritis, joint pain, abdominal pain, and muscle cramps have been shown to be related to HRQoL.[8,38,43] Fatigue is also of concern in patients with CLD.[8,36,42,44-46] Lastly, anaemia[47] and low physical activity[48] have been associated with poorer HRQoL in HCV patients.

Besides these mainly physical aspects of the illness, the association between psychological aspects of CLD and HRQoL has also received some attention. Depression, anxiety, illness understanding, social stigma, worry about family situation, fear of complications, problems with concentration and memory, and loneliness are all related to HRQoL in patients with CLD.[8,36,41,49-51] The relative impact of these psychological aspects on HRQoL has, however, not been studied. Furthermore, two important psychological concepts that deserve attention have rarely been assessed in patients with CLD: 'coping' and 'self-efficacy'. 'Coping' refers to the way people deal with stressful situations, such as having a (chronic) disease and the consequences thereof.[52] 'Self-efficacy' refers to an optimistic self-belief that one can perform difficult or new tasks, or that one can cope adequately with adversity.[53] Both coping and self-efficacy have been shown to affect HRQoL in various patient populations,[54-58] but this has never been investigated

for patients with CLD. Including measures of coping and self-efficacy in future studies on HRQoL in patients with CLD is advisable.

IMPLEMENTATION OF HRQOL MEASUREMENT IN CLINICAL PRACTICE

Interest in using HRQoL in clinical practice as more than just an outcome measure has increased.[1-4] Standardised assessment of HRQoL preceding each consultation may potentially provide physicians with valuable information for several reasons. First of all, several studies have shown that physicians vary in their ability to elicit psychosocial information, or that they underestimate patients' HRQoL.[16,59-66] Secondly, various studies have shown that when communication with the physician encompasses both physical and psychosocial issues, patients have better treatment compliance, are more satisfied with the consultation and report less symptoms.[3,59,60,65,67-73] Thirdly, timely recognition of psychosocial problems means that patients can be referred for adequate treatment such as psychotherapy or social work, whereas failure to recognise these problems often results in unexplained symptoms and over-utilisation of health care.[71,73,74]

Studies assessing routine administration of HRQoL in clinical practice have yielded positive findings: availability of HRQoL information to physicians during the consultation was generally well accepted, and physicians expressed an interest in continued use of the information. Furthermore, routine administration of HRQoL in clinical practice has been shown to increase the frequency of: 1) identification and/or discussion of HRQoL-related issues,[2,3,75-77] 2) identification of patients with moderate to severe health problems and/or anxiety,[2,78] and 3) actions being taken.[75,78] A decrease in depression, potential improvement in symptom control, and better HRQoL and emotional functioning have been observed in association with the availability of HRQoL information for the physician,[3,4,77] even though several other studies have failed to show robust evidence to suggest that routine administration of HRQoL in clinical practice is of benefit in actually improving HRQoL or psychosocial outcomes.[2,79-81] This may have been due to the lack of sensitivity of the measures used to detect small changes[82] and/or insufficient clinical relevance of measures to prompt physicians to make changes to patient management.[79] On the other hand, it may be slightly overzealous to expect HRQoL measurement in clinical practice to cause significant improvement in HRQoL since it encompasses so many dimensions.

For a successful implementation of HRQoL assessment in clinical practice, several practical and attitudinal barriers have to be overcome, or at least expected, such as general lack of time, money and human resources, impracticability of instruments, lack of IT support, disruption of clinical routine, and health professionals' lack of knowledge in this area and/or scepticism towards the validity of existing measures.[79,82-85] Efforts should be aimed at optimising practical support such as money and human resources. Furthermore, more research and subsequently additional evidence of the benefits of HRQoL measurement in clinical practice may aid in convincing health professionals of the added value. Any changes in clinical practice are to be expected to be met with some resistance.

CONCLUSION

Studies have shown HRQoL to be impaired in patients with CLD, and many physical and psychological factors have been associated with this impaired HRQoL. However, more conclusive research is desirable on the strength of the relationship of each of these factors with HRQoL in order to be able to determine the focus of treatment. This may also help the clinical decision-making of physicians who use routine HRQoL assessment in clinical practice. With regard to the implementation of HRQoL assessment in clinical practice, and the obstacles experienced in this process, it should be recognised that it is often a long process that requires patience, but the field of HRQoL research has been calling for this move into clinical practice as a logical and needed next step, which will contribute to the improvement of patient care. As long as routine HRQoL assessment is seen as an additional tool for physicians, and the emphasis remains on the clinical experience of the physician and the verbal communication with patients, these barriers should not be a reason to refrain from routine assessment of HRQoL in clinical practice, in our opinion.

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REVIEW

Prevalence and clinical significance of organ-specific autoantibodies in type 1 diabetes mellitus

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ABSTRACT

As diabetes mellitus type 1 (DM1) is associated with other autoimmune diseases, clinical tools are needed to diagnose and predict the occurrence of other autoimmune diseases in DM1. We performed a systematic search of the literature on the prevalence, and the diagnostic and prognostic significance of organ-specific autoantibodies in DM1, focusing on the most prevalent autoimmune diseases in DM1: Hashimoto's disease, autoimmune gastric disease, Addison's disease and coeliac disease.

We found 163 articles that fulfilled our selection criteria. We analysed and compared the prevalence of autoantibodies in DM1 and control populations, studied the relation between antibody prevalence and age, gender, race and DM1 duration and studied the relation between the presence of autoantibodies and organ dysfunction.

Because of the large variation in population characteristics and study design, a uniform conclusion on the relation of these autoantibody prevalences with age, gender, race, DM1 duration and target organ failure cannot be drawn easily. In addition, most studies reviewed used a cross-sectional design. Therefore, few data on the predictive value of the organ-specific antibodies in DM1 populations are present in these studies. Obviously, prospective studies are needed to fill this gap in knowledge.

Despite these restrictions, the general picture from the present review is that the prevalence of the organ-specific autoantibodies is significantly higher in DM1 than in control populations. Given the relevant risk for organ failure in DM1 patients with autoantibodies against thyroid, gastric, adrenal and intestinal antigens, we recommend checking these autoantibodies in these patients at least once, for instance at the diagnosis of DM1. For detailed advice on assessing the different organ autoantibodies and function we refer to the summaries in the results section.

KEYWORDS

Autoimmune antibodies, organ-specific dysfunction, type 1 diabetes mellitus

INTRODUCTION

Type 1 diabetes mellitus (DM1) is a clinical syndrome in which the destruction of the pancreatic islet β-cells leads to progressive insulin deficiency and hyperglycaemia, which in turn gives rise to microvascular complications such as retinopathy, nephropathy, and neuropathy as well as macrovascular complications.[1-4] The presence of autoantibodies targeted against β-cell antigens represents the autoimmune character of DM1.[5] Although the genetic risk for DM1 is considerably lower than for type 2 diabetes mellitus, certain human leucocyte antigen haplotypes are associated with an increased risk for DM1.[6-10] The significance of environmental factors is still unclear, despite recent indications for an infectious origin[2,11,12] and nutritional factors.[13] DM1 is associated with other immune-mediated disorders[14,15] such as autoimmune thyroiditis,[16-19] Addison's disease,[20,21] pernicious anaemia,[22,23] and coeliac disease.[24-26] Autoimmune disorders can be subdivided in organ-specific and non-organ-specific diseases. In organ-specific autoimmune diseases, a single organ or organ system is affected, whereas in non-organ-specific autoimmune diseases, several organs or tissues are involved. As DM1 is associated with other autoimmune diseases, clinical tools are needed to diagnose and predict the occurrence of other autoimmune diseases in DM1.

Organ-specific autoimmune diseases can be part of autoimmune polyglandular syndromes (APS), of which three types can be distinguished.[27,28] Type 1 (APS-I, also called APECED) is characterised by the triad of mucocutaneous candidiasis, autoimmune

hypoparathyroidism and primary adrenal insufficiency (Addison's disease). Other phenomena, such as DM1, primary hypogonadism, alopecia and vitiligo, may also be present. APS-II is the most common form and consists of Addison's disease, autoimmune thyroid disease, DM1, primary hypogonadism, myasthenia gravis and coeliac disease. Vitiligo, alopecia, serositis and pernicious anaemia also occur with increased frequency in individuals with this syndrome. APS-III involves autoimmune thyroid disease, DM1 and vitiligo.[2,27,29] Various other diseases including hypoparathyroidism, myasthenia gravis, stiff man syndrome, premature ovarian failure and hypergonadotrope hypogonadism may also be present.[2] Several theories exist to explain (the combinations of) these autoimmune endocrinological diseases,[30,31] but despite extensive research, their exact aetiology is still unresolved.[2,32] As DM1 is associated with other autoimmune diseases, clinical tools are needed to diagnose and predict the occurrence of other autoimmune diseases in DM1. We performed a search of the literature on the prevalence, and the diagnostic and prognostic significance of organ-specific autoantibodies in DM1, focusing on the most prevalent autoimmune diseases in DM1: Hashimoto's disease, autoimmune gastric disease, Addison's disease and coeliac disease.

METHODS

We studied antimicrosomal or antithyroid peroxidase antibodies (TPO-AB) and antithyroglobulin antibodies (Tg-AB), antiparietal cell antibodies (PCA), antiadrenocortical antibodies (ACA) and antiendomysial antibodies (EMA). We performed a search in MEDLINE up to and including December 2005, using the query 'Search diabetes mellitus, type I [MeSH] AND (antibodies [MeSH] OR autoantibodies [MeSH] OR autoimmunity [MeSH] OR antibod* OR polyendocrinopathies, autoimmune [MeSH]) NOT Case Reports [Publication Type] NOT ((diabetes mellitus, type I [MeSH] AND (antibodies [MeSH] OR autoantibodies [MeSH] OR autoimmunity [MeSH] OR antibod*)) NOT ((thyroid diseases [MeSH] OR thyroiditis [MeSH] OR thyroiditis, autoimmune [MeSH] OR hashimoto's OR TPO OR thyroglob* OR Tg-AB OR Tg AB OR thyroid microsomal antibodies OR antithyr*) OR (Gastric Mucosa [MeSH] OR Parietal Cells, Gastric [MeSH] OR Gastritis [MeSH] OR Gastritis, Atrophic [MeSH] OR Vitamin B 12 Deficiency [MeSH] OR Anaemia, Pernicious [MeSH] OR gastritis OR parietal cell*) OR (Addison's Disease. [MeSH] OR Adrenal Gland Diseases [MeSH] OR Adrenal Cortex [MeSH] OR adrenocortic* OR addison's) OR (Coeliac Disease [MeSH] OR coeliac* OR endomys* OR anti-endomys* OR villous atrophy) OR polyendocrinopathies, autoimmune [MeSH]) NOT Case Reports [Publication Type]) Limits: only items with abstracts, English, Humans'. This search produced 387 articles. From the 387 hits originally found, an abstract-based selection was made of 220 articles that appeared to investigate organ-specific antibodies in an insulin-dependent diabetes mellitus (IDDM) population. Of them, 163 were available in the libraries of Leiden University Medical Centre or the Erasmus Medical Centre in Rotterdam. The other 57 articles could not be obtained and were therefore excluded from the review. Of the 163 articles we screened, 114 investigated organ-specific antibodies in an IDDM population and provided detailed data on at least two items of age, gender, origin or racial background and DM1 duration of the original research populations. From these 114 articles, only those 40 that reported on AB prevalence in both DM1 and controls were selected for comparison of AB prevalences between DM1 and control populations. General information about the various autoantibodies and about their relation with age, gender, race, duration of DM1 and organ dysfunction were gathered from the 163 articles mentioned above.

RESULTS

Thyroid antibodies (Th-AB)

Thyroid autoantibodies (Th-AB) are directed against thyroglobulin (Tg) and thyroid peroxidase (TPO). Tg is the thyroid prohormone and contains tyrosyl residues, which serve as targets for iodination, a process that is mediated by TPO.[33] TPO-AB can activate complement and are directly or indirectly involved in the inflammatory process as observed in autoimmune thyroiditis.[34,35] Tg-AB appear to play no pathogenic role in thyroid disease, probably because of their inability to fix complement,[33] and they are merely regarded as markers of autoimmune thyroid disease.

Thyroid peroxidase antibodies (TPO-AB)

Methods

To detect TPO-AB, some authors used (haem) agglutination,[19,36-48] while others used indirect immunofluorescence,[23,49-58] ELISA,[59-63] or RIA[17,18,64-69] and Kobayashi *et al.*[70] used the microsome test. In the article by Maclaren *et al.*,[71] the antibody detection method was not mentioned. When comparing the TPO-AB prevalences obtained by (haem)agglutination, indirect immunofluorescence, ELISA and RIA, the prevalences found by (haem-) agglutination were generally lower than the prevalences found by other methods. However, this difference did not reach statistical significance.

Prevalences

In general, TPO-AB prevalences in DM1 populations varied between 5.5 and 46.2% (interquartile range (IQR) 11.3-21.2, P_5-P_{95} 5.8-34.5) and in control populations, TPO-AB were present in 0 to 27.0% (IQR 2.0-6.8, P_5-P_{95} 0.1-20).[18,23,38-41,43,47,50,53-57,59,61-66,68,70,71]

Relations

Kokkonen *et al.*[39] investigated TPO-AB prevalence and found a significant relation with age in children with DM1; the highest prevalence of 15.0% was found in 40 patients aged 10 to 14 years, *vs* 5.9% of 17 patients younger than 10, and 11.1% of 26 patients older than 15 years. The results of Trimarchi *et al.*[19] are similar to those of Kokkonen *et al.* Chang *et al.*[64] found a significantly higher TPO-AB frequency in older than in younger age groups: 43.8% in the group older than 25 years, 27.2% in the group of 10 to 25 years and 15.6% in the group younger than 10 years (p<0.01). De Block *et al.*[69] reported that TPO-AB positive DM1 patients were older than TPO-AB negative patients (30±16 *vs* 25±16 years, p=0.012). Cardoso *et al.*[72] also reported a significant correlation between age and TPO-AB (r=0.23, p<0.05). Verge *et al.*[73] found that TPO prevalence rose with age of DM1 diagnosis: 65% in the 0 to 4 years group, 66% in the 5 to 9 years group and 73% in the 10 to 14 years groups (p=0.05). In control populations, the prevalence of TPO-AB rises with age as well.[23,71] Kokkonen *et al.*[39] reported TPO-AB in none of 24 children younger than 10, in 4.1% of 73 children aged 10 to 14 and in 6.3% of 63 children older than 15 years. A female predominance was found in TPO-AB positive DM1 patients.[17,44,50,51,55-57,61,64,69,71,74-76] Some authors compared the percentage of female patients in the TPO-AB positive group with that in the TPO-AB negative group, whereas others compared the TPO-AB prevalences between the two gender groups. Percentages of female patients ranged from 63 to 91% in TPO-AB positive DM1 patients and from 26 to 52% in TPO-AB negative DM1 patients.[17,44,55,61,64,69,72,74,75] TPO-AB were present in 7 to 32% of female *vs* 3 to 18% of male DM1 patients.[50,51,55-57,71,74] Other authors, however, did not find such a relation between gender and TPO-AB prevalence.[18,38,43,59,62,67,77,78] A particular subgroup of DM1 patients are pregnant women or women post-partum. In this group of patients, not reviewed in this study, a prevalence of TPO-AB of up to 33.8% was reported.[63,79] Although some authors reported higher TPO prevalences in their white than in their black DM1 patients,[55,56,80] no clear relation between race and TPO prevalence in DM1 was found when articles studying different races were compared.[18,31,38,39-44,47,53,55,56,59,62-68,71,74,75,77,80-86] We did not find any articles about the relation between gender or race and TPO-AB prevalence in control populations. When we compared articles that studied TPO-AB prevalence in control populations of different races, we did not find a clear relation between race and TPO-AB prevalence either. When duration of DM1 was considered, some authors reported significantly higher TPO-AB prevalences in populations with longer DM1 duration.[36,38,41,69,72] Magzoub *et al.*[41] for example found a higher TPO-AB prevalence in patients with a DM1 duration of more than ten years than in those with a DM1 duration of less than ten years (21.4 *vs* 4.9%). De Block *et al.*[69] reported that TPO-AB positive DM1 patients had a longer DM1 duration than TPO-AB negative patients (11±9 *vs* 9±8 years, p=0.048). Cardoso[72] also found a significant correlation between the presence of TPO-AB and DM1 duration (r=0.553, p<0.001). Riley *et al.*, however, did not find such a relation.[23]

Thyroglobulin antibodies (Tg-AB)

Methods

To detect Tg-AB, some authors used (haem) agglutination[19,6-49,6] while others used ELISA[59-63] or RIA.[17,18,66,67,87-89] Hagglof *et al.*[53] used a 'tanned red cell assay' and Kaino *et al.*[90] an immune complex transfer enzyme immunoassay. When comparing the Tg-AB prevalences obtained by (haem)agglutination, indirect immunofluorescence, ELISA and RIA, the prevalences found by (haem)agglutination were generally lower than the prevalences found by the other methods. However, this difference did not reach statistical significance.

Prevalences

Tg-AB prevalences in DM1 populations varied between 2.1 and 40% (IQR 5.4-22.7, P_5-P_{95} 2.6-57.3) with one exceptionally high score of 78%, reported by Kaino *et al.*[90] who also found the highest Tg-AB prevalence of 40.5% in controls. The authors suggested that the sensitivity of the detection method was probably responsible for their results. The Tg-AB prevalences found in control populations varied between 0 and 20% (IQR 1.5-8.4, P_5-P_{95} 0.6-27.2),[18,38-41,43,53,59,61,63,66,90] with one exceptionally high score of 40.5%.[90]

Relations

In the same way as TPO-AB, Tg-AB prevalence in DM1 patients increases with age.[46,72] Kordonouri *et al.*[17] reported the highest Tg-AB prevalence in DM1 children within the 15 to 20 year age group (12.8%), whereas Kokkonen *et al.*[39] reported Tg-AB in none of 17 DM1 patients younger than 10, in 5% of 40 patients aged 10 to 14 and in 3.7% of 27 patients older than 15 years. In their control population Kokkonen *et al.*[39] reported Tg-AB in none of 24 children younger than 10, in 2.7% of 73 children aged 10 to 14 and in 4.8% of 63 children older than 15 years, which is exceptionally higher than the 3.7% they reported in their DM1 patients of the same age. A female predominance was found in Tg-AB positive DM1 patients by some authors including Odugbesan *et al.*[43] who reported 100% of their Tg-AB positive patients to be female and Landin-Olsson *et al.*[61] who found 84% of their Tg-AB positive patients to be of the female gender. Others, however, did not report such an association between gender and Tg-AB prevalence.[18,38,59,62,67] We did not find any articles about the relation between race and Tg-AB prevalence in DM1 patients. Cardoso *et al.*[72] found a significant correlation

between the presence of Tg-AB and DM1 duration (r=0.545, p<0.001). In controls, we did not find any articles about the relation between gender or race and Tg-AB prevalence.

