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

April 2006, Vol. 64, No. 4, ISSN 0300-2977

VAN ZUIDEN COMMUNICATIONS

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

MISSION STATEMENT

The mission of the journal is to serve the need of the internist to practice up-to-date medicine and to keep track with important issues in health care. With this purpose we publish editorials, original articles, reviews, controversies, consensus reports, papers on speciality training and medical education, book reviews and correspondence.

EDITORIAL INFORMATION

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

CITED IN

Biosis database; embase/excerpta medica; index medicus (medline) science citation index, science citation index expanded, isi alerting services, medical documentation services, current contents/clinical medicine, PubMed.

VAN ZUIDEN communications

Contents

Cover
Print by Maja Ilic. For details about the artist, her work and how to order see elsewhere in this journal.

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

Subscription fee
The annual subscription fee within Europe is € 650, for the USA € 665 and for the rest of the world € 765. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Payment method
Please make your check payable to Van Zuiden Communications B.V., PO Box 2122, 2400 CC Alphen aan den Rijn, the Netherlands or you can transfer the fee to ING Bank, account number 67.89.10.872, Castellumstraat 1, Alphen aan den Rijn, the Netherlands, swift-code: ING BNL 2A. Do not forget to mention the complete delivery address of the Journal.

Claims
Claims for missing issues should be made within two months of the date of dispatch. Missing issues will be mailed without charge. Issues claimed beyond the two-month limit must be prepaid at back copy rates.

Orders, preprints, advertising, changes in address, author or general enquiries
Please contact the publisher.

Van Zuiden Communications B.V.
PO Box 2122, 2400 CC Alphen aan den Rijn
The Netherlands
Tel.: +31 (0)172-47 61 91, fax: +31 (0)172-47 18 82
E-mail: njm@zuidencomm.nl
Internet: www.njm-online.nl

Netherlands
The Journal of Medicine

EDITORIAL

Hepatitis C: changing genotype distribution with important implications for patient management

H. van Soest[1*], G.J. Boland[2], K.J. van Erpecum[1]

Departments of [1]Gastroenterology and Hepatology, and [2]Virology, University Hospital Utrecht, the Netherlands, [*]corresponding author: tel.: +31 (0)30-250 70 04, fax: +31 (0)30-250 55 34, e-mail: h.vansoest@umcutrecht.nl

ABSTRACT

In the Netherlands an estimated 0.1 to 0.4% of the population are chronic hepatitis C (HCV) carriers (15,000 to 60,000 persons). HCV is characterised by genetic heterogeneity and six different genotypes have been identified. The distribution of HCV genotypes is relevant for the clinician, since there are important genotype-specific differences in response to interferon-α based treatment regimens. Between 1993 and 2005 a shift was observed in the Netherlands from a dominant prevalence of genotype 1 to a situation in which genotype non-1 is becoming more important.

KEYWORDS

Genotype distribution, hepatitis C, treatment responses

Since its discovery in 1989, hepatitis C has been recognised as a major worldwide public health problem. It is a life-shortening disease associated with complex and expensive morbidity and decreased quality of life. Nearly 170 million persons are infected by hepatitis C virus (HCV) worldwide (in Western Europe about 5 million).[1] In 15 to 20% of acute HCV infections, the patient recovers spontaneously, but in the large majority of cases, the disease runs a chronic course.[2] In the Netherlands an estimated 0.1 to 0.4% of the population are chronic HCV carriers (15,000 to 60,000 persons).[3] Although reported risk of disease progression in chronic hepatitis C differs in various populations, progression to liver cirrhosis is thought to occur in 20% of cases after 20 years of infection, with significant risk of decompensation (ascites, hepatocellular carcinoma and variceal bleeding).[2] In industrialised countries, HCV is held responsible for 40% of cases of end-stage liver cirrhosis, 60% of cases of hepatocellular carcinoma and 30% of liver transplants.[4] Risk factors for progressive disease are male sex, alcohol abuse, age >40 years at infection, and presence of significant fibrosis (≥F2 fibrosis score) according to histological examination of liver biopsy.[2] Recently a promising new device based on liver stiffness measurement (by transient elastography) has been developed to assess liver damage noninvasively: Fibroscan. Liver stiffness measurement results correlate strongly with biopsy findings in hepatitis C patients.[5,6]

HCV is characterised by genetic heterogeneity. On comparing the nucleotide sequences of the HCV genome, six different genotypes can be identified.[7] These genotypes differ in 30 to 35% of the nucleotide sites over the complete genome. Within the genotypes a variable number of more closely related distinct subtypes can be found that differ 15 to 20% in their nucleotide sequence.[8] HCV genotyping plays a key role in viral transmission studies and HCV epidemiology. The article by de Vries *et al.* in the current issue of the Journal yields new insights into HCV genotype distribution in the Netherlands.[9] These data are relevant for the clinician, since there are important genotype-specific differences in response to interferon-α based treatment regimens. Nowadays the standard anti-HCV treatment consists of a combination of pegylated (PEG)-interferon and ribavirin. The success of this therapy depends on both virus-related and host-related factors, such as age, histology and biochemical parameters.[10,11] HCV genotype and pretreatment serum values of HCV RNA are the most important predictive factors. In the registration trials PEG-interferon combined with ribavirin resulted in a sustained virological response (SVR) rate in 55% of patients. However patients infected with genotypes 2 or 3 demonstrated

SVR rates of 80%, while genotype 1 patients have only 44% SVR rate.[10,11] The SVR rates in genotype 4 patients vary between 55 and 69%.[12,13] All these trials evaluated a treatment period of 48 weeks that had been proven safe and effective in the PEG-interferon monotherapy trials.[14] At an earlier stage, Poynard and McHutchison had already proposed a stopping rule at 24 weeks for standard interferon treatment. The rule implicates that in patients who still exhibited detectable HCV RNA after 24 weeks of treatment, SVR would not be achieved even if antiviral treatment was continued for another 24 weeks, with the consequence that treatment should be discontinued.[15] Subsequently, viral kinetics during PEG-interferon therapy were studied. It turned out that the drop in viral load at 12 weeks has a high negative predictive value: if the patient does not reach what is known as the early virological response (EVR), defined as a drop in HCV RNA of at least 2 log after 12 weeks of treatment compared with baseline (i.e. at least 100 times decreased viral load), he will in all probability be a non-responder and the therapy can be discontinued. By this stopping rule the inconvenience and expense of unnecessary continuation of treatment can be avoided.[16,17] The assessment of a 12-week early viral response reduces antiviral treatment duration by 40 to 44% and antiviral costs by 44 to 45% compared with a full 48-week dosing.[18]

A next step in tailoring the dose and duration of PEG-interferon-ribavirin based treatment was taken by Hadziyannis. He showed that treatment should be individualised by genotype: genotype 1 infected patients generally require a treatment period of 48 weeks with a standard dose of ribavirin (SVR 52%) while genotypes 2 and 3 infected patients appear to be adequately treated with a low dose of ribavirin for only 24 weeks (SVR 84%), at least with PEG-interferon-α2a.[19] In order to further individualise the antiviral treatment, HCV RNA decline during the earliest stages of PEG-interferon-ribavirin therapy was studied. Zeuzem showed that an undetectable serum HCV RNA after four weeks of combination therapy resulted in a sustained response of 94 and 85% in genotype 2 and 3 patients, respectively.[20]

Others studied whether even shorter treatment periods can be achieved in some cases without compromising overall efficacy. In genotype 2 and 3 infected patients with a rapid virological response (HCV RNA below 600 IU/ml after four weeks of treatment), a treatment period of 16 weeks proved to be sufficient (SVR 82%).[21] In this study the SVR in HCV genotype 2 infected patients was higher than in those infected with genotype 3. This difference was mainly due to the higher relapse rate in genotype 3 infected patients with a high pretreatment viral load (>800,000 IU/ml).[21] Therefore it was suggested to treat HCV genotype 3 infected patients with a pretreatment viral load >800,000 IU/ml for a period of 24 weeks.

Mangia showed that the treatment period in genotype 2 and 3 infected patients can be shortened to 12 weeks when serum HCV RNA is negative (<50 IU/ml) after four weeks of combination therapy.[22] Although the overall SVR was better in genotype 2 infected patients than in those infected with genotype 3, the SVR response rates were similar in patients with genotype 2 and 3 who had an early virological response and who were treated for 12 or 24 weeks.[22]

Also genotype 1 infected patients with a low baseline viral load who become HCV RNA negative at week 4 may be treated for 24 weeks without compromising sustained virological response rates.[23]

These studies all confirm differences in susceptibility for PEG-interferon-ribavirin treatment between as well as within various genotypes and that these differences become clear in the first weeks of treatment. Thus HCV genotype, viral load and decline of viral load during the earliest treatment period play key roles in tailoring and optimising antiviral therapy.[24] Recently, treatment recommendations have been given by de Knegt for genotype 2 and 3, as depicted in *table 1*.[25] Genotype distribution varies geographically with genotype 1, 2 and 3 being the most prevalent in Western Europe. Considering the different treatment schedules between genotypes 1 *vs* 2 and 3, genotype distribution in the Netherlands has a significant influence on the total costs and morbidity of anti-HCV treatment.

The CIRA study is a large, double-blind, randomised controlled multicentre trial in naive chronic hepatitis C patients comparing PEG-interferon-ribavirin treatment with a triple therapy consisting of PEG-interferon, ribavirin and amantadine/placebo. In this study, HCV genotypes were determined in 391 patients, by using amplification and hybridisation of the 5' noncoding region of the genome (INNOLiPA HCV; Innogenetics S.A., Ghent, Belgium: *table 2*). A total of 177 patients (45%) were infected with genotype 1 (more than 50% with subtype 1b), while 138

Table 1. *Treatment recommendations for HCV genotype 2 and 3 infected patients*[21,22,24]

HCV genotype	Pretreatment viral load	HCV-RNA at week 4*	Treatment§ period
2	Not important	Negative	16 weeks
2	Not important	Positive	24 weeks
3	<800,000 IU/ml	Negative	16 weeks
3	<800,000 IU/ml	Positive	24 weeks
3	>800,000 IU/ml	Not important	24 weeks

*Quantitative HCV RNA test (Amplicor Monitor HCV version 2.0, Roche Molecular Systems, Mannheim, Germany; lower limit of detection of 600 IU/ml). §Treatment consists of the combination of PEG-interferon with ribavirin in a weight-based dose (800, 1000 or 1200 mg).

Table 2. *HCV genotype distribution in the Netherlands*

Population	Year of data collection	N	HCV genotype (%)			
			1	2	3	4
HCV patients[27]	1993§	62	55	18	19	6
Dialysis patients[28]	1995/1996	71	70	17	7	4
Blood donors[26]	1994§	31	58	23	16	3
HCV patients[9]	2002-2005	351	49	10	29	11
Naive HCV patients CIRA study	2000-2004	391	45	10	35	8

§Year of publication. N = total number of persons included.

patients (35%) were infected with genotype 3, almost all with subtype a. Only 38 (10%) and 30 (8%) of the patients were infected with genotype 2 and 4, respectively. These data are largely comparable with the study by de Vries *et al.*, but differ from genotype distribution data in the blood donor population in which almost 60% of the donors were infected with genotype 1 and only 16% with genotype 3.[9,26] In the early 1990s genotype distribution in Dutch chronic hepatitis C patients was found to be as follows: 55% were infected with genotype 1, 18% were infected with genotype 2 and 19% were infected with genotype 3.[26] The data from de Vries *et al.* may be criticised for several reasons: the criteria to select physicians to be invited for data contribution are not clear: only 67% of invited physicians provided some data and there was no data verification performed.[9] Also, the authors state that: 'The percentage of patients being reported by physicians from each of the provinces was according to the percentage of inhabitants of the Netherlands living in these provinces (see *table 1* of their article)'.[9] One caveat is that hepatitis C patients are not distributed over the various provinces according to the percentage of inhabitants of the Netherlands living in these provinces, but are over-represented in certain regions such as Amsterdam and surrounding areas. Also, one might hypothesise that there could be a higher relative contribution of HCV genotype non-1 in the large cities as Amsterdam, considering the higher prevalence of these non-1 genotypes in (former) intravenous drug users. Unfortunately, the authors do not provide any additional clinical information such as sex and age distribution or stage of the liver disease. Although the data presented by the Vries *et al.* may not be a reliable reflection of the true HCV genotype distribution in the Netherlands, the observed shift from genotype 1 dominant prevalence to a situation in which genotype non-1 becomes more important (see *table 2* of de Vries *et al.*) may be real.[9] This shift may have a major and beneficial impact on treatment schedules, costs and benefits of chronic hepatitis C.

It will be interesting to follow this epidemiological spread of HCV in the future. On the one hand, the prevalence of HCV genotype 1 appears to be decreasing because it is generally acquired by transfusion of blood or blood products, a transmission route that is now effectively controlled. On the other hand it could persist in the future, since it is one of the difficult-to-treat genotypes.

REFERENCES

1. Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. N Engl J Med 1999;341(8):556-62.
2. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002;36(5 suppl 1):S35-46.
3. Health Council of the Netherlands: Committee on hepatitis C. Detection and treatment of people with Hepatitis C. 1997. Ref Type: Report.
4. EASL International Consensus Conference on hepatitis C. Paris, 26-27 February 1999. Consensus statement. J Hepatol 1999;31(suppl 1):3-8.
5. Castera L, Vergniol J, Foucher J, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128(2):343-50.
6. Ziol M, Handra-Luca A, Kettaneh A, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology 2005;41(1):48-54.
7. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol 1993;74(Pt 11):2391-9.
8. Simmonds P, Bukh J, Combet C, et al. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005;42(4):962-73.
9. De Vries MJ, te Rijdt B, van Nieuwkerk CMJ. Genotype distribution amongst hepatitis C patients in the Netherlands. Neth J Med 2006;64(4):109-13.
10. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347(13):975-82.
11. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358(9286):958-65.
12. El Zayadi AR, Attia M, Barakat EM, et al. Response of hepatitis C genotype-4 naive patients to 24 weeks of Peg-interferon-alpha2b/ribavirin or induction-dose interferon-alpha2b/ribavirin/amantadine: a non-randomized controlled study. Am J Gastroenterol 2005;100(11):2447-52.
13. Kamal SM, El Tawil AA, Nakano T, et al. Peginterferon {alpha}-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. Gut 2005;54(6):858-66.
14. Heathcote J, Zeuzem S. Pegylated interferon monotherapy for chronic hepatitis C. Semin Liver Dis 2004;24(suppl 2):39-45.
15. Poynard T, McHutchison J, Goodman Z, Ling MH, Albrecht J. Is an "a la carte" combination interferon alfa-2b plus ribavirin regimen possible for the first line treatment in patients with chronic hepatitis C? The ALGOVIRC Project Group. Hepatology 2000;31(1):211-8.
16. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38(3):645-52.
17. Ferenci P, Fried MW, Shiffman ML, et al. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J Hepatol 2005;43(3):425-33.
18. Wong JB, Davis GL, McHutchison JG, Manns MP, Albrecht JK. Economic and clinical effects of evaluating rapid viral response to peginterferon

alfa-2b plus ribavirin for the initial treatment of chronic hepatitis C. Am J Gastroenterol 2003;98(11):2354-62.

19. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140(5):346-55.

20. Zeuzem S, Hultcrantz R, Bourliere M, et al. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 2004;40(6):993-9.

21. Von Wagner M, Huber M, Berg T, et al. Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129(2):522-7.

22. Mangia A, Santoro R, Minerva N, et al. Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352(25):2609-17.

23. Zeuzem S, Buti M, Ferenci P, et al. Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006;44(1):97-103.

24. Vrolijk JM, de Knegt RJ, Veldt BJ, Orlent H, Schalm SW. The treatment of hepatitis C: history, presence and future. Neth J Med 2004;62(3):76-82.

25. De Knegt RJ. Behandeling van Hepatitis C: het kan korter! Lever, Nieuwsbulletin van de Nederlandse Vereniging voor Hepatologie 2005;29(4):11-2.

26. McOmish F, Yap PL, Dow BC, et al. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. J Clin Microbiol 1994;32(4):884-92.

27. Kleter GE, van Doorn LJ, Brouwer JT, Schalm SW, Heijtink RA, Quint WG. Sequence analysis of the 5' untranslated region in isolates of at least four genotypes of hepatitis C virus in The Netherlands. J Clin Microbiol 1994;32(2):306-10.

28. Schneeberger PM, Keur I, van der Vliet W, et al. Hepatitis C virus infections in dialysis centers in The Netherlands: a national survey by serological and molecular methods. J Clin Microbiol 1998;36(6):1711-5.

Netherlands
The Journal of Medicine

EDITORIAL

A major leap in the diagnosis of pulmonary embolism

M. Levi

Departments of Internal Medicine and Vascular Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, tel.: +31 (0)20-566 21 71, fax: +31 (0)20-691 96 58, e-mail: m.m.levi@amc.uva.nl

Souhami's Textbook of Medicine states that '*The characteristic symptom of pulmonary embolism is sudden breathlessness. Lateral, usually basal pleuritic chest pain and haemoptysis, consisting of frank red blood without sputum, develop some time after the onset of breathlessness. In addition to respiratory symptoms, there may be pain or swelling of the leg, suggesting deep vein thrombosis. (...) The respiratory rate is usually raised and if infarction has taken place, there may be a pleural rub and a small pleural effusion. The most important cardiovascular sign is tachycardia. Within a few hours of pulmonary infarction fever is the rule*'.[1] Harrison's Principles of Internal Medicine is somewhat more cautious than the above-presented straightforward clinical presentation of pulmonary embolism.[2] The chapter on pulmonary embolism stresses that all of the mentioned symptoms and signs may occur, but that quite often the presentation can be highly atypical, with only one of the mentioned symptoms present, or that history and physical examination may be '*deceptively normal*' in a patient with pulmonary embolism. In fact, the notion that the clinical diagnosis of pulmonary embolism may be very difficult, and the confusion that may occur with many other clinical entities, such as myocardial infarction, pneumonia, pleuritis, or pericarditis, was already expounded decades ago in the clinical handbooks 'Bedside Medicine' by the famous Dutch internist Snapper and the 'Netherlands Textbook of Internal Medicine' by Formijne *et al.*[3,4] Indeed, most clinicians will appreciate that the manifestation of pulmonary embolism may be difficult, in some cases highly atypical, at times misleading, and sometimes leading to considerable confusion and delay of the proper diagnosis.

However, even if the clinical suspicion of pulmonary embolism has been properly raised, confirmation of this diagnosis may also pose some problems. Confirmation of a clinical suspicion of pulmonary embolism has indeed been found indispensable, as more than 70% of the patients with clinically suspected pulmonary embolism do not have this diagnosis and would be unnecessarily exposed to anticoagulant treatment if no additional accurate tests were done.[5] Accurate diagnostic tests have long been hampered by disadvantages, such as the invasiveness of the 'gold standard' test, i.e. pulmonary angiography, or limited availability, difficulty in interpretation and serious important inter-observer variability of other tests, such as perfusion scintigraphy. Recently, new diagnostic modalities for the diagnosis of pulmonary embolism have been introduced. First, spiral computed tomography has emerged as a relatively simple, generally accessible, and accurate test for the diagnosis of pulmonary embolism.[6] Secondly, measurement of plasma D-dimer levels have been shown to have a very high sensitivity for venous thrombotic disease (including pulmonary embolism).[7,8] This means that the negative predictive value of low levels of D-dimer for the absence of pulmonary embolism is strong, particularly if the pre-test likelihood of the diagnosis is low. Lastly, clinical decision guidelines, based on an incisive analysis of databases of signs and symptoms of large numbers of patients with and without pulmonary embolism, have been evaluated and were found to be able to discriminate between patients with a high, intermediate, and low risk of pulmonary embolism. The clinical decision guideline generally used is the Wells score, which identifies groups with a low risk of pulmonary embolism (3.4%), a moderate risk of pulmonary embolism (27.8%), and a high-risk group (78.4% pulmonary embolism).[9] A subsequent analysis revealed that dichotomisation of the groups (i.e. high risk or low risk) further increased the clinical utility of this score.[10]

The Dutch multicentre Christopher study group has recently combined these three new diagnostic modalities and the resulting management strategy was evaluated in a large clinical trial in patients with clinically suspected pulmonary embolism.[11] First, patients were classified as having low or high risk of pulmonary embolism based on the Wells score. In patients with a low score, a D-dimer

test was carried out. If this test was normal, pulmonary embolism was considered to be excluded, no further diagnostic tests for pulmonary embolism were carried out, and the patients were not treated with anticoagulants. Of the total study population of 3306 patients, 2206 patients (67%) had a low Wells score and of these patients 1057 (48%) had a normal D-dimer. At three-month follow-up, there were no fatal events, four nonfatal pulmonary embolisms and one deep vein thrombosis. If we consider this as an acceptable outcome (which most clinicians probably will), it means that in 32% of patients with clinically suspected pulmonary embolism this diagnosis can be safely rejected on the basis of history, physical examination, and a simple laboratory test. In patients with a low Wells score but a positive D-dimer and in all patients with a high Wells score a spiral computed tomography scan was performed. In these 2199 patients (50 patients did not undergo a CT scan), pulmonary embolism was present in 674 patients (31%), absent in 1505 patients (68%), and inconclusive in 20 patients (1%). Patients with proven pulmonary embolism were treated with anticoagulants whereas patients in whom the diagnosis was rejected or those with an inconclusive scan did not receive antithrombotic treatment. At three-month follow-up there were 21 nonfatal events (7 (recurrent) pulmonary embolism, and 14 deep vein thrombosis) and 18 patients with fatal pulmonary embolism. Importantly, the incidence of fatal and nonfatal pulmonary embolism was 0.5 and 0.2%, respectively, in patients with a CT scan excluding pulmonary embolism. Taken together, the algorithm as studied by the Christopher group represents a serious simplification of the diagnostic management of pulmonary embolism with a clinical efficacy and safety that equals previously evaluated and more complex diagnostic strategies. The proposed management strategy is highly feasible in most clinical centres and will presumably quickly find its place in routine patient evaluation for suspected pulmonary embolism.

Which additional lessons can be learned from this landmark trial for the diagnosis of pulmonary embolism? First, even the most sophisticated diagnostic algorithm is not 100% perfect. The presence of pulmonary embolism, sometimes even fatal, in patients who were thought to be free of this disease based on the diagnostic steps in this strategy was rare but did occur, which is in line with previous studies.[12] In fact, one may view this as the premium that must be paid for the large number of patients who do not require extensive additional testing or who would have unjustly received anticoagulant treatment in the absence of pulmonary embolism. In the last group alone, the number of complications that might be expected from this inappropriate over-use of anticoagulation is likely to outweigh the burden of patients in this study that had an undetected pulmonary embolism.

A complicating factor may be that the interpretation of a spiral CT scan for pulmonary embolism, which is crucial for the diagnosis in more than two thirds of the patient population, may be difficult and can only be performed by experienced radiologists. In fact, previous studies have shown that proper radiological adjudication of especially subsegmental and distal emboli may be difficult, although the interobserver variability is still acceptable.[13] Also in the Christopher study a small number of CT scans were judged as inconclusive. On the other hand, the use of the CT scan in the diagnostic algorithm carries the advantage that if pulmonary embolism is not present, an alternative diagnosis can be made on the basis of the scan result, which was often not possible with previous imaging modalities for pulmonary embolism, such as angiography or scintigraphy.[14]

Another factor that needs to be stressed is that the diagnostic algorithm can only be applied as it was evaluated in the study. This means that D-dimer has only proven to be valuable in patients with a low probability of disease and only for the exclusion of thrombotic disease. D-dimer has no role whatsoever in patients with a high suspicion of pulmonary embolism or as a marker to select patients who might have pulmonary embolism based on a positive result. Unfortunately, the subtle intricacies of the operator characteristics of D-dimer are easily forgotten in clinical practice, and abuse and misinterpretation of this test is a real threat for the accurate diagnosis of pulmonary embolism in many clinical settings.

Lastly, and importantly, the Christopher trial is one of the largest, if not the largest, trial in more than 3500 patients with suspected pulmonary embolism. The study was a multicentre enterprise, undertaken in 12 academic and nonacademic centres in the Netherlands without any form of external financial support. The investigators need to be commended for this enormous effort and their determination to complete this very important study, which is indeed likely to change the diagnostic management of patients with a clinical suspicion of pulmonary embolism.

REFERENCES

1. Souhami RL, Moxham, J. Textbook of Medicine. Churchill Livingstone, Edinburgh, 1997.
2. Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS. Principles of Internal Medicine. Mc Graw Hill, New York, 1987.
3. Snapper I. Bedside Medicine. Grune Stratton, New York, 1960.
4. Formijne P. Nederlands Leerboek der Interne Geneeskunde. Scheltema Holkema, Amsterdam, 1960.
5. The PIOPED Investigators. Value of the ventilation/perfusion scan in acute pulmonary embolism. Results of the prospective investigation of pulmonary embolism diagnosis (PIOPED). The PIOPED Investigators. JAMA 1990;263:2753-9.

6. Perrier A, Roy PM, Aujesky D, et al. Diagnosing pulmonary embolism in outpatients with clinical assessment, D-dimer measurement, venous ultrasound, and helical computed tomography: a multicenter management study. Am J Med 2004;116:291-9.

7. Kraaijenhagen RA, Piovella F, Bernardi E, et al. Simplification of the diagnostic management of suspected deep vein thrombosis. Arch Intern Med 2002;162:907-11.

8. Kruip MJ, Leclercq MG, van der HC, Prins MH, Buller HR. Diagnostic strategies for excluding pulmonary embolism in clinical outcome studies. A systematic review. Ann Intern Med 2003;138:941-51.

9. Wells PS, Ginsberg JS, Anderson DR, et al. Use of a clinical model for safe management of patients with suspected pulmonary embolism. Ann Intern Med 1998;129:997-1005.

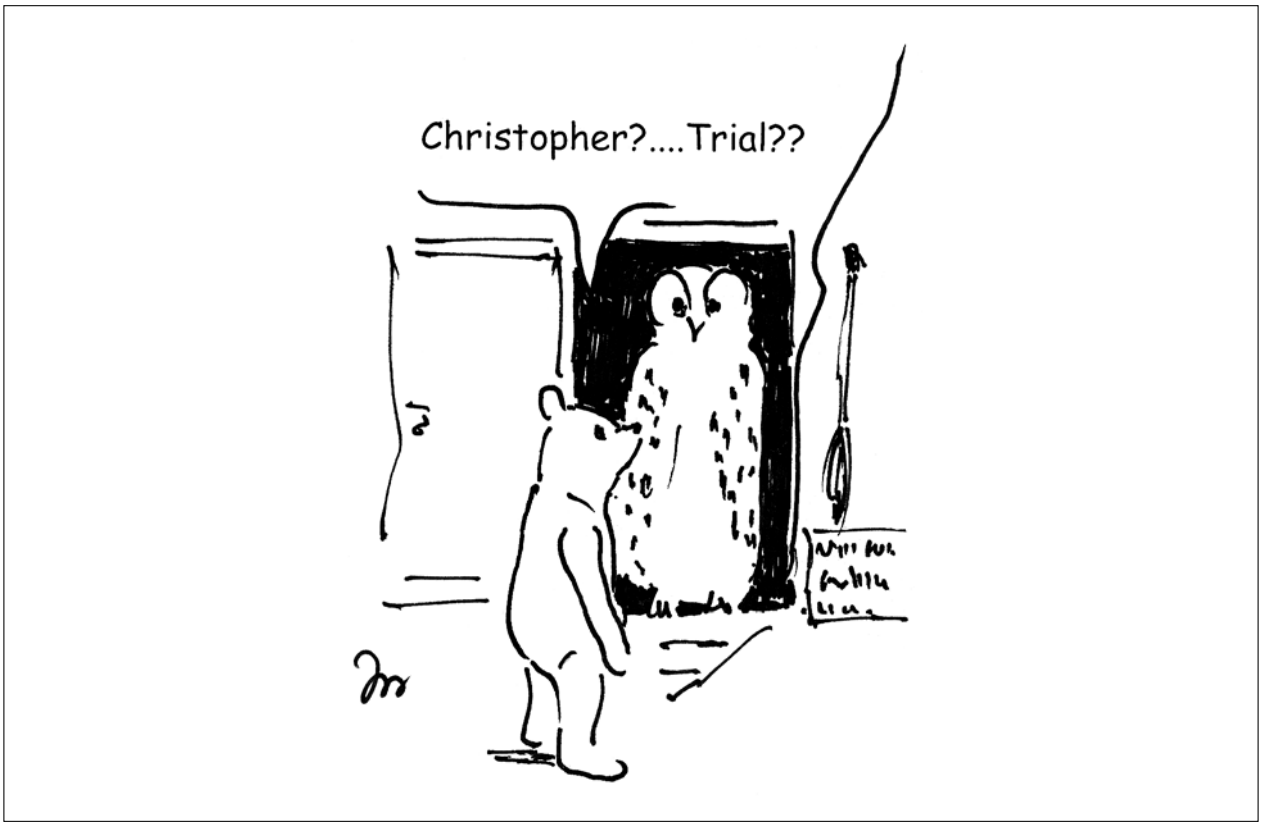
10. Wells PS, Anderson DR, Rodger M, et al. Derivation of a simple clinical model to categorize patients probability of pulmonary embolism: increasing the models utility with the SimpliRED D-dimer. Thromb Haemost 2000;83:416-20.

11. Van Belle A, Buller HR, Huisman MV, et al. Effectiveness of managing suspected pulmonary embolism using an algorithm combining clinical probability, D-dimer testing, and computed tomography. JAMA 2006;295:172-9.

12. Donato AA, Scheirer JJ, Atwell MS, Gramp J, Duszak R Jr. Clinical outcomes in patients with suspected acute pulmonary embolism and negative helical computed tomographic results in whom anticoagulation was withheld. Arch Intern Med 2003;163:2033-8.

13. Brunot S, Corneloup O, Latrabe V, Montaudon M, Laurent F. Reproducibility of multi-detector spiral computed tomography in detection of sub-segmental acute pulmonary embolism. Eur Radiol 2005;15:2057-63.

14. Musset D, Parent F, Meyer G, Maitre S, et al. Diagnostic strategy for patients with suspected pulmonary embolism: a prospective multicentre outcome study. Lancet 2002;360:1914-20.



Levi. Diagnosis of pulmonary embolism.

Netherlands
The Journal of Medicine

REVIEW

Fc gamma receptor mediated modulation of dendritic cells as a potential strategy in the battle against rheumatoid arthritis

M.H. Wenink, W.B. van den Berg, P.L.C.M. van Riel, T.R.D.J. Radstake*

Department of Rheumatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24-361 45 80, e-mail: t.radstake@reuma.umcn.nl

ABSTRACT

Autoimmune diseases such as rheumatoid arthritis (RA) result from a deregulation of immune responses culminating in immune-mediated tissue injury. In RA, this tissue injury is mainly reflected by synovitis and subsequent joint damage, although involvement of visceral organs (heart, lungs and kidneys) often leads to severe comorbidity. Accumulating evidence points towards dendritic cells (DC) as the principal regulators of the balance between immunity and tolerance. Recently, a large body of evidence has demonstrated that the balance between activating and inhibitory Fc gamma receptor (FcγR) subtypes is intricately involved in the regulation of DC behaviour. In this overview we summarise recent findings from our group and others that suggest an important role for FcγR in arthritis. Furthermore, we postulate novel mechanisms of how triggering of FcγR might be used to manipulate DC function and combat autoimmunity. When DC are envisaged as useful targets in the light of DC immunotherapy in RA, detailed knowledge on the regulatory pathways of FcγR in RA is of paramount importance.

KEYWORDS

Dendritic cells, Fc gamma receptors, rheumatoid arthritis

INTRODUCTION

Dendritic cells (DC) represent a unique set of antigen-presenting cells unrivalled in their capacity to attract and activate naive T cells and are therefore considered the most influential cells in the regulation of the immune response and the orchestration of tolerance.[1] How DC fulfil these opposing tasks is potentially hidden in the fact that many features of DC are divided in time and place and translated into two developmental stages, namely immature and mature DC.[2,3] Immature DC (iDC) are present in virtually all tissues where they encounter both self and non-self antigens. Triggered by a multitude of signals including selected pathogens and proinflammatory mediators, iDC mature after which they display an extraordinary immune-stimulatory capacity. Compared with iDC, mDC express high levels of co-stimulatory and MHC molecules, *de novo* expression of maturation markers such as DC-LAMP and CD83 and low levels of receptors involved in antigen capture. This phenotype makes mDC perfectly adept for the presentation of antigens to neighbouring T cells that were captured beforehand.[2,4,5]

Autoimmune diseases, such as RA, develop as a result of a loss of immune tolerance culminating in immune-mediated tissue injury. Since DC are believed to be key regulators in directing the fine balance between tolerance and immunity, a major goal in the treatment of autoimmune diseases could be to deflect the presentation of self-antigens by DC to T cells so that anergic T cells or regulatory T cells are induced, thereby inducing torlerance.[1,6,8] Nowadays, an important role has been suggested for DC in synovial inflammation, supporting the theory that targeting DC is likely to be beneficial in RA.[9-13] However, tuning DC function as a therapeutic target in RA has not been tested thus far. In contrast, alteration of DC function has been exploited in other pathological conditions including cancer and transplantation medicine and holds great promise as a future therapy.[14] In the battle against cancer, DC cultured with a proinflammatory phenotype and loaded with antigenic cargo originating from the tumour are generated and administered to patients eliciting antitumour responses. It is reasonable to

postulate that the potency of these 'DC vaccine therapies' might be extrapolated to create tolerogenic DC in case of autoimmunity and transplantation medicine, providing an attractive strategy to break the vicious circle of chronic inflammatory responses in autoimmune diseases such as RA.

For the recognition and processing of antigens and subsequent activation, iDC are equipped with several mechanisms of which receptor-mediated endocytosis is considered the most important. Various receptors mediate antigen uptake; the Fc gamma receptors (FcγR) form the basis of this review. FcγR constitute a group of receptors designed to recognise IgG immunoglobulins or IgG-containing immune complexes (IC) and are abundantly present in serum and synovial fluid of patients with rheumatoid arthritis (RA). In humans three classes can be distinguished, FcγRI, FcγRII and FcγRIII.[15,16] FcγRII is further divided into two subtypes: FcγRIIa and FcγRIIb. FcγRIIa, together with FcγRI and III, activate cellular responses upon triggering, whereas FcγRIIb is a unique inhibitory FcγR.[17,18] Since both activating and inhibitory FcγR are expressed on various immune cells, the concerted action of these opposing signalling systems unequivocally determines the cellular response.[19] In accordance with this, it has been clearly shown that the determination of DC phenotype and behaviour is critically determined by FcγR triggering through IC, stressing the involvement of FcγR in both autoimmunity as well as tumour immunity.[19-21] This review will mainly focus on the involvement of monocyte-derived DC (moDC), and their surface FcγR, in the pathogenesis of RA. In addition we will elaborate on the potential of moDC and FcγR as promising therapeutical targets in the treatment of RA.

ARE DC IMPLICATED IN SYNOVIAL INFLAMMATION?

Many reports demonstrate the presence of DC in inflamed synovial tissue suggesting a critical role in RA. Recently, Weyand and colleagues categorised synovial inflammation into three main groups based on the appearance of DC and their location in secondary lymphoid organs.[22] Remarkably, also fully matured DC are present in perivascular regions and ectopic lymphoid organs in synovial tissue of RA patients, which is highly suggestive of an altered maturation process during synovitis.[11,22-24] Highly interesting was the finding that synovial DC in RA reflect characteristics of cell activation such as increased expression of inducible heat-shock protein 70,[25] presentation of specific immune-epitopes,[23] RANK-RANKL interactions which mirror DC-T cell interaction in the target organ and the expression of transcription factors such as Jak3, STAT1, STAT4 and STAT6.[11,26] Interestingly, alterations in DC phenotype and behaviour are not confined to the synovium in RA. In fact, our group has demonstrated that moDC obtained from circulating peripheral blood mononuclear cells displayed an increased expression of FcγRII.[12] Further research has led to the observation that this increased expression of FcγRII resulted in an altered production of pro- and anti-inflammatory mediators (TNF-α, IL-6 and chemokines) upon triggering by immune complexes (IC).[27,28] In addition, moDC from RA patients were found to express high levels of Toll-like receptors (TLR) and were found to react more strongly upon TLR ligand stimulation than those from healthy controls. This suggests active involvement of moDC in the inflammatory process.[29] TLR are pattern recognition receptors that bind bacterial fragments and viral RNA (exogenous proteins) on the one hand, but on the other hand also 'endogenous ligands' including heat-shock proteins and cartilage degradation products.[30] Since TLR triggering provides a very potent stimulus for immune activation of DC, the finding that TLR signalling is likely to occur in RA suggests a critical role for moDC during this condition.

More evidence for the involvement of DC in synovitis originates from an elegant study from Leung *et al*. which demonstrates that DC primed with collagen are able to induce inflammatory arthritis after transfer.[9] In line with this finding, DC that were genetically modified to express the anti-inflammatory cytokine IL-4 were capable of fully inhibiting collagen-induced arthritis.[31,32] Since IL-4 is known to be a modulator of FcγR balance, this latter effect might be partially explained by the alteration of FcγR expression locally due to the presence of IL-4 producing cells. Likewise, IL-10-treated DC and exosomes derived from these DC were able to completely block the onset of a collagen-induced arthritis and even reduce the severity of an established arthritis.[33] Altogether, these data clearly indicate that DC are involved in the onset and perpetuation of the inflammatory circle of synovitis and suggest that DC targeting is a challenging approach to treat rheumatoid arthritis.

THE ROLE OF FcγR IN ARTHRITIS

The use of experimental arthritis models combined with the availability of FcγR knockout mice has lead to the appreciation of the role of FcγR in arthritis. For example, during antigen-induced arthritis and immune complex mediated arthritis the presence of activating FcγR (FcγRI and FcγRIII, mice lack FcγRIIa) was associated with chondrocyte death and cartilage erosion.[34-37] Further investigation of the FcγR subtypes revealed that the expression of FcγRIII was required for the development of collagen-induced arthritis since FcγRIII -/- mice developed virtually no inflammatory signs and subsequent cartilage

damage.[38] The absence of the inhibitory FcγR subtype IIb, on the other hand, renders mice susceptible to collagen-induced arthritis.[39] In addition, it was recently hypothesised that FcγRIIb reduces both joint inflammation and destruction by inhibition of the activating FcγR and by the efficient clearance of immune complexes.[40] An important role for the inhibitory FcγRIIb in arthritis was further substantiated by the finding that arthritis can be induced by a single injection of IgG anti-collagen type II antibody in mice lacking this receptor.[41] In addition, it was observed that IL-13 mediated upregulation of FcγRIIb prior to immune-complex arthritis inhibited chrondrocyte death and cartilage matrix degradation, two key features of joint damage.[42]

The role for FcγR in synovial inflammation in humans is less clear, but accumulating evidence suggests that these receptors are of considerable importance. Until recently a major problem in the study of FcγR on human myeloid cells was the inability to distinguish FcγRII subtypes (FcγRIIa and FcγRIIb) on the cellular surface. In the synovial tissue macrophages and DC are abundantly present and it was demonstrated that these cells express significantly higher levels of FcγRII and III when compared with those present in synovium from healthy controls.[12,43] Similarly, it was found that moDC obtained from patients with RA express FcγRII at significantly higher levels than those from their healthy counterparts.[12] Since these DC display an anti-inflammatory phenotype upon IC stimulation the increased expression of FcγRII was thought to reflect an increase in FcγRIIb. Intriguingly, this increased expression of FcγRII was unique for patients with active RA and still present after full maturation, suggesting that local factors responsible for this phenomenon are present during early moDC life as suggested previously.[44] With the recent development of a unique antibody directed specifically against the inhibitory FcγRIIb (gifted by MacroGenics. Inc), it could be demonstrated that the inhibitory FcγRIIb subtype was largely responsible for the increased expression of FcγRII as observed before (manuscript in preparation). This is conform the hypothesis that FcγRIIb is pivotal in the counter-regulatory response of proinflammatory responses in RA. It was highly interesting to see that the functional polymorphism of the FcγRIIb (I232T) was identified as the strongest prognostic factor for radiological joint damage in RA and was found to modulate moDC function, further substantiating the important role of FcγR in RA.[45]

ARE DC AND FcγR CONNECTED IN RA PATHOLOGY?

In order to prevent chronic inflammation and subsequent tissue damage, in time every established immune response has to be terminated. To this end, the immune system is provided with a multitude of inhibitory receptors that counteract the immune response.[46] The inhibitory FcγRIIb is a perfect example of such. As discussed before, the expression of FcγRIIb on moDC from RA patients is found to be increased compared with those from healthy controls. Intriguingly, this increased expression was exclusively found in patients with an active phase of disease. Therefore, we postulated that FcγRIIb might function as a counteractive mechanism to dampen proinflammatory responses. During inflammation both pro- and anti-inflammatory cytokines are generated that regulate the balance between activating and inhibitory receptors, including FcγR. TNF-α and IFN-γ are known to induce the upregulation of mainly activating FcγR whereas IL-4 and IL-13 were recently identified as factors that induce the opposite.[19,47-49] Bearing these data in mind a conceptual framework can be envisaged in which proinflammatory cytokines will upregulate activating FcγR during the early stages of inflammation. Circulating immune complexes CIC, which are omnipresent in RA, will potentate the proinflammatory reaction by inducing cytokine production and moDC maturation. To counteract this initial proinflammatory response, anti-inflammatory cytokines will be produced through which inhibitory FcγR gain the upper hand, the IC will then provide a negative feedback loop silencing the inflammatory response. In line with this conceptual framework, IL-13 was found to be increased in both serum and synovial fluid from RA patients further supporting this hypothesis.

Why then do RA patients develop chronic disease? Our previous findings along with the observations that intravenously administered immune globulins (IVIG) lack clinical efficacy in RA patients despite their success in many other inflammatory conditions including inflammatory myositis, Guillain Barré syndrome and multiple sclerosis, prompted us to hypothesise that the FcγRIIb pathway is defective in RA.[50-54] The finding that IL-13-mediated upregulation of FcγRIIb is absent in RA strongly supports this hypothesis and warrants further research.

CAN FcγRS BE USED AS THERAPEUTIC TARGETS IN RA?

Triggering of Toll-like receptors (TLR) provides a strong stimulus for DC activation. The notion that TLR are involved in the regulation of both innate and adaptive immune responses sparked a revolution of research in the potential role of these receptors in a plethora of inflammatory conditions. In RA, the role of TLR in the disease pathogenesis has only recently become a subject of intense investigation. Recent research has

demonstrated that TLR are expressed at increased levels in RA synovium,[29,55] moDC from RA patients reacted more potently with TLR agonists compared with DC from healthy controls, producing vast amounts of proinflammatory cytokines,[29] and RA synovial fibroblasts are activated by RNA released from necrotic cells.[56] All these data suggest that a role for TLR in RA is likely. Recent observations suggesting an interaction between the inhibitory FcγRIIb and TLR4 are therefore intriguing since this might provide new insights in how to modulate TLR responses *in vivo*.[49] It is tempting to speculate that local moDC might act as a counter-regulatory mechanism with the purpose to silence the inflammatory response and restore tolerance after eradication of the provocative element. DC fulfil a central role in the organisation of immune responses against pathogenic invaders and in preventing autoimmune responses harmful to the host. Their directive task in shaping the immune response makes DC excellent targets in the battle against rheumatoid arthritis and other autoimmune diseases. To suppress (auto)immune responses several potential strategies can be envisaged in which active modulation of DC function plays a prominent part. One approach would be aimed at stimulating the tolerogenic capacities of DC *in vivo*, preferably at the site of inflammation. Current research is now focussing on whether the inhibitory FcγRIIb could be targeted for this goal.