Thyroid antibodies (TPO-AB and/or Tg-AB)

Prevalences

Some authors assessed the overall Th-AB prevalence and found that 17.6% of DM1 patients had either thyroid-stimulating antibodies (TSI, associated with Graves' disease), TPO-AB or Tg-AB.[45] Others found that 11 to 46% of DM1 patients had either TPO-AB *or* Tg-AB, *vs* 1.4 to 11.5% of control subjects,[36,37,46,49,59,66,72,88,91] and that 9.5 to 11% of DM1 patients had TPO-AB *and* Tg-AB, *vs* 1.9 to 3.8% of control subjects.[59,66]

Relations

Kordonouri *et al.*[17] reported that DM1 children with Th-AB were significantly older (15 to 20 years) than those without Th-AB (<15 years). Shiau *et al.*[46] found a Th-AB prevalence of 18.4% in patients older than 18 years (with a mean age of DM1 onset of 18.0 years and mean DM1 duration of 6.9 years) *vs* 12.8% in those younger than 18 years (with a mean age of DM1 onset of 3.6 years and a mean DM1 duration of 4.5 years). Many other authors also found that the Th-AB prevalence rises with age.[41,49,71,72,75,92,93] Th-AB are associated with female gender: 58 to 78% of Th-AB positive patients were females.[17,44,46,88,93] In Th-AB negative DM1 patients, only 27 to 45% were females.[17,44] Several authors reported higher Th-AB prevalences in their white than in their black DM1 patients; Burek *et al.*[80] reported TPO-AB and/or Tg-AB in 50% of 82 white and in 16% of 72 black DM1 patients and Bright *et al.*[36] found Th-AB in 32% of 164 white and in 6% of 18 black DM1 patients. Kordonouri *et al.*[17] reported the DM1 duration of patients with either TPO-AB and/or Tg-AB to be longer than the DM1 duration of patients without Th-AB (5.2±3.9 *vs* 4.4±3.9 years, p<0.001) as did Barker (5.35 *vs* 2.62 years, p<0.0001).[93] Park *et al.*[94] also reported a longer DM1 duration in patients with Th-AB, compared with patients without Th-AB (5.9±3.8 *vs* 4.2±3.3 years, p<0.05).

Relation between thyroid antibodies and thyroid function

Various authors have investigated the relation between TPO-AB and Tg-AB, and thyroid function.[14,17,19,38,40,44,45,49,52,56,59,64,66,69,71,72,75,80,83-85,95-100] The authors of the articles we reviewed reported different types of thyroid dysfunction. We distinguish between subclinical hypothyroidism (defined as elevated serum thyrotropin (TSH) concentrations with serum free thyroxine (T_4) levels within the reference range), clinical hypothyroidism (defined as both a raised TSH and a low free T_4 level) and hyperthyroidism (defined as a raised free T_4 with a low TSH level). The prevalences of organ failure in DM1 and in control populations depend on the type of organ dysfunction reported.

Subclinical hypothyroidism

Subclinical hypothyroidism was found in 6.3 to 18.9% of DM1 patients with Th-AB. Rattarasarn *et al.*[44] reported subclinical hypothyroidism in 6.3% (and hyperthyroidism in 25%) of 16 patients who were either TPO-AB or Tg-AB positive. Roldán *et al.*[45] found subclinical hypothyroidism in 11% of 36 patients who were either TSI, TPO-AB or Tg-AB positive. Court *et al.*[96] reported subclinical hypothyroidism in 17.6% of Th-AB positive patients. Betterle *et al.*[49] investigated 37 DM1 patients with TPO-AB and/or Tg-AB and found that seven (18.9%) had subclinical hypothyroidism. Of 19 first-degree relatives of DM1 patients with TPO-AB and/or Tg-AB, four (21.1%) had subclinical hypothyroidism and three Graves' disease. In another article Betterle *et al.*[95] investigated 49 Th-AB positive DM1 patients, 24 Th-AB positive first-degree relatives of DM1 patients and 15 Th-AB positive healthy controls. Of the 49 Th-AB positive DM1 patients, nine (18.4%) had subclinical hypothyroidism. Of the 24 Th-AB positive first-degree relatives of DM1 patients, two (8.3%) had subclinical hypothyroidism. The 15 Th-AB positive healthy controls all had normal thyroid function. Presotto *et al.*[14] found subclinical hypothyroidism in 18% of 60 DM1 patients with Th-AB without clinical symptoms of thyroid disease. In their total group of 26 TPO-AB positive DM1 patients, Fernandez *et al.*[84] found five (19.2%) patients with subclinical hypothyroidism.

Change of subclinical to clinical hypothyroidism

Rattarasarn *et al.*[44] reported that two out of eight DM1 patients with Th-AB developed clinical hypothyroidism during a follow-up of 19±8 months; in the Th-AB negative DM1 group nobody developed thyroid dysfunction during 16.4±6.3 months follow-up.

Clinical hypothyroidism

In two groups of DM1 patients who were either TPO-AB *or* Tg-AB positive, clinical hypothyroidism was reported in 6.3 and 24% of the cases.[44,59] In 26% of 53 patients with Tg-AB and/or TPO-AB, Burek *et al.*[80] found hypothyroidism; those with hypothyroidism all had both TPO- and Tg-AB. Betterle *et al.*[49] investigated 37 DM1 patients with TPO-AB and/or Tg-AB and found seven patients who had clinical disease: Graves' disease (4), Hashimoto's thyroiditis (2) and idiopathic hypothyroidism (1). They did not detect clinical hypothyroidism in any of their Th-AB positive controls. Of *isolated* TPO-AB positive DM1 patients 11.5 to 72% were reported to have clinical hypothyroidism.[52,69,71,83,100] In their total group of 26 TPO-AB positive DM1 patients, Fernandez *et al.*[84] found four (15.4%) with clinical hypothyroidism. In 2.8% of 36 DM1 patients who were either TSI, TPO-AB *or* Tg-AB positive, Roldán *et al.*[45] reported clinical hypothyroidism.

Otherwise specified thyroid dysfunction

Some authors either used other definitions for thyroid dysfunction than ours,[56,66,95] did not report their criteria for thyroid dysfunction,[38,59] or did not distinguish between subclinical and clinical hypothyroidism.[49,80,97,98,101] We summarise their results as being '(sub)clinical hypothyroidism', which is found in 0 to 33% of DM1 patients with Th-AB.[42,49,59,75,80,95,98,101] Kordonouri *et al.*[17] reported (sub)clinical hypothyroidism in 16% of patients with Th-AB *vs* 8% without Th-AB (p<0.001). (Sub)clinical hypothyroidism was reported in 20% of patients with both Th-AB,[66] in 45% of patients with isolated TPO-AB,[56] and in 7.1% of patients with isolated Tg-AB.[66] Lorini *et al.*[40] found that none of five Th-AB positive DM1 patients had (sub)clinical hypothyroidism. Falorni *et al.*[97] reported (sub) clinical hypothyroidism in 44% of TPO-positive patients with latent autoimmune diabetes in adults. Trimarchi *et al.*[19] did not find any relation between circulating Th-AB and (sub)clinical hypothyroidism in DM1 patients. Some authors combined hyperthyroidism and hypothyroidism under the header 'autoimmune thyroid disease'.[78,93] Barker *et al.*[93] found autoimmune thyroid disease in 37% of 201 DM1 patients who had TPO-AB (with or without Tg-AB), compared with 10% in 20 DM1 patients with Tg-AB alone. Glastras *et al.*[78] found that 46.2% of 13 children who were TPO-AB positive at diagnosis of DM1 developed thyroid disease within 13 years, compared with 3.6% of 139 children who were TPO-AB negative at diagnosis. They recommend annual screening for thyroid disease only in DM1 patients who are TPO-AB positive at diagnosis, and TPO-AB screening at two yearly intervals in patients who are TPO-AB negative at diagnosis. Hanukoglu *et al.*[59] did not find (sub)clinical hypothyroidism in any of four non-DM1 TPO-AB positive controls. De Block *et al.*[65] reported that none of 18 TPO-AB positive first-degree relatives of DM1 patients had (sub)clinical hypothyroidism.

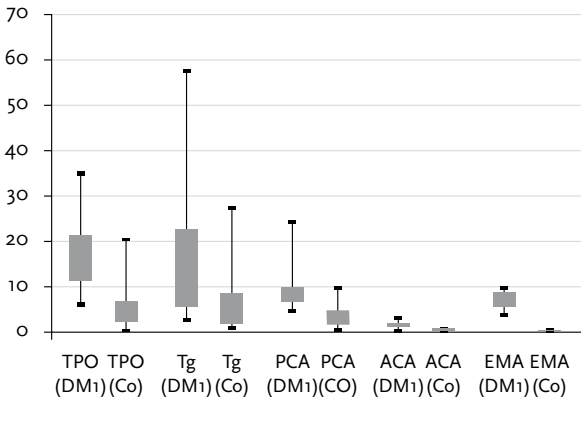
Frasier *et al.*[38] found goitre in 26% of 31 DM1 patients with isolated TPO-AB; seven were euthyroid and one hyperthyroid. Euthyroid goitre is not considered to be a form of thyroid dysfunction, but Frasier *et al.* suggested that euthyroid goitre could indicate compensated hypothyroidism. Cardoso *et al.*[72] found goitre in 53.8% of TPO-AB *and* Tg-AB positive DM1 patients. Gómez *et al.*[102] reported that otherwise healthy DM1 patients had larger thyroid volumes than healthy controls. The differences in thyroid volume were not related to thyroid dysfunction or autoimmunity, since patients and controls with previously diagnosed thyroid dysfunction, TPO-AB, or abnormal TSH had been excluded. The authors suggested that differences in body composition could be related to the differences in thyroid volumes.

Relations

In his review, Blecher[103] found autoimmune thyroiditis to be four times more common in females than in males. De Block *et al.*[65] reported that 78% of their DM1 patients with subclinical hypothyroidism and 82% of their DM1 patients with overt hypothyroidism were females. Also Chang *et al.*[64] and Fernandez *et al.*[84] reported a higher percentage of females among patients with thyroid autoimmunity: 69% hypothyroidism and 67.7% hyperthyroidism in patients with thyroid autoimmunity *vs* 52 and 33.7% in patients without thyroid autoimmunity. Hanukoglu,[59] however, found hypothyroidism to be equally divided among both sexes: of seven patients with hypothyroidism, four were male and three were female. In his review, Blecher[103] reported autoimmune thyroid disease to be four times more common in white than in black patients.

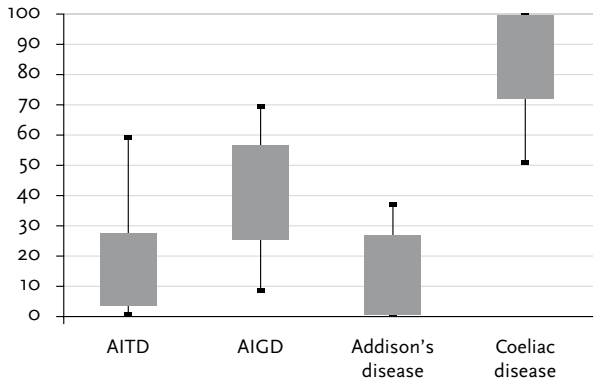
In summary, both antithyroid peroxidase (TPO-AB) and antithyroglobulin antibodies (Tg-AB) are more frequently present in patients with type 1 diabetes mellitus (DM1) than in control populations (*figure 1*): TPO-AB in 5.5 to 46% (IQR 11.3-21.2, P_5-P_{95} 5.8-34.5) of DM1 patients *vs* 0 to 27% (IQR 2.0-6.8, P_5-P_{95} 0.1-20) in controls, and Tg-AB in 2.1 to 40% (IQR 5.4-22.7, P_5-P_{95} 2.6-57.3) of DM1 patients *vs* 0 to 20% (IQR 1.5-8.4, P_5-P_{95} 0.6-27.2) in controls. Prevalences seem to be highest among females and seem to increase with age and DM1 duration. Some authors reported higher thyroid antibody (Th-AB) prevalences in their white than in their black DM1 patients, but no clear overall conclusion can be drawn with regard to race and Th-AB prevalence, when results of populations of different racial background are compared. The prevalence of subclinical and clinical hypothyroidism varied from 6 to 72% of Th-AB positive DM1 patients *vs* 0 to 25% in controls (*figure 2*), depending

Figure 1. *Autoantibody prevalences (%) in type 1 diabetes mellitus patients and controls in 50 articles that were selected according to criteria as mentioned in the methods section*



DM1 = type 1 diabetes mellitus; Co = controls; ACA = antiadrenocortical antibodies; EMA = antiendomysial antibodies; PCA = antiparietal cell antibodies; Tg-AB = antithyroglobulin antibodies; TPO-AB = antithyroid peroxidase antibodies. Bars represent interquartile ranges, lines represent P_5 and P_{95}.

Figure 2. *Prevalence (%) of organ dysfunction in autoantibody positive type 1 diabetes mellitus patients*



AITD = autoimmune thyroid disease; AIGD = autoimmune gastric disease. Bars represent interquartile ranges, lines represent P_5 and P_{95}.

on whether they had TPO-AB or Tg-AB or both. Given the estimated upper level of IQR of the prevalence of thyroid failure of 27% in Th-AB positive DM1 patients, we recommend checking thyroid function biennially in these patients. Given the upper level of IQR of the prevalence of Th-AB of 21 to 23% in DM1 patients and the possible relation to age, female gender and DM1 duration, we recommend checking Th-AB in these patients at regular intervals. Although the optimal time interval should be determined by prospective studies, a practical approach could be to check Th-AB every five years.

Parietal cell antibodies (PCA)

PCA are directed against the parietal cells in the stomach,[104-106] chronically targeting H^+/K^+ ATPase, which leads to atrophic gastritis, hypochlorhydria or achlorhydria, and a decline in intrinsic factor production, causing hypergastrinaemia, vitamin B12 malabsorption and ultimately pernicious anaemia.[106-107] Hypochlorhydria may also impair iron absorption and cause iron deficiency anaemia.[108] De Block *et al.*[22] confirmed the relation between PCA titre and the severity of corpus atrophy, earlier found by Sipponen *et al.*[109] suggesting that humoral mechanisms involving cytotoxic AB play a role in mediating mucosal damage in autoimmune gastritis. The pathogenicity of PCA, however, remains unclear, because circulating AB do not have direct access to gastric H^+/K^+ ATPase.[110] PCA in gastric secretions on the other hand might have direct access to this target. The fact that PCA is not found in every patient with autoimmune gastritis could be explained by the possible mediation of autoimmune gastritis by CD4+ T cells recognising H^+/K^+ ATPase.[111] Other explanations for the existence of autoimmune gastritis without PCA could be exhaustion of the autoimmune response as parietal cells are depleted or failure to recognise autoantibodies.

Methods

To detect PCA, most authors used indirect immunofluorescence,[23,36,39,40,41,43,49,53-55,57,60,65] whereas others used the ELISA method.[61]

Prevalences

The PCA prevalences in DM1 populations ranged from 3 to 34% (IQR 6.3-9.5, P_5-P_{95} 4.2-24.4) and in control populations from 0 to 13% (IQR 1.5-4.8, P_5-P_{95} 0-9.8).[23,36,39,40,41,43,49,53-55,57,60,61,65]

Relations

In the literature, different observations about the relation between age and PCA prevalence in DM1 patients were made. Bright *et al.*[36] found a PCA prevalence of 14.6% in 48 DM1 children younger than 13 years *vs* 30% in 40 patients older than 13 years. Kokkonen *et al.*[39] reported PCA in 5.9% of 17 DM1 children younger than 10 years, in 12.5% of 40 patients aged 10 to 14 years and in 7.4% of 27 patients aged 15 years or older. De Block *et al.*[69] found that PCA-positive DM1 patients were older than EMA-negative patients (31±17 *vs* 25±16 years, p=0.002). They also reported that according to logistic regression, PCA status was determined by age (β=0.03, p=0.002). Other authors, however, did not find a significant relation between PCA positivity and age in DM1 populations.[41,61,112] Maclaren *et al.*[71] reported a relation between PCA positivity and age in DM1 patients, close relatives of DM1 patients and controls. In healthy children, Kokkonen *et al.*[39] also related PCA prevalence to age: none of 24 children younger than ten years were PCA positive, but 6.8% of 73 children aged 10 to 14 and 4.8% of 63 children older than 15 years were PCA positive. De Block *et al.*[65] reported PCA in 11% of 397 first-degree relatives of DM1 patients, who were older than the EMA-negative first-degree relatives (26±9 *vs* 22±9 years, p=0.025). Riley *et al.*[57] found a predominance of 63% females *vs* 37% males in their PCA-positive patients. Other authors, however, did not find such a relation between gender and PCA prevalence.[36,41,49,51,61,69,76,92,113] Overall, PCA prevalences in European populations are reported in 3 to 15%,[23,36,39,49,53,54,57,60,61,65] except for Lorini[40] who found PCA in 34% of their Italian DM1 patients. As far as non-European patients are concerned, 8% of Indian-Asian DM1[43] and 6.3% of Sudanese patients had PCA.[41] Neufeld *et al.*[55] found PCA in 10% of black *vs* 8.5% of Caucasian DM1 patients. Riley *et al.*[57] reported a PCA prevalence of 9.4% in black *vs* 7.8% in Caucasian DM1 patients. We found no articles about the relation between gender or race, and PCA prevalence in control populations. Neufeld *et al.*[55] reported a PCA prevalence of 17% in islet cell antibody (ICA) positive patients with a DM1 duration of more than five years *vs* 7% in ICA-positive patients with a DM1 duration of less than five years. In ICA-negative patients, they found no relation between PCA prevalence and DM1 duration. De Block *et al.*[69]

found PCA-positive DM1 patients to have a significantly longer DM1 duration than EMA-negative DM1 patients (11±10 *vs* 9±8 years, p=0.011). Bright *et al.*,[36] however, did not find any relation between DM1 duration and PCA prevalence.

Relation between parietal cell antibodies and parietal cell function

For a good interpretation of research articles about the clinical significance of PCA, clearly described criteria are necessary to define organ failure. Frequently used criteria are the presence of atrophic gastritis, achlorhydria, hypergastrinaemia and pernicious anaemia.

Atrophic gastritis

Of PCA-positive DM1 patients, 43 to 50% had atrophic gastritis.[14,57,111] Presotto *et al.*[14] performed gastroscopy in 20 PCA-positive DM1 patients and found macroscopic atrophic gastritis in ten, of which four were mild, three moderate and three severe. Of the remaining ten patients, eight had superficial gastritis and two had a normal mucosa. De Block *et al.*[51] performed gastroscopy in 14 PCA-positive DM1 patients with symptoms of dyspepsia and found atrophic gastritis in 92.8 *vs* 56.3% in 16 symptomatic EMA-negative DM1 patients. In another study[22] they compared 47 PCA-positive and 41 EMA-negative DM1 patients and found a significant difference in prevalence of autoimmune atrophic gastritis of 57 *vs* 10%. Betterle *et al.*[49] reported that four out of six PCA-positive, non-DM1 relatives of DM1 patients had atrophic gastritis.

Hypochlorhydria or achlorhydria

Of PCA-positive DM1 patients, 25 to 73% had achlorhydria.[57,112] When de Block *et al.*[22] compared 47 PCA-positive and 41 PCA-negative DM1 patients, they found a significant difference in prevalence of hypochlorhydria of 73 *vs* 19%.

Hypergastrinaemia

De Block *et al.*[51] reported hypergastrinaemia in 27% of their PCA-positive DM1 patients. When they compared 47 PCA-positive and 41 EMA-negative DM1 patients,[22] they found a significant difference in prevalence of hypergastrinaemia of 47 *vs* 22%, which confirmed the results of Kokkonen *et al.*,[39] who reported significantly higher gastrin levels in PCA-positive than in EMA-negative DM1 patients. De Block *et al.*[65] found significantly elevated gastrin levels in 10.8% of 397 PCA-positive first-degree relatives of DM1 patients. They also reported that gastrin levels correlated inversely with the percentage of parietal cells.[22] Others[109,114] noted that gastrin levels correlated with corpus atrophy and (inversely) with peak acid output. The gastrin level therefore seems to be good indicator of atrophic gastritis and, especially in PCA-positive patients, could serve as a screening tool, although sensitivity and specificity vary between studies.[115-117]

Pernicious anaemia

For pernicious anaemia, which is seen as the end-stage of autoimmune gastritis,[104] lower prevalences of 1 to 23% were reported in DM1 patients[14,22,49,51] than for gastritis, achlorhydria or hypergastrinaemia. When de Block *et al.*[22] compared 47 PCA-positive and 41 PCA-negative DM1 patients, they found a significant difference in prevalence of pernicious anaemia of 23 *vs* 2%. In another study[65] of 397 PCA-positive first-degree relatives of DM1 patients, they detected pernicious anaemia in only two relatives.

In summary, antiparietal cell antibodies (PCA) are more prevalent in patients with type 1 diabetes mellitus (DM1) (3-34%, IQR 6.3-9.5, P_5-P_{95} 4.2-24.4) than in control populations (0-13%, IQR 1.5-4.8, P_5-P_{95} 0-9.8) (*figure 1*). In DM1 populations, non-DM1 controls and healthy relatives of DM1 patients, PCA prevalence is correlated with age. PCA prevalence also seems to be higher in patients with longer DM1 duration. Due to lack of data no conclusions can be drawn with respect to the relation of gender or race with PCA prevalence in DM1 patients or controls. The relation between PCA positivity and organ dysfunction (*figure 2*) depends on the criteria used to define organ dysfunction. Given the clinical significance of the insidious development of pernicious anaemia with a prevalence to 23% in PCA-positive DM1 patients, we recommend monitoring parietal cell function biennially in patients with PCA by measuring fasting gastrin and vitamin B 12 levels. Although there are no follow-up data for the development of PCA in EMA-negative DM1 patients, the likelihood of developing PCA with increasing age and DM1 duration makes it worthwhile to monitor PCA at regular intervals in these patients. Although the optimal time interval should be determined by prospective studies, a practical approach could be to check PCA every five years.

Adrenocortical antibodies (ACA)

Adrenocortical autoimmune disease, also called primary adrenal insufficiency or Addison's disease, is the result of humoral and cell-mediated inflammation of the adrenal cortex.[28] Adrenocortical antibodies (ACA) are directed against 21-hydroxylase, a microsomal cytochrome P450-enzyme that converts 17-α-progesterone and progesterone into 11-deoxycortisol and 11-deoxycorticosterone.[104,117] These antibodies can fix complement and mediate cytotoxicity, thus destructing the adrenal cortex.

Methods

Most authors used indirect immunofluorescence to detect ACA.[36,43,49,54,55,57,58,60,11] Others used a radio-binding assay.[119-121] No significant differences were observed when the prevalences, obtained by different assays, were compared.

Prevalences

The ACA prevalences in DM1 populations ranged from 0 to 4% (IQR 0.9-1.8, P_5-P_{95} 0-3.3) and in control populations from 0 to 0.7% (IQR 0-0.6, P_5-P_{95} 0-0.7).[36,43,49,54,55,57,58,60,120-123]

Relations

Some authors found a female predominance of ACA prevalence in DM1 patients (1.9-6% of females *vs* 1.2-3% of males) [49,55,57], but de Block *et al.*[69] did not report any differences in frequency between the sexes, neither did Betterle,[49] nor Barker. [93] De Block *et al.*[69] found no relation of ACA with age or DM1 duration. Neufeld *et al.*[55] found a positive relation between DM1 duration and ACA prevalence. They reported an ACA prevalence of 11% in ICA-positive patients with a DM1 duration of more than five years *vs* 1% in ICA-positive patients with a DM1 duration of less than five years. In ICA-negative patients, they found no relation between ACA prevalence and DM1 duration. Barker *et al.*[93] reported that patients with ACA had a longer DM1 duration than ACA-negative patients (8.92 *vs* 3.29 years, p=0.03). No articles were found in which the relation between race and ACA in DM1 patients, nor between age, gender or race, and ACA in control populations was described.

Relation between adrenocortical antibodies and adrenal function

Most authors use an abnormal response to ACTH during a test, or the clinical syndrome of Addison's disease, as their criterion for adrenal dysfunction.[14,49,122,123]

A strong relation has been found between the presence of ACA and the subsequent development of overt adrenal impairment. Among ACA-positive DM1 patients, 3.3 to 40% had Addison's disease,[49,54,93,122] although Peterson *et al.*[119] did not find Addison's disease in any of five DM1 patients with ACA. Yu *et al.*[122] reported that nine of 966 DM1 patients had known Addison's disease; seven of them had ACA, two were not tested; of the 957 DM1 patients without known Addison's disease, 15 were ACA positive, of which three had newly diagnosed Addison's disease. Betterle *et al.*[124] performed a longitudinal analysis of 15 DM1 patients with organ-specific autoimmune disease who were positive for ACA: 40% developed Addison's disease during a mean observation period of 3.2 years. In another study, Betterle *et al.*[49] found that none of two ACA positive, non-DM1 relatives of DM1 patients, had adrenal dysfunction.

In summary, antiadrenocortical antibody (ACA) prevalence, like other autoantibody prevalences, is higher in patients with type 1 diabetes mellitus (DM1) (0-4%, IQR 0.9-1.8, P_5-P_{95} 0-3.3) than in control populations (0-0.7%, IQR 0-0.6, P_5-P_{95} 0-0.7) (figure 1). The relation of ACA prevalence with age, DM1 duration and gender is not clear. Of ACA-positive DM1 patients, 3 to 40% develop Addison's disease (*figure 2*). Because of the high risk of developing overt Addison disease (to 40%), patients with ACA should undergo annual ACTH testing. Although the prevalence of ACA in DM1 patients is low, the development of ACA in ACA-negative DM1 patients is associated with a high risk of developing overt Addison disease. It may therefore be advisable to monitor ACA in these patients at regular intervals. Although the optimal time interval should be determined by prospective studies, a practical approach could be to monitor ACA every five years.

Endomysial antibodies (EMA)

Coeliac disease (CD) is a malabsorption disease, which is due to an immune-mediated destruction of the villous structure in the small intestine. The clinical manifestations depend on the extent and severity of the lesion and vary from isolated anaemia to severe malabsorption. The most common symptoms in patients with extensive disease include diarrhoea, weight loss and a malabsorption syndrome, reflected by extraintestinal symptoms such as anaemia, osteopenia, muscular atrophy, and peripheral neuropathy.[125,126] However, many patients do not have any symptoms, and are therefore said to have silent CD.[127] T cells are probably the key mechanism of villous atrophy in coeliac disease, but autoantibodies also appear to be involved in inducing villous atrophy by acting against cellular proteins in response to the presence of gliadin.[106,128] The endomysial antibodies (EMA) discussed here are IgA antibodies,[129] directed against the endomysium, the smooth muscle inter-myofibrillary substance in the gut.[130] The exact role of autoantibodies in the pathogenesis of coeliac disease remains unknown.[131]

Methods

Indirect immunofluorescence was usually used to detect EMA.[24,87,126,132-135]

Prevalences

EMA prevalences between 1.5 and 10% (IQR 5.1-8.7, P_5-P_{95} 3.4-9.8) have been documented in DM1 patients and in controls between 0 and 2% (IQR 0-0.3, P_5-P_{95} 0-1.5).[24,87,126,132-135]

Relations

Little research has been published in DM1 patients about the relation between age, gender, race or DM1 duration on the one hand, and EMA prevalence on the other. Shabazkhani *et al.*[136] reported that DM1 patients with EMA were older than DM1 patients without EMA (29.5 *vs* 18.4 years, p<0.001), but neither de Block *et al.*,[69] Aygun *et al.*[137] nor Talal *et al.*[26] found any relation between EMA prevalence and age or DM1 duration. Schober *et al.*[138] reported that EMA-positive DM1 patients had a lower age of onset of DM1 (median 5.6, range 1-12 years) than EMA-negative patients (median 8.4, range 1-15 years).[137] Barera *et al.*[139,140] found that

EMA seroconversion took place within three to five years after the onset of DM1. Crone *et al.*[141] however, reported seroconversion to occur throughout the course of DM1, and not just in the first years. Schober *et al.*[138] found a female predominance in EMA positivity: 10 out of 12 EMA-positive DM1 patients were females. No information was found about the relation between race and EMA prevalence in DM1 patients, nor about the relation between age, gender or race, and the EMA prevalence in controls.

Relation between endomysial antibodies and intestinal villous function

In EMA-positive DM1 patients, 44 to 100% have CD,[24,26,77,78,87,126,132-134,137-158] compared with 0 to 0.6% in control populations.[59,87,126,133,159-161]

Cerutti *et al.*[146] assessed the prevalence of CD in DM1 patients with and without siblings with DM1 and reported CD in 37.5% of the first group *vs* 6.1% in the second. Hanukoglu *et al.*[59] detected biopsy-proven CD in 6% of first-degree relatives of DM1 patients. Glastras *et al.*[78] reported that all four patients who had EMA at diagnosis of DM1 developed CD within the first year after diagnosis and that EMA seroconversion took place 2.8 to 10.2 years after diagnosis of DM1 in patients with negative EMA titres at diagnosis. Coeliac disease did not develop for some years after diagnosis of diabetes in patients who were EMA negative at diagnosis. They therefore recommend screening for coeliac disease only at two-yearly intervals, not annually.

Relations

Information about the relation between age, gender, race or DM1 duration, and CD prevalence was less scarce than the information about the relation between these parameters and EMA prevalence. Cerutti *et al.*[146,160] found that female gender was associated with the presence of the combination of DM1 and CD (odds ratio (OR) 1.75, 95% CI 1.35-2.29, p<0.0001). Roldan *et al.*[149] Shabazkhani *et al.*[136] and Mahmud *et al.*[156] also found a clear female predominance among their DM1 patients with CD (female:male ratio: 6:1, 6:0 and 9:2, respectively). Verge *et al.*[77] and Buysschaert[155] found no such female predominance. Roldan *et al.*[149] and Hansen *et al.*[87] reported that DM1 patients with CD generally had a younger age of onset of DM1 than DM1 patients without CD: 4.2±3.6 *vs* 8.4±4.0 years (p<0.005) and 3.2 (0.7-9.3) *vs* 7.4 (1.3-16.6) years (median (range)) (p=0.005) respectively. Cerutti *et al.*[146] reported that in comparison with age of onset being older than nine years, age of onset younger than four years conferred an OR of 3.27 (95% CI 2.20-4.85, p<0.001). They[160] also found that CD prevalence decreased from 3.3% in patients with a DM1 duration of less then one year to 0.6% in patients with DM1 duration of more than ten years. This confirmed the results of Barera *et al.*[140] who reported that all new cases of CD developed before the fourth year after onset of DM1. In contrast, Buysschaert[156] found that duration of DM1 was comparable between DM1 patients with and without CD. Ashabani *et al.*[162] reported that age, gender and DM1 duration did not help identify DM1 patients with CD.

In summary, antiendomysial antibody (EMA) prevalence is higher in patients with type 1 diabetes mellitus (DM1) (1.5-10%, IQR 5.1-8.7, P_5-P_{95} 3.4-9.8) than in control populations (0-2%, IQR 0-0.3, P_5-P_{95} 0-1.5) (*figure 1*). There seems to be little consensus about the relation of age, gender, race or diabetes duration with EMA prevalence. Of EMA-positive DM1 patients 44 to 100% have biopsy proven coeliac disease (*figure 2*). Therefore in these EMA-positive patients an intestinal biopsy should be performed annually. Although the prevalence of EMA in DM1 patients is rather low, the high predictive value of EMA for the development of CD makes it worthwhile to monitor EMA at regular intervals. Although the optimal time interval should be determined by prospective studies, a practical approach could be to monitor EMA every five years.

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ORIGINAL ARTICLE

Extreme leucocytosis: not always leukaemia

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ABSTRACT

Three patients were analysed for an extreme leucocytosis (>50 x 10^9/l) because leukaemia was suspected. In all three patients the leucocytosis proved to be caused by a leukaemoid reaction. This reaction was associated with a hepatic angiosarcoma in the first patient, with a *Salmonella* infection in the second patient and with a necrotic leg abscess in the third patient. Retrospectively, 25 patients with a leukaemoid reaction were identified in our hospital during a four-year period. Besides leukaemia, a leukaemoid reaction, which often has a dismal prognosis, should be considered in patients with an extreme leucocytosis.

KEYWORDS

Leucocytosis, leukaemoid reaction, paraneoplastic

INTRODUCTION

The causes of leucocytosis include severe infection, a (haematological) malignancy or use of certain drugs such as G-CSF (granulocyte colony-stimulating factor). A leucocyte count exceeding 50 x 10^9/l could be due to leukaemia or a leukaemoid reaction.[1] In a recent case report in the Netherlands Journal of Medicine, a patient was presented with a leukaemoid reaction in metastasised melanoma.[2] In this report, we describe three patients with an extreme leucocytosis associated with a malignant or infectious disease. Besides, we report the results of a retrospective study on the incidence and causes of possible leukaemoid reactions in a large teaching hospital during a four-year period.

CASE REPORTS

Patient A, a 74-year-old man, visited the Emergency Department because of progressive jaundice and fatigue. For four weeks he had experienced an intermittent fever of up to 38.5°C and upper abdominal discomfort. He had lost 8 kg in weight. Previous medical history revealed surgery and radiation therapy for cystic carcinoma 24 years ago. On physical examination, a dehydrated, icteric male was seen. There were no enlarged lymph nodes and no pathological findings of heart or lungs. The liver was palpable, 4 cm under the right costal margin.

Laboratory testing revealed a normocytic anaemia (Hb 5.0 mmol/l, MCV 94 fl), thrombocytopenia (platelet count 71 x 10^9/l) and an extreme leucocytosis (white blood cell count 74.7 x 10^9/l; see *table 1* for differentiation). Renal insufficiency was found (serum creatinine 195 µmol/l, serum blood urea nitrogen 22.4 mmol/l) and the liver parameters were abnormal (bilirubin 126 µmol/l (conjugated 86 µmol/l), aspartate aminotransferase 288 U/l, alanine aminotransferase 165 U/l, lactate dehydrogenase (LDH) 1162 U/l, alkaline phosphatase 520 U/l and γ-glutamyltransferase 244 U/l). Prothrombin time and activated partial thromboplastin time were increased (15.1 and 34 seconds, respectively). C-reactive protein (CRP) was elevated (175 mg/l). Because of the extremely high number of mature granulocytes and the absence of immature cells in peripheral blood, a chronic neutrophilic leukaemia (CNL) was suspected. However, the patient refused further diagnostic procedures and succumbed within 24 hours of admission.

At autopsy, an enlarged liver was found with an angiosarcoma showing diffuse growth in the right liver lobe. The liver had ruptured and blood was found in the intra-abdominal cavity. Bone marrow showed normal precursor cells with little hypercellularity of the myeloid precursor cells. It was concluded that the extreme leucocytosis with mature granulocytes was a paraneoplastic effect of the angiosarcoma.