Although still little is known regarding the signals that can induce the tolerogenic pathway in DC, accumulating evidence indicates that various immunosuppressive pharmacological substances are able to modulate DC phenotype and function and it is conceivable that some might shift the balance between immunity and tolerance by interfering in DC pathways.[57] Another strategy for the use of DC to combat autoimmune diseases might be to manipulate DC *ex vivo*, delivering 'programmed DC'. By means of *in-vitro* culture strategies (Vit-D3 or dexamethasone or IL-10) or genetic modification the DC's tolerogenic immune suppressive programme can be instigated. The potential effectiveness of such strategies has already been demonstrated in transplantation models in which moDC differentiated in the presence of vasoactive intestinal peptide, a known immunosuppressive neuropeptide, expressed an immune regulatory phenotype able to prevent acute graft-versus-host disease *in vivo*.[58] In addition, DC genetically modified to express IL-4 were able to abrogate synovial inflammation and abolish subsequent cartilage damage in experimental arthritis models.[32] Another possible way by which DC characteristics can be manipulated lies with the Toll-like receptors. Repetitive triggering of DC via TLR was shown to abrogate their proinflammatory capacity whereas combinations of TLR were found to unleash a synergistic effect on DC with respect to DC activation.[29,59,60] This suggests that stimulation of TLR, the selective blocking of TLR or the modulation of TLR responses might be used to modulate DC behaviour in such a manner that is favourable for the host. The use of such TLR-activated DC has shown promising results in the battle against melanoma.[61,62] In the case of autoimmune diseases the inhibitory FcγRIIb might prove to be a valid target since this inhibitory receptor seems interconnected with TLR signalling and is known to be important in the delicate balance between tolerance and autoimmunity.[49,63]

FUTURE PROSPECTIVE

Both DC and FcγR are implicated in the inflammatory pathways during synovitis in RA. The modulation of the balance between activating and inhibiting FcγR might provide a means to modulate the behaviour of DC. However, the upregulation and/or function of FcγRIIb seems to be defective in RA; further studies resolving the cause of this potentially altered FcγRII regulation pathway are currently being conducted. For a better understanding of DC biology in autoimmune diseases, more knowledge on this inhibitory pathway is warranted and might provide new clues regarding potential new treatment strategies to battle autoimmune diseases including RA.

REFERENCES

1. Steinman RM, Nussenzweig MC. Inaugural Article: Avoiding horror autotoxicus: The importance of dendritic cells in peripheral T cell tolerance. Proc Natl Acad Sci USA 2002;99(1):351-8.
2. Bell D, Young JW, Banchereau J. Dendritic cells. Adv Immunol 1999;72:255-324.
3. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell 2001;106(3):255-8.
4. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392(6673):245-52.
5. Fanger NA, Wardwell K, Shen L, Tedder TF, Guyre PM. Type I (CD64) and type II (CD32) Fc gamma receptor-mediated phagocytosis by human blood dendritic cells. J Immunol 1996;157(2):541-8.
6. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767-811.
7. Liu YJ. Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. Cell 2001;106(3):259-62.
8. Turley SJ. Dendritic cells: inciting and inhibiting autoimmunity. Curr Opin Immunol 2002;14(6):765-70.
9. Leung BP, Conacher M, Hunter D, McInnes IB, Liew FY, Brewer JM. A novel dendritic cell-induced model of erosive inflammatory arthritis: distinct roles for dendritic cells in T cell activation and induction of local inflammation. J Immunol 2002;169(12):7071-7.
10. Page G, Lebecque S, Miossec P. Anatomic localization of immature and mature dendritic cells in an ectopic lymphoid organ: correlation with selective chemokine expression in rheumatoid synovium. J Immunol 2002;168(10):5333-41.
11. Page G, Miossec P. RANK and RANKL expression as markers of dendritic cell-T cell interactions in paired samples of rheumatoid synovium and lymph nodes. Arthritis Rheum 2005;52(8):2307-12.

12. Radstake TR, Blom AB, Sloetjes AW, et al. Increased FcgammaRII expression and aberrant tumour necrosis factor alpha production by mature dendritic cells from patients with active rheumatoid arthritis. Ann Rheum Dis 2004;63(12):1556-63.

13. Thomas R, Davis LS, Lipsky PE. Rheumatoid synovium is enriched in mature antigen-presenting dendritic cells. J Immunol 1994;152(5):2613-23.

14. Figdor CG, de Vries IJ, Lesterhuis WJ, Melief CJ. Dendritic cell immunotherapy: mapping the way. Nat Med 2004;10(5):475-80.

15. Salmon JE, Pricop L. Human receptors for immunoglobulin G: key elements in the pathogenesis of rheumatic disease. Arthritis Rheum 2001;44(4):739-50.

16. Dijstelbloem HM, van de Winkel JG, Kallenberg CG. Inflammation in autoimmunity: receptors for IgG revisited. Trends Immunol 2001;22(9):510-6.

17. Malbec O, Fridman WH, Daeron M. Negative regulation of hematopoietic cell activation and proliferation by Fc gamma RIIB. Curr Top Microbiol Immunol 1999;244:13-27.

18. Qin D, Wu J, Vora KA, et al. Fc gamma receptor IIB on follicular dendritic cells regulates the B cell recall response. J Immunol 2000;164(12):6268-75.

19. Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, Young JW. Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. J Clin Invest 2005;115(10):2914-23.

20. Amigorena S. Fc gamma receptors and cross-presentation in dendritic cells. J Exp Med 2002;195(1):F1-3.

21. Schuurhuis DH, Ioan-Facsinay A, Nagelkerken B, et al. Antigen-antibody immune complexes empower dendritic cells to efficiently prime specific CD8+ CTL responses in vivo. J Immunol 2002;168(5):2240-6.

22. Weyand CM, Goronzy JJ. Ectopic germinal center formation in rheumatoid synovitis. Ann N Y Acad Sci 2003;987:140-9.

23. Baeten D, Steenbakkers PG, Rijnders AM, Boots AM, Veys EM, de Keyser F. Detection of major histocompatibility complex/human cartilage gp-39 complexes in rheumatoid arthritis synovitis as a specific and independent histologic marker. Arthritis Rheum 2004;50(2):444-51.

24. Pettit AR, MacDonald KP, O'Sullivan B, Thomas R. Differentiated dendritic cells expressing nuclear RelB are predominantly located in rheumatoid synovial tissue perivascular mononuclear cell aggregates. Arthritis Rheum 2000;43(4):791-800.

25. Martin CA, Carsons SE, Kowalewski R, Bernstein D, Valentino M, Santiago-Schwarz F. Aberrant Extracellular and Dendritic Cell (DC) Surface Expression of Heat Shock Protein (hsp)70 in the Rheumatoid Joint: Possible Mechanisms of hsp/DC-Mediated Cross-Priming. J Immunol 2003;171(11):5736-42.

26. Walker JG, Ahern MJ, Coleman M, et al. Expression of Jak3, STAT1, STAT4 and STAT6 in inflammatory arthritis: Unique Jak3 and STAT4 expression in dendritic cells in seropositive rheumatoid arthritis. Ann Rheum Dis 2006;65(2):149-56.

27. Radstake TR, van Lent PL, Pesman GJ, et al. High production of proinflammatory and Th1 cytokines by dendritic cells from patients with rheumatoid arthritis, and down regulation upon Fc{gamma}R triggering. Ann Rheum Dis 2004;63(6):696-702.

28. Radstake TR, Van der Voort, ten Brummelhuis M, et al. Increased expression of CCL18, CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis and regulation by Fc gamma receptors. Ann Rheum Dis 2005;64(3):359-67.

29. Roelofs M, Joosten L, Abdollahi-Roodsaz S, et al. The expression of Toll-like receptor 3 and 7 in RA synovium is increased and co-stimulation of TLR3, TLR4 and TLR7 results in synergistic cytokine production by dendritic cells. Arthritis Rheum 2005;52(8):2313-22.

30. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4(7):499-511.

31. Kim SH, Kim S, Evans CH, Ghivizzani SC, Oligino T, Robbins PD. Effective treatment of established murine collagen-induced arthritis by systemic administration of dendritic cells genetically modified to express IL-4. J Immunol 2001;166(5):3499-505.

32. Morita Y, Yang J, Gupta R, et al. Dendritic cells genetically engineered to express IL-4 inhibit murine collagen-induced arthritis. J Clin Invest 2001;107(10):1275-84.

33. Kim SH, Lechman ER, Bianco N, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol 2005;174(10):6440-8.

34. Blom AB, van Lent PL, van Vuuren H, et al. FcgammaR expression on macrophages is related to severity and chronicity of synovial inflammation and cartilage destruction during experimental immune-complex-mediated arthritis (ICA). Arthritis Res 2000;2(6):489-503.

35. Van Lent PL, van Vuuren AJ, Blom AB, et al. Role of Fc receptor gamma chain in inflammation and cartilage damage during experimental antigen-induced arthritis. Arthritis Rheum 2000;43(4):740-52.

36. Van Lent PL, Nabbe K, Blom AB, et al. Role of activatory Fc gamma RI and Fc gamma RIII and inhibitory Fc gamma RII in inflammation and cartilage destruction during experimental antigen-induced arthritis. Am J Pathol 2001;159(6):2309-20.

37. Nabbe KC, van Lent PL, Holthuysen AE, Kolls JK, Verbeek S, van den Berg WB. FcgammaRI up-regulation induced by local adenoviral-mediated interferon-gamma production aggravates chondrocyte death during immune complex-mediated arthritis. Am J Pathol 2003;163(2):743-52.

38. Diaz DS, Andren M, Martinsson P, Verbeek JS, Kleinau S. Expression of FcgammaRIII is required for development of collagen-induced arthritis. Eur J Immunol 2002;32(10):2915-22.

39. Yuasa T, Kubo S, Yoshino T, et al. Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. J Exp Med 1999;189(1):187-94.

40. Van Lent P, Nabbe KC, Boross P, et al. The inhibitory receptor FcgammaRII reduces joint inflammation and destruction in experimental immune complex-mediated arthritides not only by inhibition of FcgammaRI/III but also by efficient clearance and endocytosis of immune complexes. Am J Pathol 2003;163(5):1839-48.

41. Nandakumar KS, Andren M, Martinsson P, et al. Induction of arthritis by single monoclonal IgG anti-collagen type II antibodies and enhancement of arthritis in mice lacking inhibitory FcgammaRIIB. Eur J Immunol 2003;33(8):2269-77.

42. Nabbe KC, van Lent PL, Holthuysen AE, et al. Local IL-13 gene transfer prior to immune-complex arthritis inhibits chondrocyte death and matrix-metalloproteinase-mediated cartilage matrix degradation despite enhanced joint inflammation. Arthritis Res Ther 2005;7(2):R392-401.

43. Blom AB, Radstake TR, Holthuysen AE, et al. Increased expression of Fcgamma receptors II and III on macrophages of rheumatoid arthritis patients results in higher production of tumor necrosis factor alpha and matrix metalloproteinase. Arthritis Rheum 2003;48(4):1002-14.

44. Santiago-Schwarz F, Anand P, Liu S, Carsons SE. Dendritic cells (DCs) in rheumatoid arthritis (RA): progenitor cells and soluble factors contained in RA synovial fluid yield a subset of myeloid DCs that preferentially activate TH1 inflammatory-type responses. J Immunol 2001;167(3):1758-68.

45. Radstake TRDJ, Franke B, Wenink MH, et al. The inhibitory Fc gamma receptor IIb (CD32) modulates radiological joint damage and dendritic cell function in rheumatoid arthritis. Submitted.

46. Leibson PJ. The regulation of lymphocyte activation by inhibitory receptors. Curr Opin Immunol 2004;16(3):328-36.

47. Wijngaarden S, van de Winkel JG, Jacobs KM, Bijlsma JW, Lafeber FP, van Roon JA. A shift in the balance of inhibitory and activating Fcgamma receptors on monocytes toward the inhibitory Fcgamma receptor IIb is associated with prevention of monocyte activation in rheumatoid arthritis. Arthritis Rheum 2004;50(12):3878-87.

48. Pricop L, Redecha P, Teillaud JL, et al. Differential modulation of stimulatory and inhibitory Fc gamma receptors on human monocytes by Th1 and Th2 cytokines. J Immunol 2001;166(1):531-37.

49. Wenink MH, Ronnelid J, Bonvini E, Koenig S, van den Berg WB, Radstake TRDJ. Interleukin-13 skews the Fc gamma receptor (FcγR) balance towards the inhibitory FcγRIIb which tunes the Toll-like receptor mediated cytokine production by dendritic cells. Submitted.

50. Kanik KS, Yarboro CH, Naparstek Y, Plotz PH, Wilder RL. Failure of low-dose intravenous immunoglobulin therapy to suppress disease activity in patients with treatment-refractory rheumatoid arthritis. Arthritis Rheum 1996;39(6):1027-9.

51. Maksymowych WP, Avina-Zubieta A, Luong M, Russell AS. High dose intravenous immunoglobulin (IVIg) in severe refractory rheumatoid arthritis: no evidence for efficacy. Clin Exp Rheumatol 1996;14(6):657-60.

52. Cherin P, Pelletier S, Teixeira A, et al. Results and long-term followup of intravenous immunoglobulin infusions in chronic, refractory polymyositis: an open study with thirty-five adult patients. Arthritis Rheum 2002;46(2):467-74.

53. Van der Meche FG, Schmitz PI. A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain-Barre syndrome. Dutch Guillain-Barre Study Group. N Engl J Med 1992;326(17):1123-29.

54. Sorensen PS, Wanscher B, Jensen CV, et al. Intravenous immunoglobulin G reduces MRI activity in relapsing multiple sclerosis. Neurology 1998;50(5):1273-81.

55. Radstake TR, Roelofs MF, Jenniskens YM, et al. Expression of Toll-like receptors 2 and 4 in rheumatoid synovial tissue and regulation by proinflammatory cytokines interleukin-12 and interleukin-18 via interferon-gamma. Arthritis Rheum 2004;50(12):3856-65.

56. Brentano F, Schorr O, Gay RE, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts via toll-like receptor 3. Arthritis Rheum 2005;52(9):2656-65.

57. Hackstein H, Thomson AW. Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. Nat Rev Immunol 2004;4(1):24-34.

58. Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Ganea D, Delgado M. Vasoactive intestinal peptide induces regulatory dendritic cells that prevent acute graft-versus-host disease while maintaining the graft-versus-tumor response. Blood 2006;[epub ahead of print 17 January].