Table 1. *Differentials of peripheral leucocytes (x 10^9/l) in patients A, B and C*

	Patient A	Patient B	Patient C First admission August 2003	Patient C Second admission January 2004
Leucocytes	74.7	92.2	58.7	224.2
Eosinophilic granulocytes	0	0	0	0
Basophilic granulocytes	0	0	0	0
Band neutrophils	6.7	0	2.3	22.4
Segmented neutrophils	65.7	57.2	45.8	107.6
Lymphocytes	0.7	9.2	7.0	17.9
Monocytes	1.5	9.2	1.2	0
Others:	0	17	4	76
• Blasts	0	0	0	36
• Promyelocytes	0	1	2	7
• Myelocytes	0	10	0	18
• Metamyelocytes	0	6	2	16
• Erythroblasts	0	0	0	4

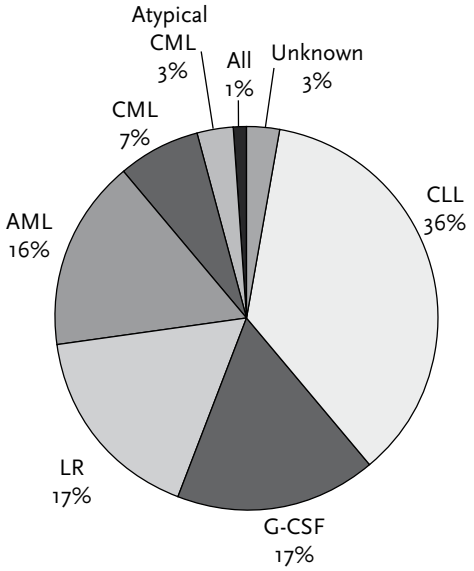
Patient B, an 89-year-old woman, was admitted to the Department of Geriatric Medicine because of severe diarrhoea based on an infection with *Salmonella B*. Her medical history revealed resection of the sigmoid colon due to an adenocarcinoma eight years ago. For a year she had received blood transfusions at regular intervals because she had an anaemia based on myelodysplastic syndrome (MDS) (type refractory anaemia, MDS-RA). During the admission, her condition deteriorated, she developed a severe inflammatory response syndrome (SIRS), and the peripheral leucocyte count increased from 11.8 x 10^9/l at admission to 92.2 x 10^9/l in several days (see *table 1* for differentiation). Laboratory testing revealed a normocytic anaemia (Hb 5.3 mmol/l), increased LDH (2099 U/l) and CRP (175 mg/l). Because an acute leukaemia was suspected, bone marrow aspiration was performed. The bone marrow showed features of MDS-RA with trilineage dysplasia and 1.5% blast cells, so no features of an acute leukaemia. It was concluded that the leucocytosis was caused by the SIRS, probably based on the *Salmonella* infection. The patient was treated with systemic antibiotic therapy and fluid resuscitation. Her condition improved and the leucocyte number decreased to 7.2 x 10^9/l in two weeks. Five weeks after admission she was able to return home.

Patient C, a 77-year-old woman, was admitted to hospital with an abscess of the left anterior tibial muscle. She had undergone coronary artery bypass grafting (CABG) ten years ago. Laboratory testing revealed a microcytic anaemia (Hb 4.9 mmol/l, MCV 72 fl), thrombocytopenia (platelet count 120 x 10^9/l) and leucocytosis (leucocyte count 46.7 x 10^9/l (with 43.0 x 10^9/l mature neutrophilic granulocytes and no immature cells)). CRP was elevated (331 mg/l). Antibiotic treatment was started. After four days, the CRP had decreased (155 mg/l) but the leucocyte count had increased to 58.7 x 10^9/l (see *table 1* for differentiation). Bone marrow aspiration and biopsy showed hypercellular bone marrow with dysplastic features such as micromegakaryocytes, decreased erythropoiesis and dysplastic myelopoiesis. The number of blast cells was not raised (0.5%). After the abscess was drained and the patient recovered, the leucocyte count dropped to 4.4 x 10^9 /l with a normal distribution. Granulocytes still showed hypogranular cytoplasm as a sign of myelodysplasia. It was concluded that she was suffering from MDS-RA and had experienced a leukaemoid reaction associated with a muscle abscess. After four months she was admitted again with a severe anaemia (3.6 mmol/l) and an extreme leucocytosis (214 x 10^9/l; see *table 1* for differentiation). Repeated bone marrow biopsy showed 6% blast cells; therefore it was concluded that there was a progression to refractory anaemia with an excess of blasts (RAEB-t according to FAB classification, RAEB-1 according to WHO classification). Shortly after admission, the patient succumbed.

RESULTS OF RETROSPECTIVE ANALYSIS

We retrospectively investigated the prevalence of an extreme leucocytosis (>50 x 10^9/l) in adult patients in the Meander Medical Centre Amersfoort during a four-year period (January 2000 to December 2003). In this period a white blood cell count >50 x 10^9/l was seen in 147 patients (*figure 1*). As no further information was available for four patients, we were able to analyse data from 143 patients. Ninety-three patients had leukaemia (63%). Twenty-five patients had received subcutaneous injections of G-CSF in order to decrease the leucopenic period after the administration of myelosuppressive chemotherapy (15 patients), or because they were being treated for a haematological malignancy according to a research protocol including the use of G-CSF (ten patients). The leukaemoid

Figure 1. *Causes of extreme leucocytosis (>50 x 10^9/l) in 147 adult patients between 1 January 2000 and 12 December 2003 in the Meander Medical Centre Amersfoort, the Netherlands*



G-CSF = use of granulocyte colony-stimulating factor after chemotherapy; CLL = chronic lymphocytic leukaemia; CML = chronic myeloid leukaemia; AML = acute myeloid leukaemia; LR = leukaemoid reaction.

reaction in the other 25 patients appeared to be associated with other diseases (*table 2*). Nine patients had positive blood cultures for micro-organisms and 11 patients had a malignant disease. Of the remaining patients, one had a biliary pancreatitis, three patients had tissue necrosis due to ischaemia (two enteric, and one leg soft tissue), and one patient had a decompensated cirrhosis. Patient U suffered severe chronic obstructive pulmonary disease and was admitted with a pneumothorax and severe dyspnoea. He died within hours. A possible explanation for the extremely high white blood cell count in this patient could be a combination of severe stress and pneumonia. In both patients with cirrhosis (Patients N and W), the cirrhosis was caused by alcohol abuse. At the time of this investigation (January 2004) only seven of 25 patients were alive (mortality 72%). Ten patients died within two weeks of the leukaemoid reaction.

DISCUSSION

In the three patients described, leukaemia was considered to be a possible cause of the extreme leucocytosis. Based on bone marrow biopsies, however, a leukaemoid reaction

Table 2. *Patients with a leukemoid reaction without use of G-CSF*

Code	Sex	Age	Leucocytes (x 10^9/l)	Died	Malignancy	Infection	Blood culture	Other
A	M	74	74.7	+	Angiosarcoma			
B	F	89	56.5	+	MDS	+		
C	F	77	58.7	+	MDS	+		
D	M	69	65.3	+	Bladder (m)			
E	F	83	63.3	+	Thyroid (m)			
F	F	34	62.9	+				Enteric ischaemia
G	F	65	62.5	+		+	*Pneumococcus*	
H	F	52	61.0	-		+		Biliairy pancreatitis
I	F	68	59.8	+	Sigmoid	+	*Pseudomonas*	
J	F	47	58.9	+	Lung (m)			
K	F	53	56.8	+	Lung (m)	+		
L	F	53	56.4	-		+	*Pseudomonas*	
M	F	93	55.5	+				Leg necrosis
N	F	37	54.9	-				Cirrhosis
O	F	84	53.6	+	Bladder			
P	F	67	52.9	+		+	*Streptococcus A*	
Q	M	35	52.5	-		+	*Streptococcus A*	
R	M	50	52.0	+		+	*Pseudomonas & E. coli*	
S	M	53	51.7	-		+	*Pseudomonas & Streptococcus A*	
T	M	69	51.6	+	Lung			
U	M	78	51.3	+				
V	F	66	50.6	-		+	*E. coli*	
W	M	68	50.2	+		+	*Pneumococcus*	Cirrhosis
X	M	89	50.1	+				Enteric ischaemia
Y	F	74	50.1	-	Lung (m)			

MDS = myelodysplastic syndrome; (m) = metastastic disease.

appeared to be the cause of the peripheral leucocytosis. A leukaemoid reaction is defined as a white blood cell count >50 x 10^9/l with a cause outside the bone marrow.[1] A raised number of white blood cells can be due to mature leucocytes (patient A), resembling a CNL. If an increased amount of immature granulocytes such as (pro)myelocytes or metamyelocytes is seen (Patient B), a leukaemoid reaction can imitate chronic myeloid leukaemia (CML). Investigation of the bone marrow including immunophenotyping may help to differentiate between leukaemia and a leukaemoid reaction. Cytogenetic abnormalities associated with leukaemia should be looked for by karyotyping and by reverse transcriptase-polymerase chain reaction (RT-PCR). The BCR-ABL protein can be found in CML and in some cases in acute lymphoblastic leukaemia or acute myeloid leukaemia. When dysplastic features are found in the bone marrow, the amount of blast cells in the bone marrow should be used to discriminate between a leukaemoid reaction and leukaemia. Because the leucocytosis disappeared upon treatment of the infection in patients B and C, both these patients seemed to have experienced a leukaemoid reaction of a dysplastic bone marrow. In Patient A, the leukaemoid reaction was associated with an angiosarcoma and rupture of the liver.

Not much is known about the incidence and course of leukaemoid reactions. Most knowledge is based on case reports.[2-8] Several known causes of leukaemoid reactions are given in *table 3*. A paraneoplastic leukaemoid reaction can be caused by increased serum levels of G-CSF or other growth factors, which are considered to be produced by the malignant cells, mostly from an endothelial tumour.[3-5] In some reports, a decrease in G-CSF levels was described after treatment of the primary tumour.[6] The leukaemoid reaction can be present even years before the diagnosis of the carcinoma.[7] McKee described a group of 21 patients with a leukaemoid reaction based on a malignant disease of whom 20 suffered a carcinoma, mostly of the lung.[8] In those patients, a leukaemoid reaction was associated with aggressive tumour behaviour and high mortality.[8]

In a retrospective analysis, we identified 50 patients (out of 147 patients with >50 x 10^9/l leucocytes) who met the definition of a leukaemoid reaction. Within this group, 25 of the cases were associated with treatment with G-CSF. In the remaining patients high numbers of malignancies, mainly epithelial, or bacteraemia, were seen, in concordance to earlier reports.

In conclusion, in one third of patients (35%) with an extreme leucocytosis (>50 x 10^9/l), leucocytosis was not caused by leukaemia but by a leukaemoid reaction. This leukaemoid reaction is usually seen in association with a malignancy or a severe sepsis and is characterised by a high mortality.

Table 3. *Several causes of a leukemoid reaction*

Infectious	Shigellosis
	Hepatic abscess
	Tuberculosis
	Sepsis
Paraneoplastic	Bronchus carcinoma
	Carcinoma of bladder, kidney and prostate
	Carcinoma of tongue and nasopharynx
	Carcinoid
	Hepatocellular carcinoma
	Carcinoma of oesophagus
	Cholangiocarcinoma
	Carcinoma of cervix or ovary
	Splenic haemangiosarcoma
	Liposarcoma and soft tissue sarcoma
	Leiomyosarcoma of the bladder
	Melanoma
	Bone metastasis
	Multiple myeloma
	Hodgkin's disease
Drug induced	Granulocyte colony stimulating factor
	Corticosteroids
	Tetracycline
	Streptokinase
Miscellaneous	Diabetic ketoacidosis
	Alcoholic hepatitis
	Ethylene glycol intoxication
	Enteric necrosis

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ORIGINAL ARTICLE

Current health status of patients who have survived for more than 15 years after liver transplantation

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ABSTRACT

Background: Liver transplantation was started in our centre as early as 1979. We have studied the clinical outcome of patients surviving longer than 15 years, with special interest for the broad range of comorbidity and the self-perceived quality of life.

Methods: All patients who underwent a liver transplantation at an adult age, between March 1979 and February 1991, and who had survived at least 15 years were eligible for the study. Data were collected from the medical records. Health-related quality of life was assessed using the Six-Dimensional EuroQol test.

Results: The five-year survival of patients alive 15 years after transplantation was 78%. Thirty-seven patients are currently alive with a median follow-up of 18.8 years (range 15.0 to 26.8) after transplantation. Comorbidity consists predominantly of overweight (57%), osteoporosis (49%), *de novo* cancer (38%, mainly skin cancer), hypertension (38%), cardiovascular events (19%), diabetes mellitus (22%), cataract (24%), and renal clearance <50 ml/min (11%). Eight patients (22%) underwent a retransplantation, and compensated cirrhosis is present in four patients (11%). The pattern of comorbidity seems to relate to the type of immunosuppression which consisted mainly of prednisolone and azathioprine. Quality of life was perceived as satisfactory (7 on a scale of 0 to 10). However, about half of the patients reported limitations in the domains mobility, usual activities and pain/discomfort. In addition a minority reported some anxiety or depression.

Conclusion. The outcome of liver transplantation in this early cohort of patients is fairly good. Improvements may be achieved by adaptations in the immunosuppressive regimen.

KEYWORDS

Comorbidity, EQ-6D, health status, liver transplantation, long-term survival, quality of life

INTRODUCTION

Liver transplantation has been the accepted therapeutic option for end-stage liver disease for more than 20 years. Over the years, survival rates have improved. A substantial number of patients now survive for more than one or even two decades. However, quality of life may be influenced by long-term side effects of immunosuppressive treatment and by the functional status of the liver graft as *de novo* liver disease or recurrent liver disease might develop. Most studies have focussed on single complications after liver transplantation, e.g. cardiovascular disease or renal disease, mainly in the first decade after the transplant, and are not concerned with the whole spectrum of comorbidity. Only two studies are known to us that report extensively on the health status in patients longer than ten years after liver transplant.[1,2] Patterns of comorbidity might differ between centres in relation to patient characteristics, duration of survival after liver transplantation, and the types of immunosuppressive drugs that are used.

The present study concerns the health status and quality of life of patients who received a liver transplant in our centre between 1979 and 1991 and were alive in February 2006.

PATIENTS AND METHODS

All patients who underwent a liver transplantation in our centre at an adult age (>17 years), between 1 March 1979 and 1 February 1991, and who survived at least 15 years were eligible for the present study.

Data collection

From the medical records the following basic data were collected: gender, age at transplant, present age, indication(s) for (re)transplant, date(s) of (re)transplant, date of death, and cause of death.

From all the patients who were alive in February 2006, the following data regarding the health status were collected: eye problems, ENT problems, neurological disease, lung disease, cardiovascular disease, hypertension, body mass index, diabetes, gastrointestinal disease, renal and urological disease, gynaecological disease, malignancies, and osteoporosis. The state of the liver was evaluated by the most recent liver pathology, radiology, and laboratory tests. Present medication, including the dosages of the immunosuppressive drugs, was noted. Most recent routine laboratory tests were noted, including haematological tests, liver tests, creatinine, creatinine clearance, and total cholesterol.

Immunosuppression

Basically, two immunosuppressive regimens have been used for long-term maintenance therapy since the start of our programme in 1979. Until 1986, immunosuppression consisted of azathioprine, 125 to 150 mg/day, and prednisolone in a starting dose of 200 mg/day, which was gradually tapered to a dose of 30 mg/day at six months, 20 mg/day at one year, and 10 mg/day at two years. In 1986 cyclosporine was added, which resulted in a triple drug regimen with lower prednisolone dosages. After the second year we aimed to taper and discontinue cyclosporine in patients with a triple drug regimen. Since 2000 we aim to reduce the prednisolone dose to 5 mg/day and the azathioprine dose to 50 mg/day in patients with long-term survival.

Quality of life

Health-related quality of life was assessed using the Six-Dimensional EuroQol test (EQ-6D).[3,4] The EQ-6D is a concise test which consists of six dimensions: mobility, self-care, usual activities, pain/discomfort, anxiety/depression, and cognitive functioning. Each dimension has three possible answers: no problems, some problems, and extreme problems. Three questions were added concerning a paid job (yes or no), paid help at home (yes or no), and a numerical expression of self-perceived health status (0 to 10, 0 = worst, 10 = best). The questionnaire was sent to the patients by post with the request to participate and to return the list by pre-paid post.

Charlson comorbidity index

The Charlson Comorbidity Index (CCI) gives a weighted score that takes into account both the number and the seriousness of a series of diseases. In addition weight is given to age.[5] We used the modified CCI according to Birim *et al.*[6] in which coronary heart disease is not limited to myocardial infarction alone. In short, one point is given for the conditions coronary artery disease, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective pulmonary disease, peptic ulcer disease, mild liver disease, and diabetes. Two points are given for hemiplegia, moderate or severe renal disease, diabetes with end-organ damage, any tumour in the last five years, leukaemia, and lymphoma. Three points are given for moderate or severe liver disease. Six points are given for metastatic solid tumour and AIDS. In addition, for each decade >40 years of age, one point is added.

In the absence of clear definitions, we defined moderate or severe renal disease as a creatinine clearance <50 ml/min, and moderate or severe liver disease as the presence of advanced fibrosis, cirrhosis, and/or portal hypertension.

Statistical analysis

The χ^2 or Fisher's exact test was used to analyse the categorical data. Survival was analysed by the Kaplan-Meier method. All data were analysed using the Statistical Package for Social Sciences 11.0 (SPSS Inc., Chicago, Illinois, USA). A two-tailed p value <0.05 was considered to indicate statistical significance. When not otherwise stated, the results are given in median and range.

RESULTS

Survival after the 15th year

Forty-nine (45.4%) of the 108 adult patients receiving a liver transplantation before 1 February 1991 survived for at least 15 years after the transplant. The median age at 15 years was 55.7 years (range 32.4 to 73.7). After the 15th year seven patients have died so far. Causes of death were cardiovascular in four patients, bacterial sepsis in relation to recurrent cholangitis and intra-abdominal abscess, respectively, in two patients, and colonic cancer in one patient.

The one- and five-year patient survival rates after the 15th year were 89 and 78%, respectively. In this respect there was no difference between patients older or younger than 55 years.

Health status of the currently alive patients

Patient characteristics

Five patients moved outside the Netherlands and are excluded from the study because of lack of detailed information. The patient characteristics of the 37 remaining patients are listed in *table 1*. Thirty patients are

Table 1. *Patient characteristics of 37 patients currently alive more than 15 years after liver transplantation (median and ranges)*

Number of patients	**37**
Gender (female/male)	30/7 (81%/19%)
Age at LT (years)	38.5 (17.3-58.7)
Diagnosis of liver disease:	
• Primary biliary cirrhosis	13 (35%)
• Primary sclerosing cholangitis	6 (16%)
• Autoimmune cirrhosis	5 (14%)
• Cryptogenic cirrhosis	4 (11%)
• Budd-Chiari	3 (8%)
• Miscellaneous	6 (16%)
Calendar year and month of LT	March 1987 (April 1979-January 1991)
Follow-up after first LT (years)	18.8 (15.0-26.8)
Re-LT:	
• Number of re-LTs	9 re-LTs in 8 patients (21.6%)
• First re-LT, years after LT	5.6 (0.0-12.9)
Reasons for re-LT:	
- HAT	2 (22%)
- De novo HCV	2 (22%)
- Chronic rejection	2 (22%)
- Acute rejection	1 (11%)
- ITBL	1 (11%)
- PNF	1 (11%)

LT = liver transplantation; HAT = hepatic artery thrombosis; HCV = hepatitis C virus; ITBL = ischaemic-type biliary lesions; PNF = primary non-function.

female. Present age is a median of 57.4 years (range 37.7 to 79.3). The median follow-up after liver transplantation is 18.8 years (range 15.0 to 26.8). Most patients were transplanted for autoimmune liver diseases. Eight patients (21.6 %) underwent retransplantations for different reasons.

Long-term medical complications after liver transplant

An overview is depicted in *figure 1*.

Eyes. Nine patients (24.3%) developed a cataract, for which five underwent surgery. One patient developed a glaucoma and one had Sjogren's disease.

ENT. Two patients (5.4%) needed ENT surgery for recurrent sinusitis.

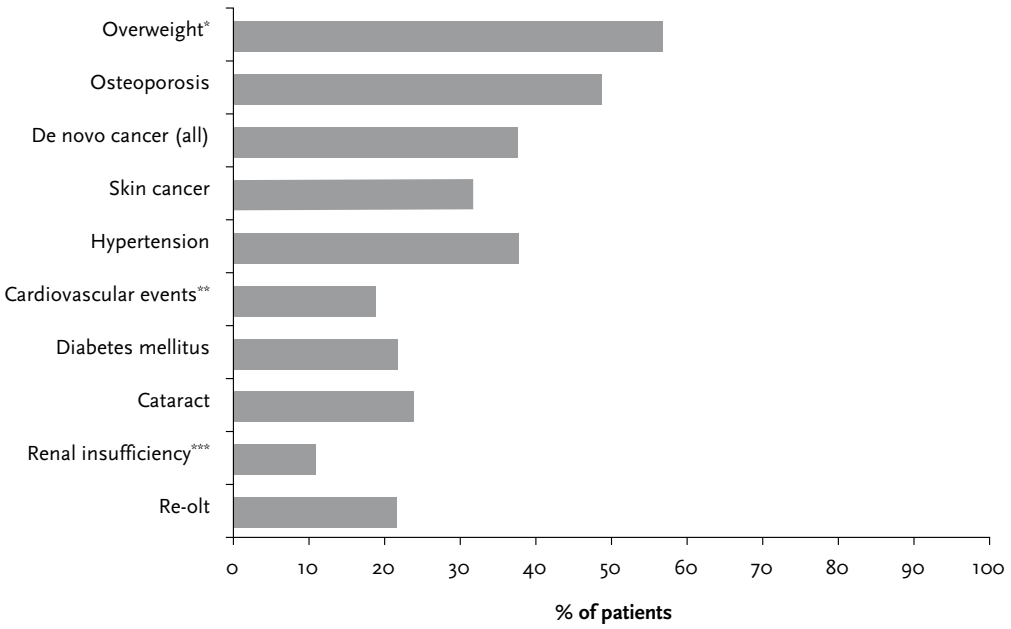
Oral cavity. None of the patients developed (pre)malignancy in the oral cavity.

Lungs. No major lung problems have occurred except that eight patients have had more than one episode of bacterial infection. One patient is suffering from COPD.

Breast. None of the patients developed breast cancer. In one patient a benign tumour was removed; and one patient underwent corrective surgery.

Neurological disorders. One patient suffered from a stroke, peroperatively, with minor long-term sequelae. Two patients had transient ischaemic attacks (TIA). One patient is being treated for epilepsy, after having developed a reversible coma associated with the use of cyclosporine in the first year after transplantation.

Figure 1. *Prevalence of the main comorbidity in 37 patients currently alive more than 15 years after liver transplantation*



***BMI ≥25; ****including myocardial infarction, angina, claudication and TIAs; *****creatinine clearance <50 ml/min.**

Cardiovascular system. Fourteen patients (37.8%) are receiving treatment for hypertension. Two patients suffered a myocardial infarction, after which one of them underwent coronary bypass surgery. Another two patients are being treated for angina pectoris. Two patients are being treated for intermittent claudication. Overall, including the patients with TIAs, 15 patients (40.5%) developed symptomatic cardiovascular disease. In addition one patient suffered from an episode of rheumatic pericarditis. Ten patients (27%) are receiving lipid-lowering drugs. The most recently measured serum cholesterol level is 5.31 mmol/l (3.30 to 9.60).

Body mass index. Overweight, defined as BMI ≥25, is currently present in 21 patients (56.8%). Seven patients (18.9%) are obese, with a BMI >30.

Diabetes mellitus. Six patients developed *de novo* diabetes mellitus type 2 after liver transplant. Including two patients who already had diabetes before the liver transplant, eight patients (21.6%) are currently being treated for diabetes mellitus.

Upper gastrointestinal tract. Two patients had peptic ulcers. Four patients developed recurrent asymptomatic oesophageal varices. Nineteen patients (51.4%) are taking proton-pump inhibitors or H2 blockers. One patient is being treated for exocrine pancreatic insufficiency.

Liver disease. See below, under liver graft.

Colon. One patient developed a colon cancer and underwent a hemicolectomy. Another eight patients had adenomatous polyps removed during screening colonoscopies. Four patients had documented inflammatory bowel disease before the transplant. One of them underwent colectomy after the liver transplant because of severe dysplasia.

Kidneys and urinary tract. Two patients developed a renal carcinoma (detected by routine ultrasound) for which a nephrectomy was performed. One patient with extensive uro-genital condylomata acuminata underwent a cystectomy with an uretero-ileostomy. Eight patients (21.6%) suffered from urinary tract stones. Ten patients were treated more than once for bacterial urinary tract infection.

The serum creatinine is 82 µmol/l (42 to 133), and the creatinine clearance 80 ml/min (24 to 148). Four patients have a clearance <50 ml/min.

Gynaecological disorders. Four of the 30 women had undergone a hysterectomy before liver transplantation. After the transplant, one patient who had surgery is still being monitored closely for extensive condylomata acuminata. Three patients were treated for meno-metrorrhagias. Another three patients were treated for cervical dysplasia. Three patients had successful pregnancies.

Haematological disorders. One patient developed a non-Hodgkin's lymphoma (Epstein-Barr virus negative), which was successfully treated with chemotherapy and anti-CD20. After five years this patient is doing well without signs of recurrence and on low-dose immunosuppression. One patient has anaemia in relation to erythropoietic protoporphyria. Recent laboratory tests show the following blood counts: haemoglobin 8.6 mmol/l (4.0 to 9.9), mean corpuscular volume 94.8 fl (70.9 to 102.1), leucocytes 7.7 10^3/l (range 2.2 to 12.7), platelets 224 10^9/l (61 to 504).

Bone disease. Overall 18 patients (48.6%) are suffering from osteoporosis, defined as a T value <2.5 SD, as measured by bone densitometry. Fifteen of these patients developed the osteoporosis after liver transplant. In 11 patients vertebral osteoporotic fractures occurred. Eight patients suffered from fractures of an arm or leg. Two patients have advanced arthrosis of the hip and ankle, respectively. One patient received a total hip arthroplasty.

Skin. Actinic keratosis is documented in 17 patients and Bowen's disease in five patients. Skin cancer developed in 12 patients (32.4%): basocellular in five patients, planocellular in four patients, and both in three patients. One patient is taking acetretine (Neotigason).

De novo cancer. Overall 16 *de novo* cancers developed in 14 of the 37 patients (37.8%). Excluding the patients who developed skin cancer, four of the 37 patients (10.8%) developed *de novo* cancer at other sites: renal cancer (two patients), colon cancer (one patient), and lymphoma (one patient).

The liver graft

Eight of the 37 patients were retransplanted for a variety of reasons (*table 1*). The current graft function in the 37 patients is as follows.

Most recent liver pathology shows cirrhosis in four patients (10.8%), and fibrosis in a greater or lesser degree in another ten patients (27.0%). Four patients (16.2%) have oesophageal varices. None of these patients, however, have decompensated liver disease, defined as the absence of ascites.

Recurrent disease is present in seven patients (18.9%). Recurrent primary biliary cirrhosis (PBC) in an early stage is present in four of the 13 PBC patients (30.7%), recurrent primary sclerosing cholangitis (PSC; non-anastomotic strictures; as judged by MRCP and histology) in two of the six PSC patients (33.3%), and one patient had signs of recurrent Budd-Chiari syndrome early after liver transplantation.

Three patients have hepatitis C infection; in all three the virus was acquired in the perioperative period either from the (first) donor liver or from blood products. Two patients have nonanastomotic strictures in the biliary tree.

Eight patients (21.6%) are on ursodeoxycholic acid.

Recent laboratory tests reflecting the function of the liver show the following: alkaline phosphatase 70 U/l (38 to 791), aspartate aminotransferase 30 U/l (15 to 105), alanine aminotransferase 22 Ul (8 to 88), γ-glutamyltransferase 49 U/l (9 to 742), bilirubin 13 µmol/l (6-44), total protein 69 g/l (58 to 83) and albumin 41 g/l (28 to 46).

Medication

At present, the medication includes a median of seven drugs (3 to 20) for a variety of conditions. Per patient a median of five conditions (2 to 12) are being treated with drugs. The immunosuppressive regimen consists of prednisolone/azathioprine in the majority of patients (31 patients, 83.8%). The other patients are taking prednisolone as monotherapy (one patient) or in combination with mycophenolate mofetil (one patient), cyclosporine (one patient), tacrolimus (one patient) or azathioprine/cyclosporine (two patients). The median dose of prednisolone is 10 mg (5 to 10), and of azathioprine 100 mg (50 to 125). The combination of prednisolone 5 mg and azathioprine 50 mg, which is currently the lowest dose we aim for after liver transplantation, is being taken by four patients (10.8%).

Other drugs are mainly for cardiovascular disorders and for the prevention or treatment of osteoporosis. See *figure 2* for an overview.

Quality of life

The interview on self-perceived quality of life was completed and returned by 35 patients (94.6%). The results of the EQ-6D are listed in *table 2*. It is shown that a large majority of patients have no problems with respect to self care, do not feel anxious or depressed, and have no cognitive symptoms. The majority of patients have no difficulties with their usual daily activities, but a substantial number do have problems. Most patients have some problems with mobility, and suffer from at least some pain and discomfort. Full inability to perform daily activities and serious pain is reported by 11 and 14% of the patients, respectively. Of the patients, 20% have a paid job and 20% make use of paid help at home.

On the scale of 0 to 10, the self-perceived health status was scored as 7 (4 to 10).

In all these aspects no differences were found between patients older or younger than 55 years, except that more younger patients had a paid job (37.5 *vs* 5.3 %) (p=0.032).

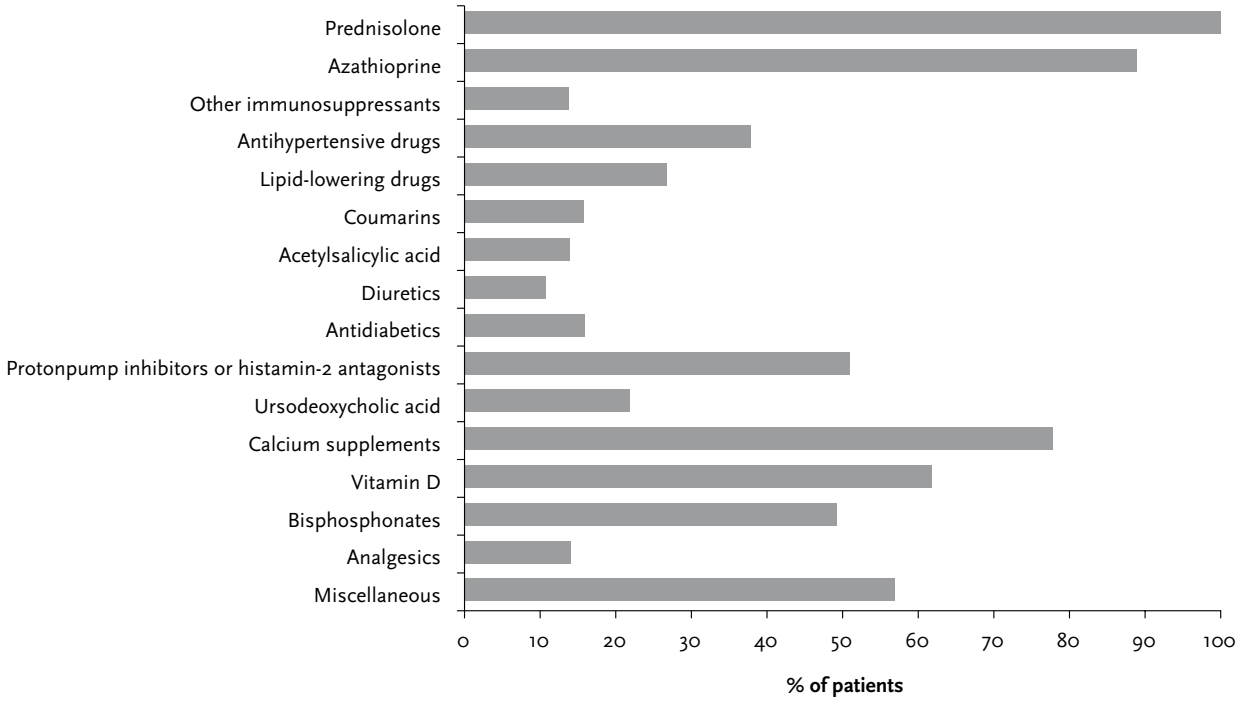
The Charlson Comorbidity index (CCI) in these 35 patients was 3 (0 to 7). No relation was found between the six

Table 2. *Quality of life as measured by the Six-Dimensional EuroQol in 35 patients (number of patients (%))*

	No problems	Some problems	Extreme problems
Mobility	15 (42.9)	19 (54.3)	1 (2.9)
Self-care*	29 (82.9)	4 (11.4)	1 (2.9)
Usual activities	19 (54.3)	12 (34.3)	4 (11.4)
Pain/ discomfort	13 (37.1)	17 (48.6)	5 (14.3)
Anxiety/ depression	27 (77.1)	8 (22.9)	0
Cognition	25 (71.4)	10 (28.6)	0

*Result from one patient is missing.

Figure 2. *Medication use in 37 patients currently alive more than 15 years after liver transplantation*



domains of the EQ-6D and the CCI. Usual activities and the need for help at home tended to relate to the presence of osteoporosis ($0.05 < p < 0.10$).

DISCUSSION

Liver transplantations are usually performed in chronically ill patients. As a result of cirrhosis, the use of drugs (e.g. prednisolone) or the cause of the disease (e.g. alcohol), many patients are already biologically old and suffer from more extrahepatic disease than age controls at the time of transplant. After transplantation, the continuous use of immunosuppressive drugs adds especially to cardiovascular and cancer risk. Although the aim of liver transplantation is to reach long-term survival well beyond 15 years, especially in the younger age group, many do not reach this goal. On the other hand, taking into account all the risks for hepatic and extrahepatic disease one might fear that the quality of life and the overall health status of the long-term survivors is much less than optimal. Liver transplantation was started in our centre as early as 1979 as the fourth regular programme in the world. We evaluated the clinical outcome of patients surviving longer than 15 years, with special interest for the broad range of comorbidity and the self-perceived quality of life. Reports on this subject have been scarce so far.[1,2]

The five-year survival of patients still alive 15 years after transplantation was 78%, with cardiovascular disease as the principle cause of death. It is interesting to note that even after 15 years, age was still not a prognostic marker for survival, and death was determined by comorbidity.

Comorbidity in the currently surviving patients, as listed in *figure 1*, consisted mainly of overweight, hypertension, cardiovascular disease, diabetes mellitus, osteoporosis, and *de novo* cancer. Renal insufficiency defined as clearance <50 ml/min was present in 11% of patients. Looking at this spectrum, two things are remarkable. First, although we did not compare the patient group with a gender- and age-matched control group, comparison with prevalence data in the Dutch population shows lower percentages in the general population.[7-10] From other studies, which focussed on one particular complication, we know that cardiovascular disease and cancer occur more often in organ transplant recipients than in controls.[11-15] Second, the spectrum of comorbidity we found appears to differ from that reported in the studies of Kisilisik *et al.*[1] and Cicarelli *et al.*[2] in patients surviving more than ten years after transplantation. Our patients more often suffered from osteoporosis (prevalence 49 *vs* 4 and 9%, respectively), skin cancer (32 *vs* 4 and 7%), overweight (56 *vs* 49 and 13%), and cataract (24 *vs* unknown and 8%). However, we less often observed hypertension (38 *vs* 64 and 48%), and end-stage renal disease for which haemodialysis or renal transplantation was indicated (0 *vs* 4 and 9%), and serum creatinine levels were significantly lower in our patient group.

Most likely, these differences in comorbid conditions reflect the different immunosuppressive regimens that were used in these patient cohorts. In the early years of our programme, patients were only taking prednisolone and azathioprine. Prednisolone was given in dosages that are, by today's standards, excessively high. A minority of patients started on cyclosporine-based triple therapy, but cyclosporine was tapered and discontinued after the second or third year in most patients. As a result, most of our long-term survivors are still being treated with prednisolone and azathioprine. In contrast, Kizilisik *et al.*[1] used cyclosporine, in combination with low-dose steroids, in most patients, and azathioprine in a minority of them. Cicarelli *et al.*[2] have used cyclosporine or tacrolimus in almost all patients, with prednisolone and/or azathioprine in some patients. The high prevalence of osteoporosis, cataract and overweight in our patients may well be the result of the continued use and high cumulative doses of steroids. Skin cancer might relate to the long-term use of especially azathioprine.[16-18] On the other hand, the limited and short-term use of calcineurin blockers in our patients seems to have led to a lower rate of hypertension and a virtual absence of renal insufficiency in comparison to the other groups. This underscores the importance of the immunosuppressive regimen as a determinant of future complications.