59. Menges M, Rossner S, Voigtlander C, et al. Repetitive injections of dendritic cells matured with tumor necrosis factor alpha induce antigen-specific protection of mice from autoimmunity. J Exp Med 2002;195(1):15-21.

60. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol 2005;6(8):769-76.

61. De Vries IJ, Lesterhuis WJ, Scharenborg NM, et al. Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. Clin Cancer Res 2003;9(14):5091-100.

62. Nestle FO, Alijagic S, Gilliet M, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 1998;4(3):328-32.

63. McGaha TL, Sorrentino B, Ravetch JV. Restoration of tolerance in lupus by targeted inhibitory receptor expression. Science 2005;307(5709):590-3.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Genotype distribution amongst hepatitis C patients in the Netherlands

M.J. de Vries[1*], B. te Rijdt[1], C.M.J. van Nieuwkerk[2]

[1]Roche Nederland BV, Beneluxlaan 2a, 3446 GR Woerden, the Netherlands, [2]VU Medical Centre, Department of Gastroenterology and Hepatology, PO Box 7057, 1007 MB, Amsterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)348-43 81 62, fax: +31 (0)348-43 80 06, e-mail: martin.de_vries@roche.com

ABSTRACT

Background: The prevalence of the genotypes of the hepatitis C virus (HCV) differs according to geographical location. In the United States and in European countries, the majority of patients are infected with genotype 1, 2 or 3. There is a lack of data on the distribution of HCV genotypes in the Netherlands.

Methods: The current survey determined the distribution of HCV genotypes amongst recently genotyped patients seen by physicians treating hepatitis C in the Netherlands.

Results: Almost half of the 351 patients (49.3%) were infected with genotype 1. Genotype 3 was the second most dominant genotype with a prevalence of 29.3%. Genotypes 2 and 4 were found in 9.7 and 10.5% of the patients, respectively. For 61.5% of the patients (n=216), the subtype was available. For genotype 1 the prevalence of subtype 1a and 1b was very similar, while for genotype 3 a large majority of patients were infected with subtype 3a.

Conclusion: This survey gives the first estimation of the distribution of HCV genotypes amongst unselected HCV patients in the Netherlands.

KEYWORDS

Genotype, hepatitis C, subtype, the Netherlands

INTRODUCTION

Chronic hepatitis C infection is a major cause of mortality, morbidity and liver transplantation. Worldwide, an estimated 170 million people are infected with the hepatitis C virus. The virus is a positive-sense, single-stranded RNA (9.6 kb) virus of the *Flaviviridae* family and shows considerable variability in its genomic structure.[1] The commonly used classification system proposed by Simmonds *et al.* is based on this heterogeneity and classifies different genotypes with multiple subtypes on the amount of nucleotide variation.[2-4] The prevalence of the HCV genotypes differs according to geographic location.[3-5] Some genotypes such as genotypes 1, 2 and 3 show a worldwide distribution, whereas others such as 4 and 5 are relatively restricted to certain geographical regions.[5] In the United States and Europe, the majority of HCV patients are infected with genotype 1, 2 or 3.[2,4] In 1996, Blatt *et al.* determined the genotype for 6807 patients and found that genotype 1 was predominant in all regions of the United States, with a nationwide prevalence of 73%.[6] Of the patients, 14% had genotype 2 and 8% genotype 3. In recent screenings in Germany and Belgium, genotype 1 was also predominant.[7,8]

Patients infected with different genotypes respond differently to treatment. Just over 50% of patients with genotype 1 respond with a sustained viral response to a course of 48 weeks of peginterferon-α and ribavirin.[9] Genotype 4 is also considered difficult-to-treat since treatment must be given for 48 weeks. With this treatment duration, however, the majority of patients respond to therapy.[10,11] The large majority of patients with genotype 2 or 3 respond to 24 weeks of treatment.[9] In view of the differences in response rate and treatment duration, the genotype distribution is of particular importance. In the Netherlands the distribution amongst unselected HCV patients was assessed for two smaller groups of patients more than ten years ago.[12,13] The current survey was therefore performed to determine the distribution of the HCV genotypes amongst unselected, recently genotyped patients in the Netherlands.

MATERIAL AND METHODS

In the Netherlands, testing for HCV RNA and genotyping of HCV-seropositive patients is requested by hospital-

based physicians. Out of some 200 physicians in internal medicine and gastroenterology known to treat hepatitis C, a selection treating substantial numbers of patients were asked to participate in this survey. Physicians were visited by representatives of the pharmaceutical company of which two of the authors are employees and were asked to report the genotype, date of genotyping and, if available, the subtype for the five most recently genotyped HCV patients. However, as we wanted to assess the current distribution, data from before 1 January 2002 should not be reported. Physicians were asked to report only on patients for whom the genotyping had been requested by the reporters themselves and to exclude patients for whom the genotype had already been determined by a referring physician. They were asked to report data on the last five patients irrespective of whether the patient received treatment or had comorbidities. However, as patients co-infected with HIV are often treated by different physicians, participants were asked to exclude these patients. Physicians considering participation were given documentation specifying the data being collected as well as a record form to report the data. The completed form could be submitted by mail or given to the representative at a subsequent visit. Non-respondents were reminded to submit the data by the representatives at subsequent visits. Physicians who intended to participate but who did not submit the data, or for whom no subsequent visit was planned during the survey, were reminded by telephone or letter. Data collection was undertaken from September 2004 to June 2005. Physicians were offered an incentive for their participation and investment of time. To assess whether the data obtained was distributed representatively over the country, we compared the percentage of survey patients being reported by physicians from each of the provinces and the percentage of inhabitants of the Netherlands living in these provinces.[14]

RESULTS

A total of 111 physicians treating hepatitis C were asked to participate in the survey. Of these, 74 physicians provided their data (66.7%). Most physicians (n=68) reported data for five patients, while six physicians provided data for two to four patients. Results for 360 patients were reported. However, nine patients (reported by seven physicians) were excluded since genotyping had been performed before 1 January 2002. Genotyping of the 351 patients had taken place between February 2002 and June 2005. The participating physicians were based in 53 hospitals located in 11 of the 12 Dutch provinces. The only province without participants was Flevoland (*table 1*). The percentage of patients being reported by physicians from each of the provinces was according to the percentage of inhabitants of the Netherlands living in these provinces (*table 1*).[14]

Table 1. *Distribution of patients in the current survey*

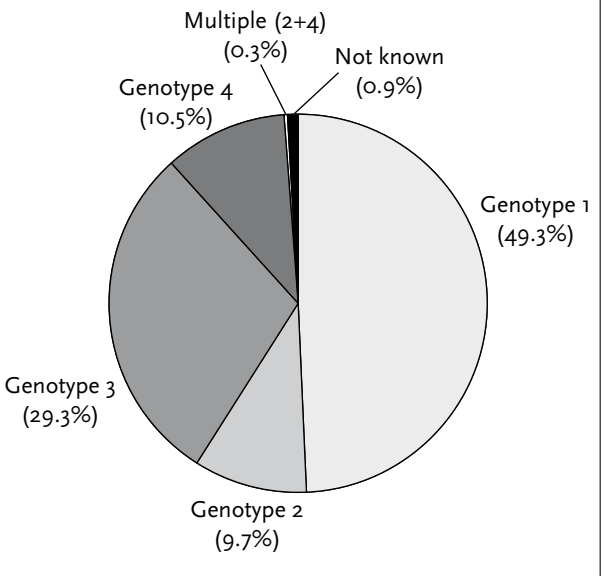
Province	No. of patients	% of the survey patients	% of Dutch inhabitants*
Flevoland	0	0	2.2
Zeeland	10	2.8	2.3
Drenthe	4	1.1	3.0
Groningen	31	8.8	3.5
Friesland	5	1.4	3.9
Overijssel	15	4.3	6.8
Limburg	37	10.5	7.0
Utrecht	42	12.0	7.1
Gelderland	38	10.8	12.1
Noord-Brabant	34	9.7	14.8
Noord-Holland	53	15.1	15.9
Zuid-Holland	82	23.4	21.2

*Statistics Netherlands, 2004.[14]

Almost half of the 351 patients (49.3%, 95% confidence interval (CI) 44.1 to 54.5%) were infected with genotype 1 (n=173) (*figure 1*). Genotype 3 was the second most dominant genotype with a prevalence of 29.3% (CI 24.6 to 34.1%, n=103). Genotypes 2 and 4 were found in 9.7% (CI 6.6 to 12.8%, n=34) and 10.5% (CI 7.3 to 13.8%, n=37) of the patients respectively. One patient showed evidence of multiple genotypes (2+4). No other genotypes were reported. For three patients, the genotype could not be determined.

For 61.5% of the patients (n=216) the subtype was available. It was not available for 35.3, 26.5, 35.0 and 67.6% of the patients with genotypes 1, 2, 3 and 4 respectively. For genotype 1, the prevalence of subtype 1a and 1b amongst

Figure 1. *Distribution of hepatitis C genotypes amongst 351 patients in the Netherlands*



the subtyped patients was 46.4 and 49.1% respectively (*table 2*). Some patients had both subtypes. The majority of the genotype 2 patients had a combination of subtypes 2a and 2b, while a large majority of genotype 3 patients had subtype 3a (94.0%). For a minority of genotype 4 patients the subtype was available, half of these patients were infected with the combination of 4c and 4d.

Table 2. *Distribution of hepatitis C subtypes amongst 351 patients in the Netherlands*

Genotype	Subtype	No.	%*
1	a	52	46.4
	b	55	49.1
	a + b	5	4.5
	Not available	61	
2	a	2	8.0
	b	8	32.0
	a + c	14	56.0
	a or c	1	4.0
	Not available	9	
3	a	63	94.0
	b	1	1.5
	c	2	3.0
	h	1	1.5
	Not available	36	
4	a	2	16.7
	c	1	8.3
	c + d	6	50.0
	e	1	8.3
	h	2	16.7
	Not available	25	
2 + 4	Not available	1	
Untypeable		3	

*Distribution of subtypes per genotype amongst subtyped patients.

DISCUSSION

This survey assesses the distribution of HCV genotypes amongst unselected HCV patients in the Netherlands by collecting data from physicians treating hepatitis C. Physicians from more than 50% of Dutch hospitals, located in 11 of the 12 provinces, participated. It can be assumed that most patients visiting a specialist for their HCV for the first time will visit a hospital in the province in which they live. The percentage of survey patients reported by physicians from each of the provinces was according to the percentage of inhabitants of the Netherlands living in these provinces (*table 1*).[14] Therefore, as long as there are no data available on regional differences in the HCV prevalence in the Netherlands, our results can be considered representative for the Netherlands. The number of patients reported by physicians from each of the provinces is too small to test for regional differences in the genotype distribution in the Netherlands.

We did not collect data on the type of assay used to determine the genotype and subtype for the patients. However, both assays used for HCV genotyping (the INNO-LiPA HCV II/VERSANT HCV Genotyping Assay (LiPA) and the Truegene HCV 5'NC Genotyping kit) can identify the six genotypes.[15] One patient showed evidence of infection with multiple genotypes. The genotype for three patients could not be determined. We do not know whether they could not be genotyped because of sensitivity problems (low viral load) or because they were infected with an unknown genotype. Nevertheless, as only three patients could not be genotyped, the validity of the data is not affected. The genotyping assays identify a large number of subtypes, but errors may occur and for a large number of patients the subtype was not available.[15] The data on the distribution of subtypes may therefore not be completely correct. Although this limitation of the survey must be taken into consideration, it has no clinical relevance as the length and the success of therapy depend on the genotype and not on the subtype.[9-11]

The distribution was assessed for HCV patients irrespective of whether treatment had been initiated. As genotypes are related significantly to the source of infection, patients with comorbidities are often infected with specific genotypes.[16] These patients could also be included; the only selection was that patients were not co-infected with HIV. A limitation of the survey is that we have no data on the number of patients with comorbidities who were included, and therefore cannot assess to what extent the results are influenced by patients from selected patient groups. However, infection with HCV in patients with comorbidities by currently known modes of transmission is nowadays excluded and most patients have been screened for HCV longer ago. We do not therefore believe that many such patients were included in the survey and hence they will not affect the results significantly.

About half of the patients were infected with genotype 1 and a third with genotype 3 (*figure 1*). Genotype 4 was found to occur at roughly the same frequency as genotype 2. This prevalence differs from that found in small groups of patients more than ten years ago, as well as from the prevalence in selected patient groups and patients with comorbidities (*table 3*).[12,13,17,18] This is to be expected as the genotype prevalence is changing over time and differs in patients with specific comorbidities.[19,20]

The data also differ from those of clinical studies in which many Dutch patients were included.[21,22] However, data from studies are for a selected group for whom treatment has been initiated and with the limitations of inclusion and exclusion criteria. In addition, patients from Belgium and Luxembourg also participated in these studies, while the prevalence of HCV genotypes may differ between countries.

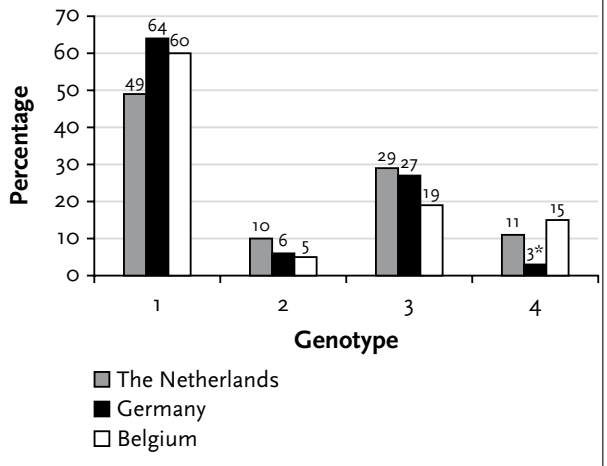
Table 3. *Distribution of hepatitis C genotypes in the Netherlands*

Patient group	Year of data collection	No.	Genotype 1 (%)	Genotype 2 (%)	Genotype 3 (%)	Genotype 4 (%)	Genotype 5 (%)	Multiple (%)
HCV patients[12]	1993+	62	55	18	19	6	-	2
HCV patients[13]	1993+	54	57	15	19	6	2	2
Dialysis patients[17]	1995-1996	71	70	17	7	4	-	6
Blood donors[18]	1994	31	58	23	16	3	-	-
Blood donors[27]	1997-2002	81	48	11	28	9	-	-
Study patients#[21]	1990-1993	322	68	10	14	5	2	1
Study patients#[22]	1996-1997	295	71	21*			8**	-
HCV patients, current survey	2002-2005	351	49	10	29	11	-	-

+Year of publication of manuscript. #Includes patients from Belgium and Luxembourg. *Genotypes 2 and 3. **Genotypes non-1,2,3.