In earlier studies, including the patients presented in this study, we have shown that bone loss occurred mainly in the first year after transplantation, despite the preventive use of daily 1-alpha-hydroxycholecalciferol and 1 gram calcium, with no significant deterioration or even improvement afterwards.[19,20] In the present era development of osteoporosis before and after transplantation is a less serious problem due to preventive strategies with biphosphonates, which became available in the 1990s, in combination with calcium and vitamin D.[21]

A second important finding concerns the graft. In total, 22% of our patients received a retransplant. We have previously reported that the cumulative retransplantation rate rises from 10% at one year to 22% at 15 years after the first transplantation. This figure does not differ from that of most centres.[22] It shows that retransplantation is feasible with good outcome. Currently, compensated cirrhosis is present in 11% of patients, but overall liver function is good. Recurrence of disease without major consequences as yet was found in a minority of patients transplanted for primary biliary cirrhosis, primary sclerosing cholangitis, and hepatitis C, as expected.

A third important finding concerns quality of life. We found that the patients were generally satisfied with their present health status, rating it on average as 7 on a scale of 0 to 10 (with 0 as lowest and 10 as optimal). However, as measured by the EQ-6D, about half of the patients reported limitations in the domains mobility, usual activities, and pain/discomfort. In addition a minority report some anxiety or depression. In a study by Hoeymans *et al.*[23] Dutch adults in the general population scored better on all

EQ-6D domains. Our findings seem to be in agreement with those from other centres. In general, quality of life has been shown to improve after a successful transplantation, but in the long run remains lower than that of the general population.[24-28] Kizilisik *et al.*[1] report an equal or even better quality of life in comparison with age-matched controls, but they restricted their questionnaire to a self-perceived health score, satisfaction with life, self care, and activity level. Also our patients scored high on satisfaction and self care, but probably lower on activity level.

Quality of life as measured by the EQ-6D did not relate to the level of comorbidity as measured by the CCI. However, caution is warranted here because the CCI and its variants were originally developed and used for prediction of outcome after breast cancer,[5] lung cancer,[6] peritoneal dialysis,[29] kidney transplantation,[30] and other.[31,32] Caution is also called for with respect to the value of these self-assessments. Having been chronically ill, patients may accept physical problems without complaining. Many patients are grateful that they received this opportunity for survival, and tend to regard remaining problems as 'minor'.

To conclude, our data show that patients ultimately have to pay a price for long-term immunosuppression. Several strategies may be useful to keep this price as low as possible. Nowadays, the availability of a wide spectrum of immunosuppressive agents allows individualised selection of drugs, thereby avoiding specific side effects. Knowledge of regimen-specific long-term toxicities should prompt adequate monitoring for side effects and timely adaptation of the regimen, and the use of prophylactic measures (e.g. biphosphonates, lipid lowering drugs). In this way, we may achieve a better long-term health status in future patients.

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CASE REPORT

Spontaneous remission of acute myeloid leukaemia after recovery from sepsis

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ABSTRACT

Spontaneous remission of acute myeloid leukaemia (AML) is extremely rare and usually of short duration. We report two patients with documented AML who developed spontaneous remission of their leukaemia shortly after an episode of severe sepsis and respiratory failure requiring mechanical ventilation. The underlying mechanisms of spontaneous remission remain unclear but an association with preceding blood transfusions and severe systemic infections has been reported. An overwhelming immune response due to sepsis and leading to raised levels of TNF-α, INF-γ, IL-2 and an increased activity of NK cells, cytotoxic T-cells and macrophages are thought to play an important role. Better insights into the mechanisms of spontaneous remission of AML after recovery from sepsis could help in developing new therapies for AML.

KEYWORDS

Acute myeloid leukaemia, critically ill, immune response, mechanical ventilation, sepsis, spontaneous remission

INTRODUCTION

Treatment of haematological malignancies is still accompanied by a high therapy-related morbidity and mortality rate.[1] If admission to an intensive care unit (ICU) is indicated because of respiratory failure, it often predicts a poor prognosis with a mortality rate of 60 to 90%.[2,3] This high mortality rate is mainly due to multiple organ failure as a result of severe sepsis caused by opportunistic infections related to prolonged neutropenia. In addition, administration of chemotherapy is often postponed until patients are discharged from the ICU, resulting in an important delay in the treatment of the underlying disease, which also affects the prognosis negatively. For these reasons, there is much controversy about whether or not patients with haematological malignancies should be admitted to the ICU when they require mechanical ventilation. Here we describe two patients with acute myeloid leukaemia (AML) who were given the benefit of the doubt and were admitted to our ICU because of severe sepsis and respiratory failure in the very beginning of their illness, just before or just after the diagnosis of AML was made. The clinical course showed an unexpected and surprising twist leading to a spontaneous remission of the AML.

CASE REPORTS

Patient A, a 29-year-old Iraqi male, was transferred to our hospital in September 1997 because of a suspected AML. Laboratory results showed a mild leucocytosis of 12 x 10^9/l, with 21% blasts. Bone marrow biopsy showed an AML, classified as French-American-British (FAB) M2. Cytogenetic analysis of the bone marrow demonstrated a t(8;21) translocation and deletion of the Y chromosome. Two days after admission, a superimposed infection was suspected and the patient was immediately treated with broad-spectrum antibiotics (cefpirom), and chemotherapy was postponed. After three days, progressive respiratory failure developed, showing bilateral infiltrative abnormalities on a chest X-ray. He was transferred to our ICU and mechanical ventilation was started. According to our protocol, empiric antifungal (itraconazole) and antiviral (aciclovir) therapy was added to broad-spectrum antibiotics (imipenem-cilastatin). Microbiological examinations, including repeated broncho-alveolar lavage (BAL), gave no additional diagnostic clues. The patient was intermittently ventilated in prone position for two weeks. Mechanical ventilation was complicated twice by an acute tension pneumothorax. Laboratory tests showed a persistent pancytopenia, necessitating frequent

transfusions with erythrocytes and platelets. Over time, the patient's clinical condition deteriorated with development of severe cachexia, and the clinical picture of an ongoing sepsis. Extensive microbiological examinations yielded no causative micro-organism. Three weeks after admission to the ICU, a spontaneous rise in the white blood cell count and platelets heralded a dramatic improvement in the patient's clinical condition, resulting in successful weaning off the ventilator followed by extubation. A repeated bone marrow examination showed a hypercellular bone marrow without the presence of blasts, suggesting a spontaneous cytological remission of the AML. This was confirmed cytogenetically by a normal karyogram without t(8;21) translocation and the presence of a normal Y chromosome. A few days later, he was transferred to the haematology ward. Bone marrow examination was done repeatedly and showed a persistent cytological remission (<5% blasts) which was confirmed by polymerase chain reaction (PCR). The patient refused a consolidation course with chemotherapy and unfortunately, after three months, PCR again showed the presence of (8;21) translocation and after six months, bone marrow examination also showed a cytological relapse of the AML. During the second induction chemotherapy according to the HOVON protocol (www.hovon.nl), the patient died of a massive intracranial haemorrhage.

Patient B, a 28-year-old Dutch male, was admitted to the ICU in January 2006 because of a septic shock. The patient's history revealed no prior diseases. He complained of having a sore throat and fever for one week and had developed diffuse bruising of his skin. The patient was seen in the emergency room of another hospital and a diagnosis of streptococcal infection was suspected. On admission, we saw a critically ill young man with high fever up to 41°C and an altered mental state. He complained of headache, fatigue and dyspnoea. On physical examination a petechial rash and bruising of the skin was seen on the trunk and lower parts of the body. He was haemodynamically unstable with a systolic blood pressure of 80 mmHg. Laboratory tests showed a pancytopenia with a white blood cell count of 1.3 x 10^9/l, Hb 5.0 mmol/l and thrombocytes of 33 x 10^9/l. The differential showed 26% blasts. Blood gas analysis showed a mild respiratory alkalosis, initially without hypoxia. Sepsis in combination with an acute leukaemia was suspected. He was treated with broad-spectrum antibiotics (imipenem/cilastatin). After a few hours his clinical condition deteriorated with progressive respiratory failure and mechanical ventilation was started. Blood cultures revealed group G β-haemolytic *Streptococci* and penicillin G was administered. Bone marrow aspirate showed an acute monoblastic leukaemia, classified as FAB-M5B, without chromosomal abnormalities. According to our protocol, antifungal (voriconazole) and antiviral (aciclovir and gancyclovir) therapy was added empirically. As a result of the patient's septic condition, cytostatic drugs were not applied. Transfusions with erythrocytes and platelets had to be given every other day. The clinical course was further complicated by a leucocytoclastic vasculitis of the skin, and a central venous catheter related bloodstream infection. Two weeks after admission, a spontaneous rise in white blood cell count and platelets developed. Surprisingly, the repeated bone marrow examination showed a complete cytological remission of the AML. The condition of the patient improved remarkably and he was extubated shortly after spontaneous remission was confirmed. The patient was transferred to the haematology ward and received remission-induction chemotherapy followed by myeloablative allogeneic stem cell transplantation without noticeable complications. Unfortunately, four weeks after he was transplanted, a full-blown relapse of the AML was diagnosed.

DISCUSSION

Spontaneous remission (SR) of AML is rare in adults. Since the first description in 1878[4] about 100 cases have been reported.[5] SR became even more infrequent when effective therapeutic strategies for acute leukaemia became widely available. SRs in AML are of short duration (mean 7.7 months; range 1 to 36); however, long-term remissions and even complete cytogenetic remissions have been documented.[6] The mechanisms inducing SR are thought to be diverse and may partially be mediated by cellular immune phenomena, but remain unclear in most cases.[7] An association of SR in AML with preceding transfusion of blood products and/or concomitant infections has been noted.[8] Mostly bacterial, but also fungal or viral infections are observed. Infections have been argued as triggering an immune response causing SR. In particular severe systemic infections appear to precede SR.[5,7,9,10] Severe sepsis is characterised by a profound increase in cytokine levels such as tumour necrosis factor-α (TNF-α), interleukin-2 (IL-2) and interferon-γ (IFN-γ) as well as an increase in natural killer (NK) cells and cytotoxic T cells. Also hypergammaglobulinaemia is frequently seen in these patients[5,8,11-14] and might suggest a potential role of humoral immune events in SR: increased antibody-mediated cytotoxicity of NK and cytotoxic T cells and of activated macrophages due to better recognition, opsonisation or adhesion could play a role in this phenomenon.[9] In our two patients, the presence of a severe systemic inflammation could have played an important and possibly a causal role in developing the observed SR.

The association between transfusions of blood components and SR has been reported.[5,8,12-14] Cytotoxic antibodies against leukaemic cells, and allogeneic lymphocytes

might play a role, representing mechanisms similar to those described as graft-versus-leukaemia (GVL) effect in allogeneic transplanted patients.[15] However, at present, neither of the theories have been proven and nearly all AML patients receive multiple leucocyte-depleted transfusions, usually without achieving SR.

It can be hypothesised that overwhelming sepsis leading to an exuberant activation of the immune system causes containment of the leukaemia. This mechanism is used as a paradigm for designing new antileukaemic therapies. For instance TNF-α, a cytokine that regulates cell proliferation and differentiation, and takes part in the immune response, has been demonstrated to cause inhibition of blast cell proliferation *in vitro*.[13,16,17] Promising effects of IL-2 have already been shown in patients with refractory or relapsed AML who were not suitable for further chemotherapy or as postconsolidation therapy after induction chemotherapy.[18,19] The principle of using the immune system to fight the cancer is being investigated in solid tumours, lysing tumour cells or preparing tumour specific peptides, followed by uptake of tumour antigens by dendritic cells and presentation to the immune system.[20] Based on this mechanism, several groups are trying to refine the method of antigen presentation, for example by dendritic cell vaccination.[21] However, these studies are still preliminary and further large randomised trials are needed to address this issue.

Although it would be very intriguing to solve the puzzle about SR in AML, prospective studies regarding this issue are difficult to carry out. The rarity of this phenomenon asks for a multicentre approach, and the complexity of the situation asks for a systematic approach, determining all possibly involved factors (cytokines etc), so that the culprit can be pinpointed and used for targeted therapy. All this has prevented such a study from actually taking place, so that we only have case reports to help us in solving the causative mechanism of SR in AML, but these have given us ideas about causative factors which are now being investigated.[13,16-21]

CONCLUSION

We have described two patients diagnosed with AML who were admitted to our ICU because of severe sepsis and respiratory failure requiring mechanical ventilation. Despite clinical deterioration and a predicted poor prognosis, SR of the AML occurred. SR in AML is very rare but it has been reported before. It is in particular associated with severe infection, although this association is hard to prove. Soluble factors such as TNF-α, IL-2, INF-γ, hypergammaglobulinaemia, cross-reactive antibodies, direct cytotoxic antibodies in donor serum as well as cellular factors such as an elevated number of NK cells or allogeneic lymphocytes with a GVL effect in blood transfusions have been considered to be involved in SR. Nevertheless, the exact mechanisms of SR still remain unclear. Though most remissions are of short duration, long-term remissions and even complete cytogenetic remissions have been documented. Better insights into mechanisms underlying spontaneous remission could help us to develop new therapeutic approaches.

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CASE REPORT

Acromegaly caused by a growth hormone-releasing hormone secreting carcinoid tumour of the lung: the effect of octreotide treatment

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ABSTRACT

In acromegaly, the overproduction of growth hormone is usually caused by a pituitary adenoma. We report a 74-year-old woman with acromegaly caused by ectopic overproduction of growth hormone-releasing hormone (GHRH), a rare diagnosis. The GHRH appeared to be produced by a carcinoid tumour of the lung. Treatment with monthly long-acting octreotide resulted in a reduction in the symptoms and normalisation of the insulin-like growth factor-I, which has been maintained for more than two years now. A review of literature concerning causes and treatment of ectopic GHRH-producing tumours is presented.

KEYWORDS

Acromegaly, ectopic GHRH-producing tumours, octreotide treatment

INTRODUCTION

Acromegaly is a clinical syndrome caused by overproduction of growth hormone.

The excess of growth hormone is nearly always produced by a pituitary adenoma.

In less than 1% of the cases, however, the normal pituitary is stimulated by either eutopic or ectopic overproduction of growth hormone-releasing hormone (GHRH).

Clinical, biochemical and radiological features are often indistinguishable between growth hormone-producing adenomas and ectopic GHRH-producing tumours, making it difficult for the latter to be recognised. In this case report we present a patient with a GHRH-producing carcinoid tumour of the lung, whose pituitary MRI and the presence of a lesion in the lung led us to the possibility of this rare cause of acromegaly. The response to a long-acting somatostatin analogue will be reported as well.

CASE REPORT

A 74-year-old woman was referred to the hospital with anaemia caused by iron deficiency (haemoglobin 3.6 mmol/l, normal 7.2-9.0, MCV 59 fl, normal 81-96, ferritin 2 µmol/l, normal 14-150). Further diagnostic work-up showed an adenocarcinoma of the proximal colon; a right-sided hemicolectomy was performed. Microscopic evaluation showed a $T_3N_0M_0$ tumour. On physical examination there were clear signs of acromegaly. She had coarsening of facial features, protrusion of the lower jaw and macroglossia. In retrospect these symptoms had been progressively present over approximately the last 15 years. Laboratory investigation showed a random growth hormone (GH) concentration of 85 mU/l (normal <20) and an elevated insulin-like growth factor-I (IGF-I) of 605 ng/ml (normal for age and gender <200), already confirming the clinical diagnosis of acromegaly.

The levels of the other pituitary hormones were prolactin 1795 mU/l (normal <500), luteinising hormone (LH) 2.4 U/l (normal for age and gender 20-100), follicle-stimulating hormone 5.8 U/l (normal for age and gender 30-120), thyroid-stimulating hormone 1.2 mU/l (normal 0.3-4.0). The free thyroxine (fT_4) concentration was 12 pmol/l (normal 11-24) and a random cortisol value was 312 nmol/l. After intravenous injection of thyrotrophin-releasing hormone as well as of LH-releasing hormone a more than

50% increase in growth hormone concentration was noted, a typical response of a pituitary growth hormone producing adenoma. An intravenous dose of 50 μg octreotide caused a steep decline of the growth hormone concentration from 80 mU/l to less than 5 mU/l after 90 minutes.
The MRI, however, did not show a demarcated tumour. Instead a symmetrically enlarged pituitary gland was visible (*figure 1*). This raised the possibility of a GHRH-secreting tumour leading to hyperplasia of the somatotrophic cells causing enlargement of the pituitary and overproduction of growth homone. Since our patient had had a stable solid lesion of the right lung for more than 15 years, a carcinoid tumour was considered (*figure 2*). In order to strengthen this hypothesis somatostatin receptor scintigraphy using ^{111}In pentetreotide was performed. The lesion in the right lung appeared to be the only spot with increased uptake of labelled octreotide (*figure 3*). A transthoracic cytological fine needle aspiration showed atypical cells compatable with a carcinoid tumour.

Figure 1. *Coronal section showing a symmetrically enlarged pituitary gland*

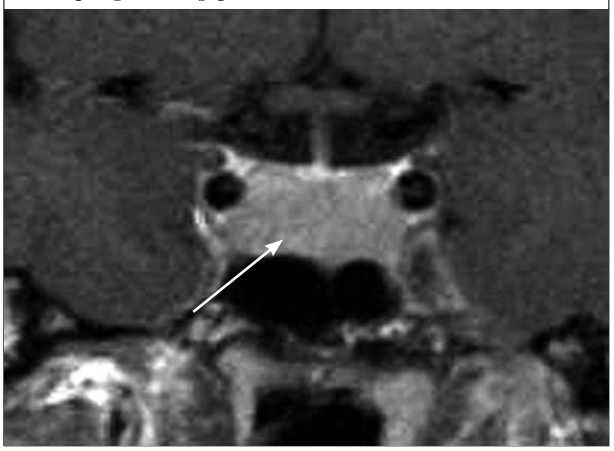


Figure 2. *Chest X-ray with a rounded-shaped tumour in right lung*

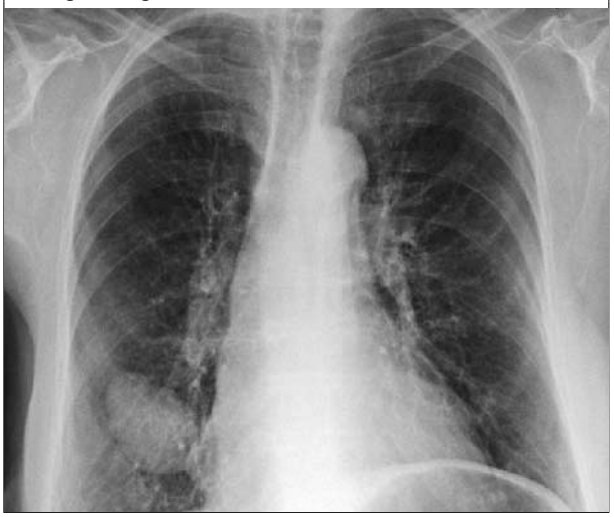
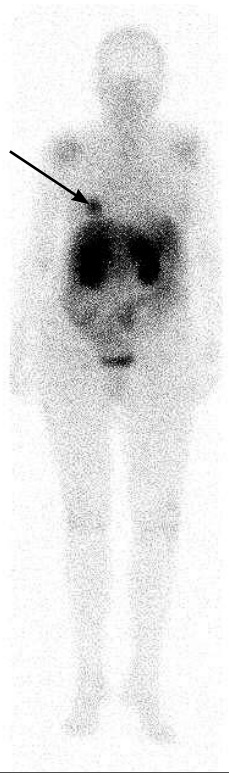


Figure 3. *^{111}In labelled pentreotide scintigraphy showing increased uptake of the radiopharmacon in a lesion in the right lung (arrow)*

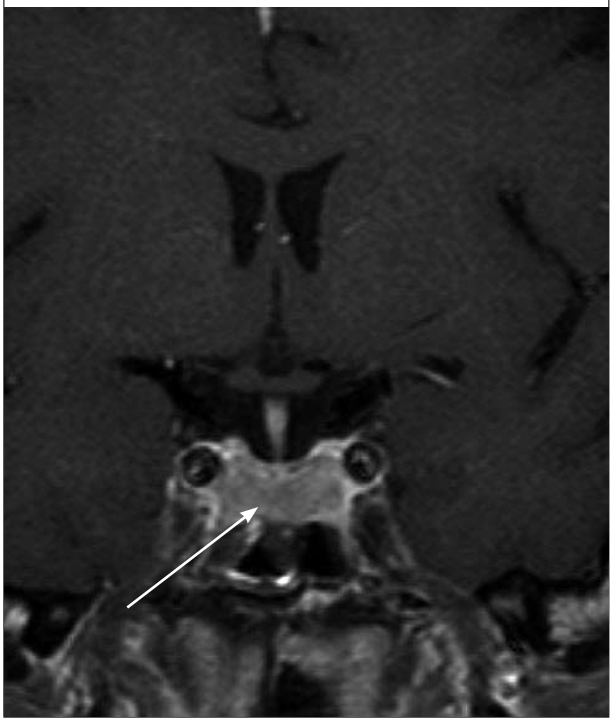


Successively measurement of fasting plasma GHRH was performed showing a high concentration of 3155 pg/ml (Quest Diagnostics, California, normal <49), as well as a simultaneously measured IGF-I level of 599 ng/ml (normal <200). We therefore concluded that the acromegaly was caused by ectopic overproduction of GHRH likely due to the carcinoid tumour of the right lung. Our patient was very reluctant to undergo surgery. We therefore started treatment using a monthly intramuscular long-acting somatostatin analogue, octreotide (Sandostatin LAR 20 mg). This led to a clinical reduction in the signs of acromegaly, a normalisation in IGF-I (177 ng/ml) and GH (2.2 mU/l) and a reduction in the fasting GHRH concentration (805 pg/ml). One year after the initiation of therapy a MRI showed a reduction in size of the pituitary gland (*figure 4*). At the moment, two years after the start of medical therapy, our patient is still being biochemically controlled with the long-acting octreotide and the lung tumour has remained unchanged in size without evidence of metastases elsewhere.

DISCUSSION

Acromegaly caused by a GHRH-secreting tumour is very rare. Only about 65 cases have been reported in the literature. Thorner measured GHRH in plasma of

Figure 4. *Coronal section showing decrease in volume of the pituitary gland one year after the start of octreotide treatment*



177 consecutive cases of acromegaly and found no cases of GHRH overproduction.[1] The frequency of GHRH excess causing acromegaly is estimated to be less than 1%.[2] Cases can be divided into eutopic and ectopic. Eutopic excess of GHRH secretion is caused by hypothalamic hamartomas, choristomas, gliomas or gangliocytomas. Ectopic causes are neuroendocrine tumours. Two-thirds are believed to be of carcinoid origin usually bronchial, but gastrointestinal and pancreatic carcinoids have also been reported. Pancreatic islet cell tumours, thymic tumours, tumours associated with multiple endocrine neoplasia type 1 (MEN-1) syndrome, small cell lung cancer, adrenal adenoma or pheochromocytoma have also been reported.[3,4-17]

Clinically and biochemically eutopic and ectopic GHRH overproduction are virtually indistinguishable from a pituitary GH-producing adenoma. Losa, however, reported that in dynamic testing all GHRH-producing tumours showed a hyperresponse (more than 50% increase from basal value) to TRH and mostly a blunted response to GHRH administration.[2] Hyperprolactinaemia is much more often present as GHRH is also known to stimulate the pituitary lactotrophic cells to increase their production of prolactin. Furthermore, in ectopic GHRH production a strikingly elevated GHRH level in peripheral blood samples is usually present, disappearing after removal of the ectopic origin.[3,18] In ectopic acromegaly there may be symptoms of mass effect of the primary tumour and in some cases of the co-secreted hormones, such as insulin, gastrin, somatostatin, glucagon, calcitonin, pancreatic polypeptide, serotonin, dopamine or norepinephrine.[5]

On pituitary imaging using MRI, there is also a wide range of abnormalities, ranging from a normal pituitary gland, to hyperplasia or adenoma.[5,19] Our patient showed both an elevated GHRH level and a symmetrical enlargement of the pituitary on MRI.

Most carcinoid tumours can be visualised with somatostatin-receptor scintigraphy. Kwekkeboom *et al.* found a sensitivity of 86% in a group of 37 patients with histologically proven carcinoid tumours.[20] ^{111}In-pentetreotide is injected intravenously and imaging is performed 24 hours later. When necessary, additional images are taken 48 hours after injection, for example if interpretation of the images of the abdomen is difficult because of physiological bowel activity. Planar images are taken routinely, single photon emission computed tomography (SPECT) can be performed for a more accurate localisation of the abnormalities. Somatostatin receptor scintigraphy can be carried out for tumour localisation or for staging purposes. Another indication is demonstration of somatostatin receptor positivity of a tumour, in order to select patients who are likely to respond favourably to somatostatin analogue therapy.[7,20]

A definitive diagnosis of ectopic GHRH production can be made either by showing an arteriovenous concentration gradient of GHRH in the region of the tumour or by normalisation of GHRH, IGF-I and GH-levels after removal of the tumour.[2] Although in our patient these conditions are not met, we feel confident with the diagnosis considering the biochemical, radiological, cytological and scintigraphic data and the response to therapy.

Surgical removal of the tumour is the usual therapy. Differentiation from pituitary adenomas is thereby essential, avoiding unnecessary pituitary surgery. Although carcinoids are believed to be slow-growing malignancies with insidious development of acromegaly over many years, by the time the diagnosis is made they have frequently metastasised, thus prohibiting curative surgery.[21] Pituitary irradiation, sometimes used in acromegaly for patients who are not cured by surgery and/or medical therapy, is of little use in ectopic GHRH-secreting tumours.

Until the end of the 1980s metastatic GHRH-producing tumours were essentially untreatable, since dopamine agonists such as bromocriptine could not suppress the GH secretion and IGF-I levels sufficiently due to a continued peripheral GHRH overproduction. The introduction of (long-acting) somatostatin analogues, octreotide and

lanreotide, suppressing both GH and the peripheral carcinoid GHRH production, changed this situation dramatically. With the start of treatment several patients experienced sudden relief of their symptoms, with a drop in GH and IGF-I levels following some months later. The effect on tumour shrinkage, also in the long term, appeared to be dose-dependent in several cases, with follow-up ranging from 3 to 120 months.[5,17,22,23]

Our patient has responded very well to the treatment and has sustained a reduction in the symptoms and acceptable biochemical marks for more than 2 years now. An MRI one year after initiation of the octreotide therapy showed regression of the size of the pituitary gland.
In conclusion, we present a patient with a very rare cause of acromegaly due to an ectopic overproduction of GHRH by a carcinoid tumour of the lung. Treatment consisted of monthly intramuscular long-acting octreotide. This induced a reduction in the symptoms with acceptable biochemical values in the follow-up of more than two years to date.

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CASE REPORT

Open biopsy: the ultimate test for pulmonary embolism

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KEYWORDS

CT angiography, pulmonary embolism, surgical biopsy

INTRODUCTION

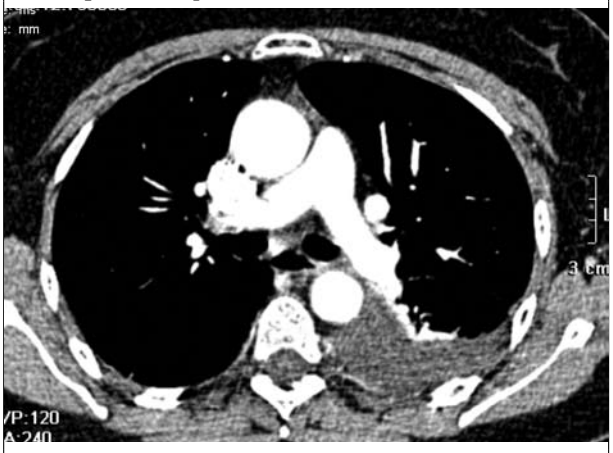
Pulmonary embolism (PE) affects 0.5 to 1 per 1000 people in the general population each year and is one of the most common preventable causes of death among hospital inpatients.[1] The overall mortality rate of PE is high with approximately 15% at three months.[2] Although anticoagulant therapy is highly effective in preventing death, it is frequently either not administrated, or administrated too late, because the diagnosis of PE has not been entertained.[3]

The diagnosis of PE remains difficult because the symptoms are not specific and all the available tests have substantial limitations. Computed tomography angiography (CTA) is readily available at most institutions and is rapidly becoming the first-line imaging test for the assessment of patients with suspected acute PE.[4] This case illustrates that also CTA has its false-negative results and how the diagnosis PE can be established alternatively.

CASE REPORT

A 58-year-old woman was admitted to our hospital because of severe chest pain and progressive shortness of breath for one week. Her medical history showed hypertension, constipation, diverticulitis, and Raynaud's phenomenon. At presentation, she had no fever and physical examination showed no abnormalities. Routine blood examination revealed high serum levels of C-reactive protein (35; normal < 10 mg/l), a normal leucocyte count and a normal troponin I level. The D-dimer assay (D-dimer Cardiac Reader; Roche Diagnostics GmbH, Germany) was negative. The electrocardiogram was normal and the chest X-ray showed some left-sided pleural effusion with minor atelectasis. The clinical suspicion of PE was considered moderate (i.e. '3' on the modified score) according to the criteria of Wells *et al.*[5] and an additional multislice CTA was performed. It confirmed the left-sided pleural effusion and showed no other abnormalities and no direct signs of PE (*figure 1*).[6] CTA was performed on a four detector row CT scan (Siemens Volume Zoom, Erlangen, Germany), 1.25 collimation, increment 0.6. The scan was obtained in a caudal to cranial direction during suspended inspiration with an automated bolus triggering technique using a 120 ml bolus of contrast agent (Ultravist 300, Schering, Germany), injected at 4 ml/sec. As there were no direct signs of PE, the heparin (Fragmin) was stopped. The patient was treated for a urinary tract infection (*E. coli*) and was discharged from hospital after being admitted for seven days.

Figure 1. *Pulmonary CTA showing no filling defects in the pulmonary vasculature*



There is pleural fluid located dorsally on the left side together with partial atelectasis of the left lower lobe.

After being home for only three days, she was readmitted in a very poor condition with progressive dyspnoea. Chest X-rays showed a large amount of pleural effusion, again on the left side. Despite extensive examination (CTA of the chest, bronchoscopy, pleural punction, rheumatoid arthritis serology, ANA and ENA determination, tuberculosis cultures, serology for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*) no cause was found for this exsudative pleuritis. It was not until after these diagnostic tests that the pleural effusion was drained and flushing of the pleural cavity with streptokinase was started.

Furthermore, intravenous antibiotics (Augmentin) were given. She soon recovered and routine chest X-rays showed further improvement. The drains were removed and the patient could leave the hospital after 19 days in a good clinical condition.

After being back home for 80 days she became ill again, showing the same symptoms that she had had before. She was admitted to hospital for the third time, one week after the start of symptoms. Also this time, extensive examination (including CTA) was performed and the cause of this left-sided pleural effusion could not be found. Because of the repeated character of the disease we decided to perform a thoracotomy for pleural drainage and open biopsy of lung and pleura. Excision biopsies of the left upper and lower lobe both revealed wedge-shaped subpleural infarctions explaining the pleural effusion. Some arteries showed intimal thickening and in one artery signs of an old thromboembolus with organisation and recanalisation was found (*figures 2* and *3*). The pleural surface of the involved area was covered with fibrin. A biopsy of the parietal pleura showed chronic inflammation and fibrin deposition. The patient received analgesics and oral anticoagulation (acenocoumarol) and could soon leave the hospital. On ambulatory check-ups her recovery was favourable. She had not encountered any further episodes of chest pain or dyspnoea. Additional blood tests for coagulopathy, including plasma homocysteine levels and lupus anticoagulans, did not reveal any abnormalities. After six months, the anticoagulation therapy was stopped.

DISCUSSION

Because there are no specific diagnostic clinical features of PE, the role of the clinical assessment is to formulate the patients presenting symptoms and signs into an estimate of the probability of PE.[3] Wells *et al*.[5] developed a simple clinical model to predict the probability of PE that is implemented in our diagnostic work-up of patients with suspected PE.

Laboratory tests are helpful to establish an alternative diagnosis or to exclude the diagnosis PE. The role of D-dimer testing in patients with suspected PE is to exclude the diagnosis: normal concentrations of D-dimer have a high negative predictive value for PE, particularly in patients with a low clinical probability.[6,7] If the D-dimer result in that group is negative, diagnostic imaging is not required and it is safe to withhold treatment, as the three-month cumulative incidence of subsequent venous thromboembolism in untreated patients is low (0.5%).[3] Contrast pulmonary angiography is the traditional reference test for the diagnosis PE, but is rarely used: it is invasive (thus associated with morbidity and mortality), requires a high level of expertise, and is not widely available.[8] So, ventilation-perfusion (V-P) scanning or CTA are generally performed instead, for the confirmation or exclusion of PE. Studies have shown that CTA, especially multislice, has greater discriminatory power than V-P scanning.[6,8] Furthermore, in up to 65% an alternative diagnosis can be made by CTA when PE is not present as other structures such as lung parenchyma and mediastinum are visualised as well.[6]

Figure 2. *Lung tissue infarcted on the left side, vital tissue on the right side (H&E staining)*

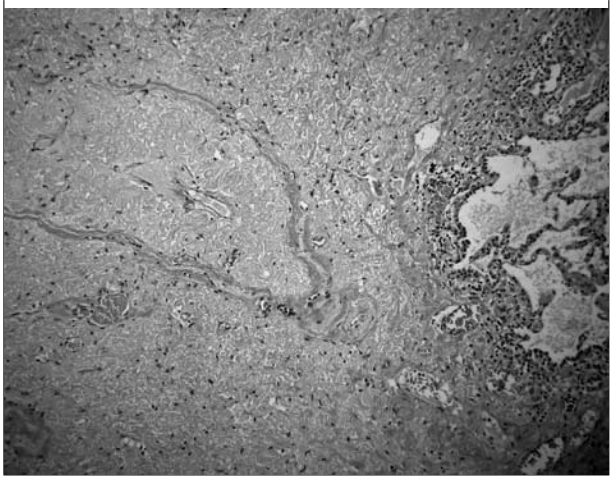
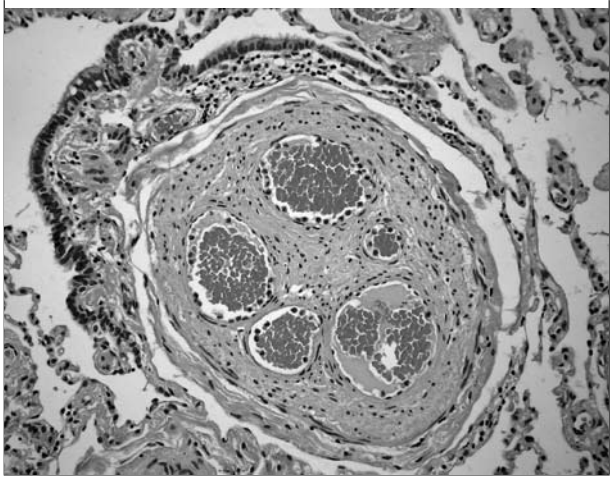


Figure 3. *Cross-section of an artery with an old thrombus with recanalisation (H&E staining)*



Although the patient in this case had a low probability for PE with a negative D-dimer test, an additional CTA was performed. Because pleural effusion and atelectasis are only indirect and nonspecific signs of PE, the diagnosis of PE could not be confirmed on either of the three CT angiograms. As the patient did not have clinical signs of a deep venous thrombosis and the additional value of serial ultrasonography of the legs is low,[9] ultrasonography was not performed. Catheter pulmonary angiography was not considered in this patient, since CTA was thought to be conclusive because of the excellent quality of the images. Moreover, catheter angiography is invasive, carries risks and also has its false-negative results: after a negative catheter angiography fewer than 2% of patients develop PE. In fact, these results are in the same range as reported after a negative CTA.[10,11]

Finally, diagnosis of PE was made by histopathological examination of an open biopsy. As small peripheral PE could have been missed on the first reading, all CT angiograms were reviewed by an experienced radiologist who was aware of the results of the lung biopsy. Nevertheless, all scans were still considered negative for PE.