Recent data are available for two countries bordering the Netherlands. In a screening of 2996 patients in Germany, the genotype was determined for 95.4% of the patients, while in Belgium it was determined for 265 patients.[7,8] The distribution in the Netherlands was found to be considerably different from that of Belgium and Germany (*figure 2*). The higher existence of genotype 2 in the Netherlands may be related to the presence of a large population of inhabitants from the former Dutch colony of Surinam where genotype 2 is dominant.[23] The higher presence of genotype 4 in the Netherlands and Belgium may indicate a larger immigration from Middle Eastern and Central African countries, where genotype 4 is predominant.[4,5] It was recently reported, however, that genotype 4 has become increasingly prevalent in several European countries, being prevalent in younger patients, with a short duration of infection, as well as in infected injection drug users (IDUs).[20,24] The current data indicate that the observation of 1995 that genotype 4 was found only sporadically in countries outside Africa is also outdated for the Netherlands.[4]

Figure 2. *Distribution of hepatitis C genotypes in the Netherlands, Germany and Belgium*



*Includes genotype 5 and 6. In Belgium 2% was infected with genotype 5.

Genotype 1b is mainly found amongst patients infected by blood transfusion and was the most prevalent subtype amongst genotype 1 patients in Europe in the past.[2,3,16] The majority of infected IDUs in Western countries have genotypes 1a and 3a. The current data indicate a comparable prevalence of subtypes 1a and 1b amongst genotype 1 infected patients. This may indicate that of the current HCV-genotyped patients, more have been infected by (prior) injection drug use than in the past. Indeed, surveillance of hepatitis C infection in the Netherlands indicates that injection drug use was the main route of transmission under patients diagnosed in 1999 to 2002.[25] From 1997 to 2002, a comparable prevalence of subtypes 1a and 1b was also found amongst Dutch HCV-positive, asymptomatic blood donors.[26] As the genotype distribution in voluntary blood donors, considered a low-risk population for HCV, is very similar to the distribution found amongst HCV patients in our survey, the mode of infection amongst HCV patients in general and HCV-positive blood donors may be quite similar.

In order to increase our knowledge of the transmission of HCV genotypes in the Dutch population, further research should focus on the genotype in association with epidemiological data, such as age, ethnic background, and duration and source of infection.

ACKNOWLEDGEMENTS

We would like to thank all the physicians for providing their data and making it possible to estimate the prevalence of the HCV genotypes in the Netherlands. We would also like to thank Tamara Hilhorst and Sanne Hoiting of Roche Nederland BV for the data collection. This survey was funded by Roche Nederland BV, Woerden, the Netherlands. Conflict of interest: M.J. de Vries and B. te Rijdt are employees of Roche Nederland BV.

REFERENCES

1. Robertson B, Myers G, Howard C, et al. Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. Arch Virol 1998;143:2493-503.
2. Simmonds P, Alberti A, Alter HJ, et al. A proposed system for the nomenclature of hepatitis C viral genotypes. Hepatology 1994;19:1321-4.
3. Simmonds P. Variability of Hepatitis C Virus. Hepatology 1995;21:570-83.
4. Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: quasispecies and genotypes. Semin Liver Dis 1995;15:41-63.
5. Nguyen MH, Keeffe EB. Epidemiology and treatment outcomes of patients with chronic hepatitis C and genotypes 4 to 9. Rev. Gastroenterol Disord 2004;4 (Suppl 1):S14-21.
6. Blatt LM, Mutchnick MG, Tong MJ, et al. Assessment of hepatitis C virus RNA and genotype from 6807 patients with chronic hepatitis C in the United States. J Viral Hepat 2000;7:196-202.
7. Zehnter E, Hüppe D, Alshuth U. Epidemiology and clinical appearance of hepatitis C patients in Germany. Hepatology 2004;40(suppl 1):393A.
8. De Maeght S, Bourgeois N, de Galocsy C, et al. A pilot observational survey of Hepatitis C in Belgium. Acta Gastroenterol Belg 2005;68:A33.
9. Hadziyannis SJ, Sette H, H, Morgan T, et al. Peginterferon alfa-2a and ribavirin combination therapy in chronic hepatitis C. A randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-55.
10. Khuroo MS, Khuroo MS, Dahab ST. Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. Aliment Pharmacol Ther 2004;20:931-8.
11. Diago M, Hassanein T, Rodes J, et al. Optimized virologic response in hepatitis C virus genotype 4 with peginterferon-alpha2a and ribavirin. Ann Intern Med 2004;140:72-3.
12. Kleter GE, van Doorn LJ, Brouwer JT, et al. Sequence analysis of the 5' untranslated region in isolates of at least four genotypes of hepatitis C virus in the Netherlands. J Clin Microbiol 1994;32:306-10.
13. Van Doorn LJ, Kleter B, Stuyver L, et al. Analysis of hepatitis C virus genotypes by a line probe assay and correlation with antibody profiles. J Hepatol 1994;21:122-9.
14. Statistics Netherlands (CBS), Regional Key Figures 2004 (Regionale Kencijfers Nederland). http:/www.cbs.nl/nl-NL/menu/cijfers/statline.
15. Pawlotsky J-M. Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36:S65-73.
16. Pawlotsky J-M, Tsakiris L, Roudot-Thorval F, et al. Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. J Infect Dis 1995;171:1607-10.
17. Schneeberger PM, Keur I, van der Vliet W, et al. Hepatitis C virus infections in dialysis centers in the Netherlands: a national survey by serological and molecular methods. J Clin Microbiol 1998;36:1711-5.
18. McOmish F, Yap PL, Dow BC, et al. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. J Clin Microbiol 1994;32:884-92.
19. Pol S, Thiers V, Nousbaum JB, et al. The changing relative prevalence of hepatitis C virus genotypes: evidence in hemodialyzed patients and kidney recipients. Gastroenterology 1995;108:581-3.
20. Schröter M, Zöllner B, Schäfer P, et al. Epidemiological dynamics of hepatitis C virus among 747 German individuals: new subtypes on the advance. J Clin Microbiol 2002;40:1866-8.
21. Brouwer JT, Nevens F, Kleter B, et al. Efficacy of interferon dose and prediction of response in chronic hepatitis C: Benelux study in 336 patients. J Hepatol 1998;28:951-9.
22. Brouwer JT, Nevens F, Bekkering FC, et al. Reduction of relapse by 18-month treatment in chromic hepatitis C. A Benelux randomised trial in 300 patients. J Hepatol 2004;40:689-95.
23. Kleter B, Brouwer JT, Nevens F, et al. Hepatitis C virus genotypes: epidemiological and clincal associations. Liver 1998;18:32-8.
24. Van Asten L, Verhaest I, Lamzira S, et al. Spread of hepatitis C virus among European injection drug users infected with HIV: a phylogenetic analysis. J Infect Dis 2004;189:292-302.
25. Op de Coul E, Bosman A, van de Laar M. Surveillance of hepatitis C infection in the Netherlands, 1992-2002 [Article in Dutch]. Inf Bull 2003;14:323-8.
26. Van de Laar, TJW, Koppelman MGHM, Zaaijer HL, et al. Diversity and origin of hepatitis C virus (HCV) among voluntary Dutch blood donors. J Hepatology, 2005;42(suppl 2):168-9.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Prevalence and clinical characteristics of insulin-treated, anti-GAD-positive, type 2 diabetic subjects in an outpatient clinical department of a Dutch teaching hospital

T.E.H. Römkens[1], G.C.M. Kusters[2], M.G. Netea[3], P.M. Netten[1*]

[1]Department of Internal Medicine, [2]Laboratory of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands, [3]Department of General Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands,
[*]corresponding author: e-mail: P.Netten@jbz.nl

ABSTRACT

Background: In clinical practice, type 1 and type 2 diabetic patients are sometimes difficult to distinguish. Type 1 diabetes has an immune-mediated pathogenesis, resulting in a loss of insulin-secreting β-cells. Type 2 diabetes mellitus is characterised by a relative insulin insufficiency, without the presence of an autoimmune aetiology, initially due to insulin resistance and later also accompanied by defective insulin release. Latent autoimmune diabetes of the adult (LADA) is a subgroup of diabetes, somewhere on the borderland between type 1 and type 2 diabetes. LADA is characterised by a late-age onset and relatively mild progression, but with unmistakable signs of autoimmunity, such as the presence of the autoimmune antibodies anti-GAD65, anti-insulin antibodies, or anti-Ia-2ab.

Objective: To establish the prevalence of anti-GAD in a diabetic outpatient clinic of a Dutch, non-university, teaching hospital and to describe these patients clinical and laboratory features, especially of the metabolic syndrome.

Methods: We evaluated GAD65 antibodies and other parameters in 244 selected diabetic patients, who had been on oral therapy for at least three months before becoming insulin-dependent.

Results: Twenty-six patients (11.6%) were positive for GAD65 antibodies. These patients had a significantly lower BMI (27.8 ± 4.5 *vs* 31.1 ± 4.9; p <0.01); less often cerebrovascular accidents (19.2 *vs* 34.9%; p<0.01) and a higher HDL cholesterol (1.73 ± 0.53 *vs* 1.21 ± 0.38; p<0.05). In contrast, anti-GAD patients had a significantly higher prevalence of hypothyroidism (23.0 *vs* 6.6%; p<0.05).

Conclusion: Anti-GAD-positive patients represent a sizable proportion of type 2 diabetes in a second-line outpatient clinic, and they are characterised by lower parameters of the metabolic syndrome, but higher prevalence of other autoimmune phenomena such as hypothyroidism.

KEYWORDS

Anti-GAD, autoimmunity, LADA, type 2 diabetes

INTRODUCTION

Classification of adult-onset diabetes mellitus (DM) into type 1 or 2 based on the clinical presentation may be difficult.[1,2] Diagnosis of the main forms of diabetes depends on clinical judgment based mainly on the age of the subject and the severity of insulin deficiency at presentation, as well as the presence or absence of features of the metabolic syndrome. In the WHO classification of 1998, an aetiological classification was chosen to subgroup the different types of diabetes.[3]

Type 1 DM refers to a loss of insulin-secreting β-cells of the islets of Langerhans, in most cases by immune-mediated pathogenic mechanisms highlighted by the presence of islet cell autoantibodies and by an altered frequency of immune-regulated genes in the HLA region. In European patients with type 1 DM 95% have positive glutamic acid decarboxylase (GAD65) and/or IA2 antibodies to antigens of the islets of Langerhans; especially the finding of GAD65 antibodies seems a quite stable finding after the age of 10 to 15 years in autoimmune diabetes.[4,5] Type 2 DM is characterised by a relative insulin insufficiency, without the presence of an autoimmune aetiology, initially due to

insulin resistance and later also accompanied by defective insulin release due to amyloid deposition in the pancreas.
Both of these major diabetes types include different stages ranging from not requiring insulin to diabetes that does require insulin for control or survival. It is recognised that the type 2 DM process does not invariably lead to insulin dependency nor, on the other hand, is total insulin deficiency exclusively classified as type 1 DM.
Furthermore a subgroup of diabetic patients can be distinguished with evidence of autoimmunity but who clinically resemble type 2 DM at diagnosis. Autoantibody positivity together with subsequent development of insulin deficiency led to the introduction of the eponym latent autoimmune diabetes in adults (LADA) for this subgroup,[6] type 1.5 diabetes,[7] or latent type 1 DM.[8] However, there is still an ongoing debate as to whether these names cover the same subgroup of diabetes.[9] LADA exits somewhere on the borderland between type 1 and type 2 diabetes, and exemplifies the difficulties we have in telling type 1 from type 2.[10] Because of its slow progression to insulin therapy, early identification of anti-GAD-positive patients could lead to implementation of preventive measures to protect residual β-cell function.

There is no consensus regarding diagnostic criteria of LADA. The grounds for designating LADA as a distinct aetiological entity are insubstantial. Several studies have used different criteria especially concerning the age of onset of diabetes and the duration of the insulin-free period.[11-13] Mainly because of these different criteria the prevalence of LADA patients varies in published studies, from 2.8%[14] to 22.3%.[15] In a large study, not restricted to hospital outpatients as the United Kingdom Prospective Diabetes Study (UKPDS) is, a prevalence of 10% was found.[16]
The aim of the present study was to establish the prevalence of anti-GAD in a diabetic outpatient clinic of a Dutch, non-university, teaching hospital and to describe these patients clinical and laboratory features of the metabolic syndrome.

PATIENTS AND METHODS

Patients

During a four-month period, we studied consecutive patients with DM type 2 who visited the outpatient clinic of the Jeroen Bosch Hospital, where 1600 patients with diabetes are monitored annually. Each year we see 200 new diabetic patients, 70% of whom start on insulin therapy. Inclusion criteria were DM type 2 (nonketotic diabetes without insulin treatment over at least three months of observation), who were treated with insulin at the start of the study or treated with maximal oral therapy and supposed to start on insulin in the next month.

Exclusion criteria were malignancy, autoimmune diseases, known abnormal thyroid function at the time of the study, use of NSAIDS or acetylsalicylic acid, or infections in the previous two weeks before the start of the study. These exclusion criteria were adopted because of further cytokine studies in the LADA population. From all the patients, blood tests were taken, including fasting glucose and lipids, $HbA_{1c,}$ thyroid function and microalbuminuria (abnormal: >30 mg albumin/24 hours). They all underwent a standard physical examination for late diabetic complications and cardiovascular risk factors and diseases. Hypertension was defined as use of antihypertensive drugs or blood pressure >140/80 mmHg. Peripheral neuropathy was scored as positive when vibration sense by a 128 Hz vibration fork at both hallux was absent, combined with a disturbed 10 g Semmes-Weinstein monofilament test and absent ankle reflexes. Macroangiopathy was present when a patient had coronary disease, defined as one or more coronary events, PTCA or CABG, or when a patient had proven cerebrovascular damage. Peripheral vascular disease was defined as symptoms of claudication and absence of foot pulses and/or a toe pressure <30 mmHg or proven peripheral vascular disease, either by radiography or vascular intervention. The diagnosis of retinopathy was based on fundoscopy after pharmacological mydriasis by an ophthalmologist. Hypothyroidism was defined as treatment with thyrax or a TSH concentration >6 mU/l and an fT4 level <13 umol/l.

Determination of autoantibodies

Ia-2a antibodies and GAD65 antibodies were determined by radiobinding assays with *in vitro* translated recombinant human ^{35}S-GAD65. GAD65-ab were expressed as relative indices, using one positive standard serum from one type 1 diabetic subject and two negative control sera from healthy subjects in each assay, and an upper level of normal of 0.035 U (mean +3SD from the indices observed in healthy individuals). The diagnostic sensitivity of the GAD65-antibody assays was 85%, the analytic specificity 100%.[17]
Informed consent was obtained from all patients.