To our knowledge, no other similar cases have been published to date. We suggest that, as a last resort, biopsy by video assisted thoracoscopy (VATS) or thoracotomy is a viable option for patients with persistent pulmonary illness without a definite diagnosis.

POSTSCRIPT

After writing this manuscript the patient was readmitted to the hospital with the same symptoms as before, two months after she had stopped taking oral anticoagulation. On this occasion, CTA did confirm the diagnosis of PE. Consequently, she is on lifelong anticoagulation therapy.

ACKNOWLEDGEMENT

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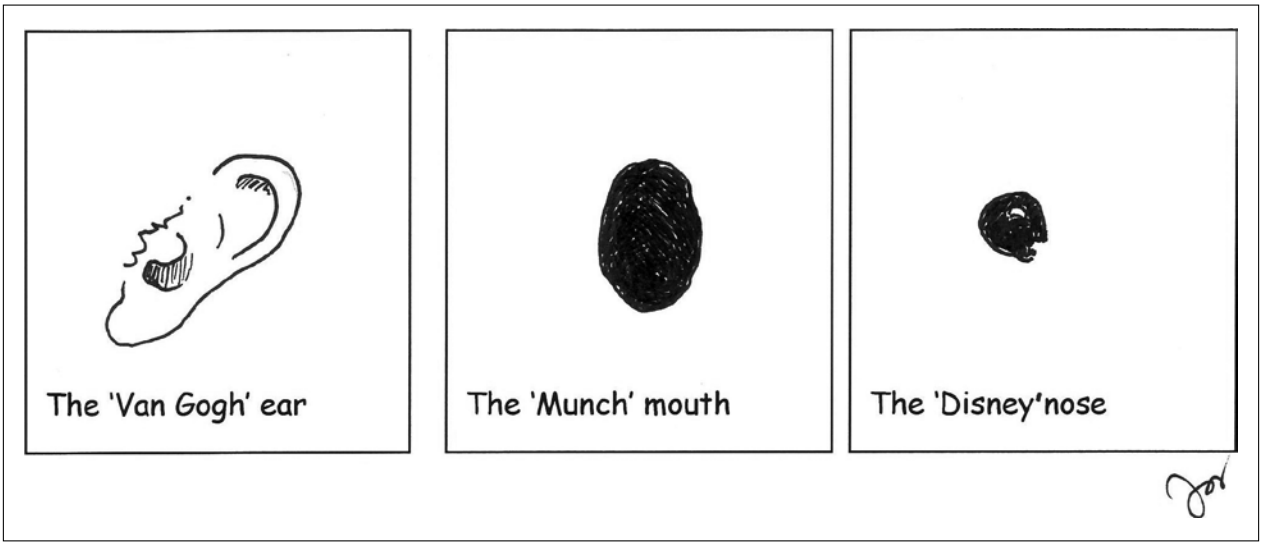

Schönhuth, et al. The ultimate test for pulmonary embolism.

Netherlands
The Journal of Medicine

CASE REPORT

The Modigliani head

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We report a case of a 55-year-old man with a diagnosis of tuberculous meningitis.

The magnetic resonance image of the brain strongly recalled to our memory a Modigliani's head sculpture. Although we promptly recognised that this was a purely incidental finding, not resulting from any pathological involvement of the brain, we were struck by the amazing coincidence; indeed, Modigliani was born in our city and died from the same disease.

A 55-year-old man with a history of alcohol abuse and pulmonary tuberculosis presented because of headache, vomiting and fever. On neurological examination, he was confused and had neck tenderness.

Contrast-enhanced T_1-weighted axial magnetic resonance imaging showed diffuse asymmetric meningeal enhancement extending deep into the base of the sulci with thick, and sometimes nodular enhancement, presumably due to inflammation.

These findings are highly suggestive of tuberculous meningitis but may also be observed in patients with sarcoidosis, Wegener's granulomatosis, or chronic meningitis.[1]

During apical scan (*figure 1*, left) a form of a mask strongly resembling the Modigliani stone sculpture heads (*figure 1*, right) appeared.

Cerebrospinal fluid analysis revealed pleocytosis (550 white cells x 10^9/l, 85% lymphocytes), elevated protein levels (2.8 g/l), and low glucose levels (1.38 g/l). The cerebrospinal fluid culture was positive for *Mycobacterium* tuberculosis after five weeks, confirming the diagnosis of tuberculous meningitis.

Amedeo Modigliani was born in Livorno (Tuscany, Italy) on 12 July 1884. He became the most famous Italian impressionist painter (impressionists were also known by the Tuscany term Macchiaioli). In 1910, he dedicated himself solely to stone sculpture, influenced by the archaic forms of idols and primitive masks.

On 22 January 1920, an unconscious Amedeo was brought to the 'Ospedale della Charité' in Paris where he died of tuberculous meningitis without having regained consciousness.[2-5]

On the contrary, our patient began treatment with a standard antituberculosis regimen, and his condition slowly improved.

Figure 1. *A magnetic resonance imaging of the brain (left) showed an image mimicking the Modigliani stone sculpture head (right)*

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PHOTO QUIZ

Posterior mediastinal masses in a patient with beta-thalassaemia intermedia

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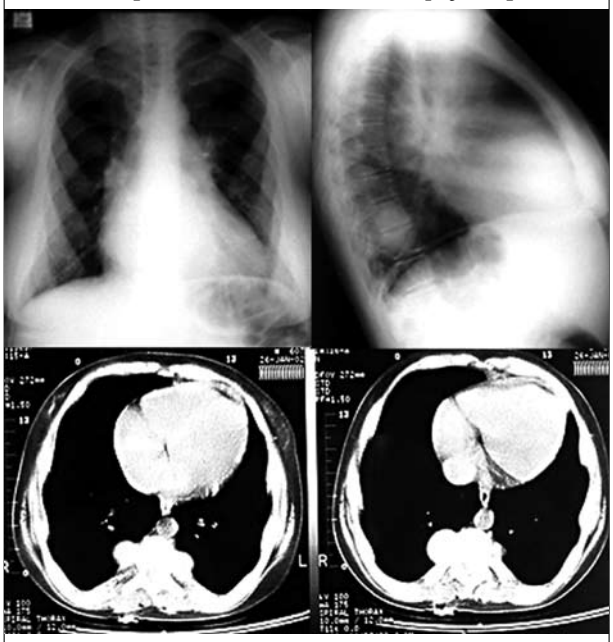
KEYWORDS

Beta-thalassaemia intermedia, extramedullary haematopoiesis, posterior mediastinal mass

CASE REPORT

A 23-year-old female patient was admitted for evaluation of a posterior mediastinal mass on her chest X-ray. She had been suffering from dyspnoea on effort and chest pain for three months. Beta-thalassaemia intermedia was diagnosed when she was four years old. Later a splenectomy was performed. She had been on treatment with folic acid and iron supplements for 13 years. Because of transfusion reactions she had only had four units of blood transfusion in her lifetime. On examination, the patient had a small stature, a typical thalassaemic face and spoon nails. Haemoglobin electrophoresis revealed levels of HbF of 99.7% and HbA_2 of 0.3%. The chest X-ray demonstrated a mass silhouette behind the cardiac shadow and widened ribs. The mass could be seen posteriorly on the lateral chest X-ray. The chest computed tomography disclosed bilateral paravertebral masses and expansion of especially the posterior ribs (*figure 1*).

Figure 1. *A mass shadow and widening of the ribs were seen in the posterior-anterior chest X-ray of our patient*



The lateral chest X-ray revealed the posterior location of the mass. Computed tomography of the chest revealed bilateral paravertebral masses.

WHAT IS YOUR DIAGNOSIS?

See page 273 for the answer to this photo quiz.

SPECIAL REPORT

2007 Award for the best article published in the Netherlands Journal of Medicine in 2006

Editors, the Netherlands Journal of Medicine

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In 2004, the Editors of the Netherlands Journal of Medicine decided to award prizes for the best articles in the Journal.[1-3] It is always extremely difficult to select recipients for awards because invariably there are more worthy candidates than there are awards. It is fortunate that the burden of deciding between the many excellent nominees for the Netherlands Journal of Medicine Best Paper Award is borne collectively by the members of the jury. This year, the jury consisted of Peter W. de Leeuw, Marc J. Bonten, and Aart Mudde. In contrast to previous years when we had three prizes to be awarded, one for each type of article, this time there was only one single award. The best paper had to be creative and clinically relevant, and had to meet rigorous scientific standards. In view of these selection criteria, the jury awarded the Netherlands Journal of Medicine Best Paper Award to Nancy Keeken from Bernhoven Hospital in Oss. As a medical student she worked under the tutelage of Dr W.A. de Boer and performed a highly relevant study on a novel, cheap and accessible test for the diagnosis of *Helicobacter pylori*.[4] She established that the accuracy of the dry rapid urease test (GUT test) appeared to be good, and that it is a reliable alternative for the widely used Clo test in diagnosing *H. pylori* infection. In the accompanying editorial, Dr Laheij commended her and concluded that the new more rapid urease test seems a promising new diagnostic test with equal diagnostic performance but considerably lower costs and a faster availability of the test results, in comparison with other biopsy-based *H. pylori* tests.[5]

The article by Dr Keeken has attracted widespread attention and was downloaded 434 times from the Journal web servers.[6] The prize was awarded at the 2007 Convention for Internal Medicine (Internistendagen) in Maastricht, the Netherlands. The prize consists of one of the works of art that have been published on the cover page of the Journal. We think that it is safe to say that we have established a fine tradition here at the Netherlands Journal of Medicine, and we really hope that the awards will contribute towards the quality of our Journal. We encourage prospective authors to submit their work to our Journal, not only because of the best paper award but also to benefit from the open access policy of the Journal.[7]

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ANSWER TO PHOTO QUIZ (ON PAGE 271)

POSTERIOR MEDIASTINAL MASSES IN A PATIENT WITH BETA-THALASSAEMIA INTERMEDIA

DIAGNOSIS

There were three paravertebral masses, the largest of which was 3 x 4 cm in diameter, located at T6 to T9. No distortion in the vertebral bodies and no extension to the spinal cord were seen on magnetic resonance imaging of the chest (*figure 2*). The nature of the masses was established by the bone marrow scan with ^{99m}Tc sulphur colloid, which revealed intense uptake of the radioisotope by the paravertebral masses, which was identical to that of bone marrow. Posterior mediastinal mass lesions of the patient were considered to be caused by extramedullary haematopoiesis (EMH).

EMH, a rare cause of posterior mediastinal mass, may occur in response to insufficient erythrogenesis. Thalassaemia major or intermedia, spherocytosis, sickle cell anaemia and congenital haemolytic anaemia are responsible in most cases of EMH.[1-4] Commonly involved organs are the liver and spleen where red cells are produced in the foetus during gestation. Intrathoracic involvement is rarely seen and the most common localisation is the lower paravertebral region.[1,4,5] It is thought to arise from the remains of primitive blood-forming precursor cells present particularly in the posterior thoracic epidural space.[6] EMH is usually asymptomatic since the organ involvement is most often microscopic; however, it may progress to an organomegaly or sometimes a mass-like lesion. It may even cause spinal cord compression or haemothorax.[3,4,7]

The radiological diagnostic clues of thoracic EMH are widening of the ribs and lobulated paravertebral masses with no calcification. MRI may be useful in establishing a diagnosis especially by demonstrating the presence of adipose tissue within the mass and by confirming that the bony cortex is intact.[1] On MRI, EMH masses appear as an isointense mass with high signal intensity on T1-weighted images and a mass of high signal intensity on T2-weighted images.[6]

It is important to recognise the possibility of intrathoracic EMH in the differential diagnosis of posterior mediastinal masses, especially in patients with chronic haemolytic anaemia, before attempting to undergo invasive procedures for the evaluation of the nature of a posterior mediastinal mass; otherwise, it may cause life-threatening bleeding into the pleural space.

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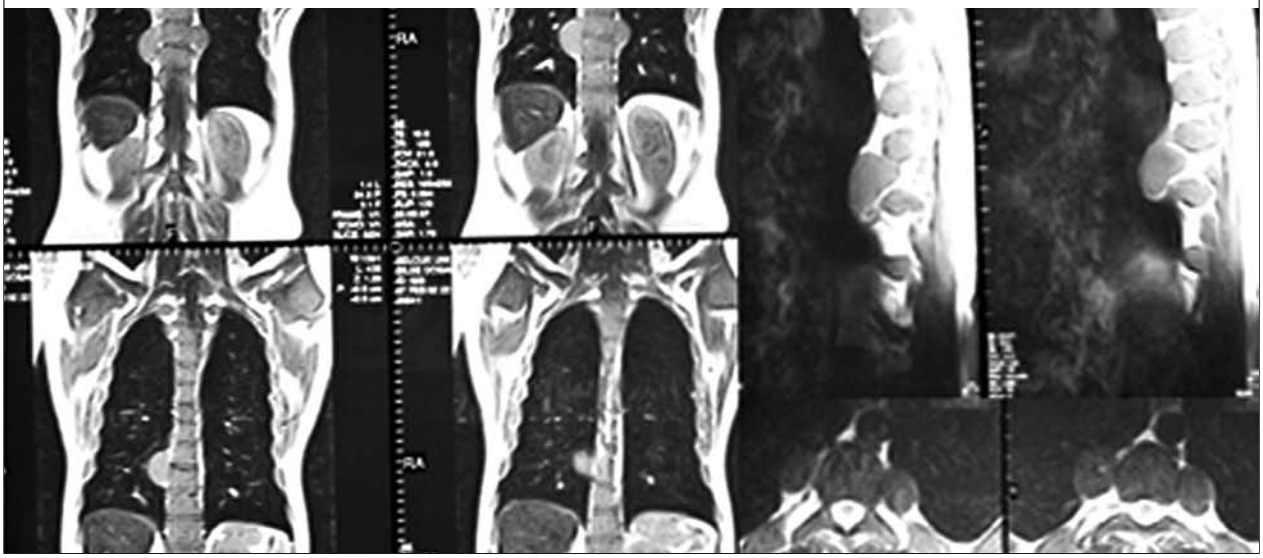
Figure 2. *MRI of the chest revealed bilateral lobular paraspinal masses from T6 to T9 that were isointense to bone marrow*



Netherlands
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Seizures and loss of vision in a patient with systemic lupus erythematosus

ABSTRACT

Posterior reversible encephalopathy syndrome (PRES) is a rare neurological condition identifiable by clinical presentation and MRI appearance.[1] Patients present with headache, seizures, loss of vision and altered mental function.[1] The pathogenesis of the syndrome is poorly understood. One hypothesis is that cerebral vasospasm results in cerebral ischaemia and subsequent development of T2 hyperintensity, and the other is a temporary failure of the autoregulatory capabilities of the cerebral vessels, leading to hyperperfusion, breakdown of the blood-brain barrier, and consequent vasogenic oedema. It is believed that a rapid rise in blood pressure overcomes cerebral autoregulatory mechanisms with abrupt dilatation of cerebral arterioles.[2,3] We report a patient with systemic lupus erythematosus and PRES after recurrent spontaneous abortion.

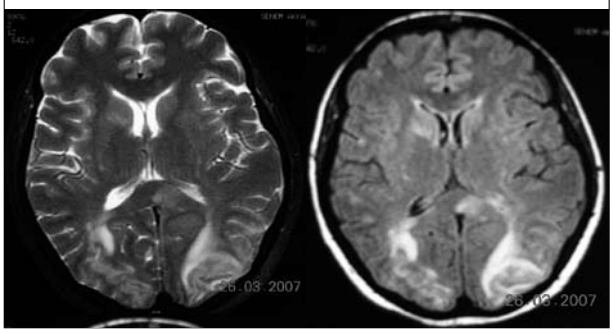
CASE REPORT

A 31-year-old woman who was 15 weeks' pregnant was admitted to our ICU with respiratory failure after her 14th recurrent abortion. Steroid therapy had been initiated previously for suspected immunological syndrome. Arterial pressure was 186/112 mmHg and esmolol infusion was initiated. She had oliguric renal failure. On the second day, the diagnosis of disseminated intravascular coagulation was made, and plasmapheresis was carried out every day for six days. On the third day, a haemodialysis programme was initiated. Based on the immunological laboratory tests, the diagnosis of systemic lupus erythematosus and antiphospholipid antibody syndrome was made. On the 11th day she began to complain of headache and loss of vision and she developed generalised tonic-clonic seizures. MRI revealed high-intensity signals in bilateral occipitoparietal regions (*figure 1*) compatible with PRES. Epanutin 100 mg three times a day orally was started to control the seizures. MRI, performed ten days later, showed a complete resolution of the lesions. She was discharged without any complications.

CONCLUSION

PRES has usually been described in association with hypertensive encephalopathy, eclampsia, renal failure, or following immunosuppressive or cytotoxic therapy; associations with connective tissue diseases, thrombotic thrombocytopenic purpura, porphyria, and organ transplantation are rarely seen.[4] Treatment consists of lowering the mean arterial blood pressure to <125 mmHg and anticonvulsant therapy in addition to other supportive measures. Early diagnosis and treatment is essential because irreversible neurological deficits or death may occur. Efforts need to be aimed at educating patients and health care workers to report symptoms early since a prompt diagnosis and treatment may result in complete resolution.

Figure 1. *High-intensity signals in bilateral occipitofrontoparietal regions, predominantly involving the white matter*



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Total	1459

ERRATUM

Vervoort G, Tack CJ. Do we need new drugs for the treatment of type 2 diabetes mellitus? Neth J Med 2007;65(5):157-9.

On page 159 the following sentence, on the second to fourth line; 'Distinct from GLP-1 analogues, DPP-IV inhibitors do not appear weight neutral and to lack gastrointestinal side effects.' is incorrect, it should read 'Distinct from GLP-1 analogues, DPP-IV inhibitors do not cause weight loss, but appear weight neutral. DPP-IV inhibitors also seem to lack the gastrointestinal side effects observed with GLP-1 analogues.'

INFORMATION FOR AUTHORS

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

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Language
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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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