STATISTIC ANALYSIS

Data will be given as mean ± standard deviation (SD) or as median and range. Student t-test was used for continuous variables, χ^2 test for dichotomous variables. Level of significance was p value <0.05.

RESULTS

A total of 407 type 2 DM patients visited the outpatient clinic during the study period. Of these patients, 183 were

excluded from the study because of refusal (1 patient), missing variables (6) or because of the following exclusion criteria: malignancy (10), autoimmune disease (31), acetylsalicylic acid use (84), NSAID use (49), and known abnormal thyroid function (2).

Of the remaining 224 patients, 26 had positive GAD65 antibodies (11.6%). The median anti-GAD level was 0.55 U (range 0.12 to 2.3 U). Of the 26 GAD65-positive patients, five had positive Ia-2A antibodies (19.2%). In the other 198 (anti-GAD-negative) type 2 diabetic patients there were no positive Ia-2A antibodies. Demographic characteristics of the anti-GAD population in comparison with the anti-GAD-negative type 2 diabetic population are mentioned in *table 1*. There was a female predominance in the anti-GAD population (73%) compared with the non-anti-GAD population (57%). The body mass index in the non-anti-GAD population was significantly higher than in the anti-GAD population ($p<0.01$). The prevalence of hypertension was similar in both groups, as were macroangiopathic complications, with the exception of cerebrovascular accidents, which were seen more frequently in the non-anti-GAD type 2 diabetic population ($p<0.01$). The prevalence of microvascular diabetic complications did not differ between the two groups. In 23% of the anti-GAD patients there was a hypothyroidism, compared with 7% in the non-anti-GAD population ($p<0.05$). The laboratory results of the two diabetic populations are shown in *table 2*. HbA_{1c} was similar in both groups. Total cholesterol results were comparable in the two groups, but in the anti-GAD patients the high-density lipoprotein (HDL) cholesterol value was significantly higher ($p<0.05$): the triglycerides were lower in the anti-GAD patients, but this was not statistically significant.

DISCUSSION

The main finding of our study is that the prevalence of anti-GAD-positive patients in a Dutch non-university, teaching hospital was 11.6 % of the total type 2 diabetic population and that in the anti-GAD population less features of the metabolic syndrome were present. The prevalence found is comparable with other studies, for instance the UKPDS.[16] If only type 2 diabetic patients were included with at least a six-month insulin-free period at diagnosis, recommended by Fourlanos *et al.* for diagnosing LADA, the prevalence would have been almost the same: 12.7% (23/181).[13] However, our study results have some limitations concerning the selection of the study population. No type 2 diabetic patients on only oral diabetic medication were included. It is possible that we overestimated the prevalence of anti-GAD-positive patients in our population because of the exclusion criteria, especially the exclusion of patients who were on acetylsalicylic acid: 84 of the 183 excluded patients (45.9%). Patients usually take acetylsalicylic acid because of macrovascular complications, a result of the metabolic syndrome. In this subgroup the prevalence of anti-GAD is supposed to be small. On the other hand, if LADA is a slow-developing autoimmune diabetes, by excluding patients with autoimmune diseases, we may have underestimated the prevalence. In line with this hypothesis is the high prevalence (23%) of hypothyroidism found

Table 1. *Demographic characteristics of the anti-GAD-positive population in comparison with the anti-GAD-negative type 2 diabetic population*

Variable	Anti-GAD positive (n=26)	Anti-GAD negative (n=198)	P
Sex (% female)	73	57	ns
Age (years) (mean ± SD)	59.3 ± 16.3	63.6 ± 12.8	ns
BMI (kg/m²) (mean ± SD)	27.8 ± 4.5	31.1 ± 4.9	0.006
Time to starting insulin after DM diagnosis (mean ± SD) (months)	13.2 ± 9.1	14.7 ± 8.0	ns
Smoker (%)	23.1 (6/26)	24.7 (47/190)	ns
Neuropathy (%)	42.3 (11/26)	59.2 (103/174)	ns
Retinopathy (%)	26.9 (7/26)	34.7 (65/188)	ns
Microalbuminuria (%)	32.0 (8/25)	29.4 (40/136)	ns
Hypertension (%)	46.2 (12/26)	43.5 (84/193)	ns
Cerebrovascular incident (%)	19.2 (5/26)	34.9 (55/166)	<0.01
Peripheral vascular disease (%)	30.8 (8/26)	27.1 (52/192)	ns
Coronary damage (%)	11.5 (3/26)	19.7 (38/193)	ns
Hypothyroidism (%)	23 (6/26)	6.6 (13/198)	<0.05

GAD = glutamic acid decarboxylase; BMI = body mass index; DM = diabetes mellitus; ns = not significant.

Table 2. *Laboratory results of the anti-GAD-positive population in comparison with the anti-GAD negative type 2 diabetic population*

Variable	Anti-GAD positive (n=26)	Anti-GAD negative (n=198)	P
Positive GAD65 antibodies (N)	26	0	-
Anti-GAD65 level (U) (median/range)	0.55 (0.12 - 2.3)	-	-
Positive IA-2Ab (%)	19.2%	0	-
IA-2Ab level (U) (median/range)	0.001 (-0.6 - 2.62)	-	-
HbA_{1c} (mmol/l) (mean ± SD)	8.3 ± 1.6	8.5 ± 1.4	ns
Total cholesterol (mmol/l) (mean ± SD)	5.4 ± 1.06	5.2 ± 0.9	ns
HDL cholesterol (mmol/l) (mean ± SD)	1.73 ± 0.53	1.21 ± 0.38	<0.05
LDL cholesterol (mmol/l) (mean ± SD)	2.88 ± 1.16	3.08 ± 0.8	ns
Triglycerides (mmol/l)	1.63 ± 1.88	2.07 ± 1.52	ns

GAD = glutamic acid decarboxylase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ns = not significant.

in our anti-GAD population. The association between autoimmune thyroid disease and type 1 diabetes has been reported in the past.[18,19] Recent studies have also found a higher prevalence of thyroid autoantibodies in adult diabetic patients with positive GAD65 antibodies.[17,20] The fact that 73% of the anti-GAD population is female is in accordance with an autoimmune pathogenesis of LADA. In older studies, the presence of islet autoantibodies was also associated with female sex.[8,21] In the study by Gambelunghe *et al.* the same high female prevalence in the anti-GAD patient group was found.[17]

The patients with anti-GAD in this study also had a better lipid profile and lower body mass index (BMI), compared with the type 2 diabetic patients without anti-GAD.[22] There was a tendency of less cardiovascular events in the anti-GAD population, which only reached statistical significance in the prevalence of cerebrovascular accidents.[22] The prevalence of hypertension did not differ between the two groups, as was found in several other studies.[22,23] Concerning the long-term diabetic complications there was no difference in prevalence of retinopathy, neuropathy and microalbuminuria.

These phenotypic differences of anti-GAD-positive patients compared with type 2 DM are more subtle and there seems to be less evidence of the metabolic syndrome. Probably this anti-GAD population of diabetic patients are type 1 diabetics diagnosed at an earlier phase or there is a true difference in the progression rate of insulin deficiency.

Hosszufalusi showed similar clinical characteristics and a high prevalence of predisposing risk alleles (HLA-DQB1*0302, -DR4, -DR3, -DR3/DR4) and risk haplotypes (DR4-DQB1*0302) in patients with LADA and adult-onset type 1 diabetes with rapid progression.[24] But he also showed that patients with LADA often had single positive islet cell-specific autoantibodies, in contrast to those with adult-onset type 1 diabetes with rapid progression. Others found a decreased frequency of the protective HLA type 0602 in the type 1 DM population.[25] Preliminary data of a prospective study on 22 newly diagnosed diabetic patients showed after a median follow-up of 2.3 years an unchanged fasting and glucagon stimulated C-peptide concentration in 11 LADA patients compared with 11 type 1 diabetic patients.[26] These facts pointed to a difference in the rate of deterioration of β-cell function between the two groups.

Assuming that LADA is a slow progressive type 1 form of diabetes, prevention of β-cell destruction should be attempted.[27] A pilot trial in 1996 comparing insulin and sulphonylureas in LADA patients showed that insulin-treated patients maintained higher B-cell function than those treated with sulphonylureas.[28] Early use of insulin could preserve endogenous insulin secretion and probably delay or prevent the decline of B-cell function. Cabrera-Rode *et al.* showed in 2002 that exclusion of sulphonylureas in treatment of slowly progressing type 1 DM patients partially decreases specific autoimmunity against endocrine pancreatic cells and improves metabolic control.[29] This suggests that initial insulin monotherapy is a good choice for the treatment of LADA patients, but this hypothesis has, beside the study by Kobayashi,[28] never been proved. The UKPDS confirmed that patients with LADA are more likely to progress to insulin, but it also showed that after ten years, or indeed at any point in between, those initially randomised to diet or sulphonylurea therapy did not differ in any respect from those initially randomised to insulin.[30] Another possibility is medication with anti-inflammatory properties, as shown by the reduction of cytokines such as tumour necrosis factor. Rosiglitazone has been shown to possess such effects *in vitro* and reduced the incidence of autoimmune diabetes in NOD mice.[31] More and prospective studies are needed to confirm the superiority of this suggested treatment regime and to implement it in daily care.

In daily practice this means that we could measure GAD antibodies in early type 2 diabetic patients (lower BMI, better lipid profile, hypothyreoidism, women). In case of positive antibodies, an earlier start of insulin is often needed. This anti-GAD-positive diabetic population with slow progression to insulin therapy compared with type 1 diabetes gives the opportunity of testing therapeutic modalities to protect the β-cell.

BIBLIOGRAPHY

The preliminary results of this study were presented as an oral presentation at the 38th Annual Meeting of the European Association for the Study of Diabetes, 1 to 5 September 2002, Budapest. Latent autoimmune diabetes of the adult: Epidemiology in the Netherlands. Römkens TEH, Netea MG, Schouten J, et al. Diabetologia 2002;45(suppl 2):A61.

ACKNOWLEDGEMENT

P. Candeloro, A Falorni and P. Brunetti from the Department of Internal Medicine, University of Perugia, Perugia, Italy are gratefully acknowledged for their participation in the Ia-2a and GAD65 antibody determination.

REFERENCES

1. National diabetes data group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039-57.
2. Report the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 2003;26:S5-S20.
3. Alberti KG, Zimmet PZ, for the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. I. Diagnosis and classification of diabetes mellitus: provisional report of a WHO Consultation. Diabet Med 1998;15:539-53.
4. Verge CF, Giani R, Kawasaki E, et al. Prediction of type 1 diabetes in first degree relatives using a combination of insulin, GAD, and ICA512bdc/IA2-autoantibodies. Diabetes 1996;45:926-33.
5. Seissler J, de Sonnaville J, Morgenthaler N, et al. Immunological heterogeneity in type 1 diabetes: pressence of distinct autoantibody patterns in patients with acute onset and slowly progressive disease. Diabetologia 1998;41:891-7.
6. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR. Antibodies to glutamic acid decarboxylase reveal latent autimmune diabetes mellitus in adults with a non-insulin-dependent onset of disease. Diabetes 1993;42:359-62.
7. No authors listed. Insulin-dependent? Lancet 1985;2:809-10.
8. Groop LC, Bottazzo GF, Doniach D. Islet cell antibodies identify latent type 1 diabetes in patients aged 35 - 75 years at diagnosis. Diabetes 1986;35:237-41.
9. Palmer JP, Hirsch IB. What' s in a name; Latent autoimmune diabetes of the adult, type 1.5, adult onset, and type 1. Diabetes Care 2003;2:536-7.
10. Gale EAM. Latent autoimmune diabetes in adults: a guide for the perplexed. Diabetologia 2005;48:2195-9.
11. Tuomi T, Carlsson A, Li H, et al. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes 1999;48:150-7.
12. Zimmet P, Turner R, Mc Carty D, Rowley M, Mackay I. Crucial points at diagnosis: type 2 diabetes or slow type 1 diabetes. Diabetes Care 1999;22:59-64.
13. Fourlanos S, Dotta F, Greenbaum C, et al. Latent autoimmune diabetes in adults (LADA) should be less latent. Diabetologia 2005;48:2206-12.
14. Bosi EP, Garancini NT, Poggiali F, Bonifacio E, Gallus G. Low prevalence of islet autoimmunity in adult diabetes and low predictive value of islet autoantibodies in the general adult population of northern Italy. Diabetologia 1999;42:840-4.
15. Bruno G, De Salvia A, Arcari R, et al. Clinical, immunological, and genetic heterogenity of diabetes in an Italian population-based cohort of lean newly diagnosed patients aged 30 - 54 years. Diabetes Care 1999;22:50-5.
16. Turner R, Stratton I, Horton V, et al. UKPDS 25: autoantibodies to islet cytoplasm and glutamic acid decarboxylase for prediction of insulin requirements in type 2. Lancet 1997;350:1288-93.
17. Gambelunghe G, Forini F, Laureti S, et al. Increased risk for enocrine autoimmunity in Italian type 2 diabetic patients with GAD65 autoantibodies. Clin Endocrinol 2000;52(5):563-73.
18. Lorini R, d'Annuncio G, Vitali L, et al. IDDM and autoimmune thyroid disease in the pediatric age group. J Pediat Endo Met 1996;9:89-94.
19. Hansen D, Bennedbaek FN, Hansen LK, Hoier-Madsen M, Jacobsen BB, Hegedus L. Thyroidfunction, morphology and autoimmunity in young patients with insulin dependent diabetes mellitus. Eur J Endocrinol 1999;140:512-8.
20. Falorni A. Immunologic and genetic aspects of latent autoimmune diabetes in the adult. Minerva Endocrinol 2003;28(4):297-312.
21. Irvine WJ, Mc Callum CJ, Gray RS, et al. Pancreatic islet-cell antibodies in diabetes mellitus correlated with the duration and type of diabetes, coexistent autoimmune disease, and HLA-type. Diabetes 1977;26:138-47.
22. Grasso YZ, Reddy SSK, Rosenfeld CR, et al. Autoantibodies to IA-2 and GAD65 in patients with type 2 diabetes mellitus of varied duration: prevalence and correlation with clinical features. Endocrine Practice 2001;7(5):339-45.
23. Isomaa B, Almgren P, Henricsson M, et al. Chronic complications in patients with slowly progressing autoimmune type 1 diabetes (LADA). Diabetes Care 1999;22:1347-53.
24. Hosszufalusi N, Vatay A, Rajczy K, et al. Similar genetic features and different islet cell autoantibody pattern of latent autoimmune diabetes in adults (LADA) compared with adult-onset type 1 diabetes with rapid progression. Diabetes Care 2003;26:452-7.
25. Li H, Lindholm E, Almgren P, et al. Possible human leukocyte antigen-mediated genetic interaction between type 1 and type 2 diabetes. J Clin Endocrinol Metab 2001;86(2):574-82.
26. Lethagan A, Groop L, Lindholm E, et al. Metabolic comparison of rapid and slow onset autoimmune diabetes in newly-diagnosed patients older than 35 years at onset. Diabetologia 2002;45(suppl 2):A155.
27. Pozzilli P, Di Mario U. Autoimmune diabetes not requiring insulin at diagnosis (Latent Autoimmune Diabetes of the Adult). Diabetes Care 2001;24:1460-7.
28. Kobayashi T, Nakanishi K, Murase T, Kosaka K. Small doses of subcutaneous insulin as a strategy for preventing slowly progressive B-cell failure in islet cell antibody positive with clinical features of NIDDM. Diabetes 1996;44:622-6.
29. Cabrera-Rode E, Perich P, Diaz-Horta O, et al. Slowly progressing type 1 diabetes: persistence of islet cell autoantibodies is related to glibenclamide treatment. Autoimmunity 2002;35:469-74.
30. Davis TME, Wright AD, Mehta ZM, et al. Islet autoantibodies in clinically diagnosed type 2 diabetes: prevalence and relationship with metabolic control (UKPDS 70). Diabetologia 2005;48:695-702.
31. Beales PE, Rosignoli G, Liddi R, Barchelor K, Pozzili P. The prevention of diabetes using rosiglitazone in the non-obese diabetic mouse. Diabetes Res Clin Pract 2000;50(suppl1):1518.

Netherlands
The Journal of Medicine

CASE REPORT

Fibrillary glomerulonephritis in a patient with type 2 diabetes mellitus

G.A.L. Gielen[1], J.F.M. Wetzels[2], E.J. Steenbergen[3], A.H. Mudde[1*]

[1]Department of Internal Medicine, Slingeland Hospital, Doetinchem, the Netherlands, Departments of [2]Nephrology and [3]Pathology, Radboud University Nijmegen Medical Centre, the Netherlands, [*]corresponding author: tel.: +31 (0)314-32 99 11, fax: +31 (0)3-432 9 150, e-mail: a.mudde@slingeland.nl

ABSTRACT

We report a 62-year-old man with documented type 2 diabetes mellitus and hypertension, who presented with a rapid deterioration in renal function. The sudden decrease in renal function in this well-controlled diabetic patient prompted us to consider a nondiabetic and nonhypertensive cause. The urinary sediment showed a glomerular haematuria suggestive of glomerulonephritis. A diagnosis of fibrillary glomerulonephritis was made on renal biopsy. Fibrillary glomerulonephritis is a rarely diagnosed disease with clinical manifestations such as proteinuria, microscopic haematuria, nephrotic syndrome and impairment of renal function. A diagnosis of fibrillary glomerulonephritis can only be made by electronmicroscopy of the renal tissue. In this case report the spectrum of this disease is reviewed.

KEYWORDS

Fibrillary glomerulonephritis, diabetes mellitus, fibrillary glomerulopathy

INTRODUCTION

The development of proteinuria and deterioration in renal function in a patient with diabetes mellitus and hypertension usually suggests diabetic nephropathy. However, other causes of renal failure should also be considered. In this report we present a case of fibrillary glomerulonephritis, a rare form of immunoglobulin deposit glomerulopathy, in a patient with diabetes. Our patient illustrates that other causes of proteinuria must be carefully considered, also in patients with diabetes. Furthermore, we discuss the entity of-fibrillary glomerulonephritis.

CASE REPORT

A 62-year-old male appeared at his scheduled six-monthly outpatient visit for monitoring of his diabetes mellitus and hypertension. He reported nonspecific tiredness for about three weeks. There was no gross haematuria, no dysuria and no fever. The relevant medical history revealed diabetes mellitus type 2 since 1979, necessitating insulin treatment since 1998; a coronary artery bypass graft in 1986; a nephrectomy in 1992 because of a complicated pyelonephritis; hypertension diagnosed in 1992 and treated since then. There was no evidence of microvascular complications of the diabetes, specifically there was no diabetic retinopathy. The patient was treated with a basal/prandial insulin regimen. Additional medical treatment consisted of phenprocoumon, bisoprolol, quinapril, pravastatin, ranitidine and transdermal nitroglycerine. Over the past years his blood pressure had been well controlled, values averaging 150/70 mmHg, and renal function had been stable with serum creatinine values ranging from 110 to 130 μmol/l. On presentation, the physical examination was unremarkable, except for a blood pressure of 174/98 mmHg. Laboratory testing revealed renal insufficiency, with a serum creatinine amounting to 284 μmol/l, implicating a creatinine clearance of 27 ml/min. The HbA1c was 7.2%. Proteinuria averaged 2.2 g/24 hours. Because of the rapid deterioration in the renal function, the sudden increase in proteinuria (*figure 1*) and the absence of microvascular complications (retinopathy), diabetic nephropathy was considered an unlikely cause. The presentation was not compatible with secondary focal glomerulosclerosis either, due to hyperfiltration injury in a single kidney. Therefore, other causes were considered. Microscopic examination of the urine sediment showed red cells (84% dysmorphic, 16% monomorphic), granular casts and red cell casts. This finding is suggestive of a glomerular disease. Tests for antinuclear antibodies,

antibodies to extractable nuclear antigens, antineutrophil cytoplasmic antibodies, cryoglobulins, antiglomerular basement membrane antibodies, antistreptolysin-O titre and complement consumption were all negative. Immunofixation did not reveal an M-protein in the serum and there were no immunoglobulin light chains in the urine. Because of the fact that the patient had a single kidney, a renal biopsy was initially not performed and the patient was treated with a short course of high-dose prednisone. However, because of further deterioration in renal function a renal biopsy was done to obtain a diagnosis.

The biopsy (*figures 2 to 4*) contained on average 12 glomeruli per cross section, of which approximately 30% showed abnormalities. There was segmental endocapillary hypercellularity with both mononuclear cells and neutrophils. Mesangial areas and the glomerular basement membrane mostly appeared unremarkable, although at high magnification a 'moth eaten' appearance of the mesangium and glomerular basement membrane was seen. Immunofluorescence (IF) revealed deposits of IgG, C3, C1q, kappa and lambda light chains, mostly in the mesangium with some involvement of the peripheral capillary walls as well. The depositions were homogeneous rather than granular in nature. Electron microscopy revealed the presence of straight fibrils measuring approximately 20 nm in diameter in mesangial areas, subendothelially and within the glomerular basement membrane. On the basis of these findings we diagnosed a focal segmental endocapillary proliferative glomerulonephritis with deposition of polyclonal IgG in the form of 20 nm wide fibrils, consistent with fibrillary glomerulonephritis.

Because of further deterioration in the renal function, the patient became dependent on haemodialysis and is currently awaiting renal transplantation.

Figure 1. *Course of renal function (expressed as 1000/serum creatinine) and urinary albumin excretion (expressed as mg albumin/mmol creatinine) in the patient*

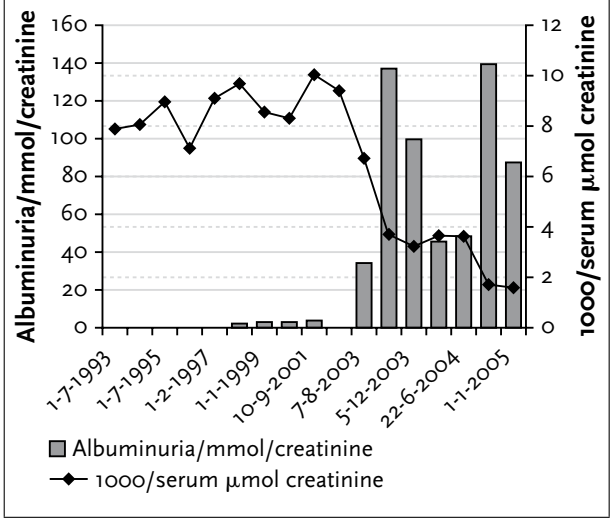
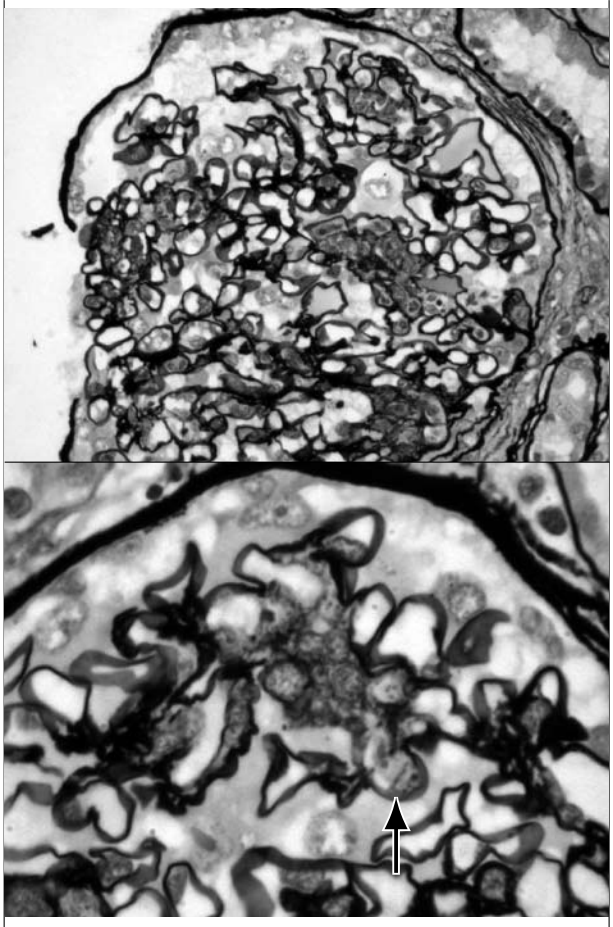


Figure 2. *Light microscopy, methenamine silver staining*



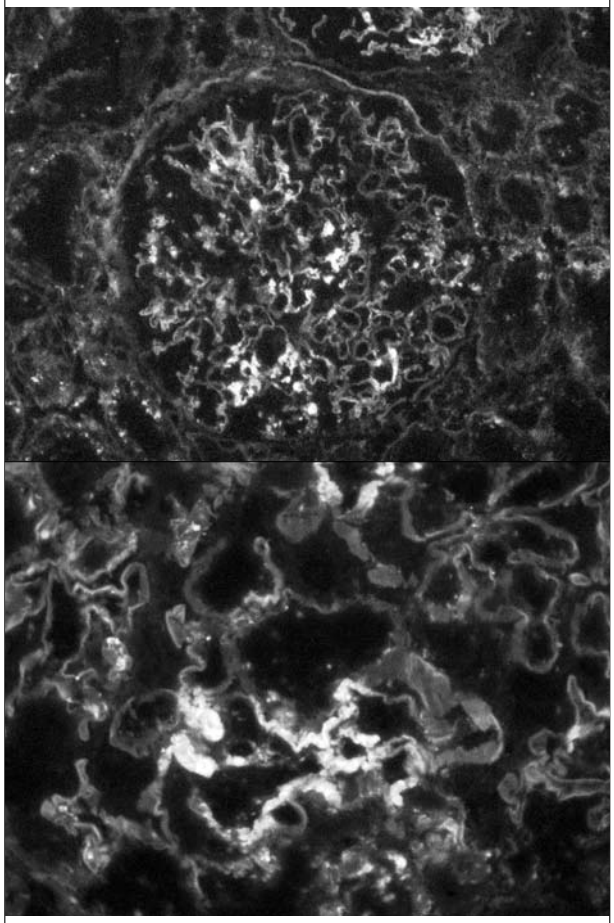
There is segmental endocapillary hypercellularity with mononuclear cells and neutrophils and some mesangiolysis. At high magnification a lucent ('moth eaten') appearance of the mesangium can be appreciated. Also the glomerular basement membrane appears locally thickened and structurally altered (arrow).

DISCUSSION

Progression from microalbuminuria to overt nephropathy occurs in 20 to 40% of Caucasian patients with type 2 diabetes within a ten-year period.[1-3] Diabetic nephropathy typically begins with the urinary excretion of small amounts of protein (microalbuminuria). Although microalbuminuria may sometimes be temporary,[4] it often heralds the development of overt diabetic nephropathy, characterised by a slowly increasing proteinuria and a gradual deterioration of renal function. This process may take many years. Most patients with diabetic nephropathy have evidence of other microvascular complications such as diabetic retinopathy.

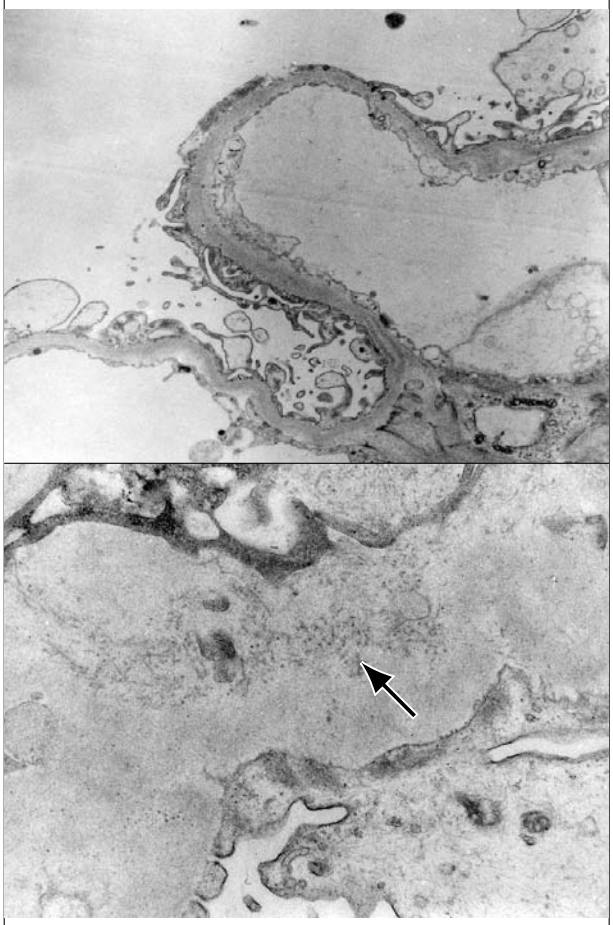
In our patient several points argued against a diagnosis of diabetic nephropathy, specifically the rapid deterioration in renal function, the sudden increase in proteinuria

Figure 3. *Immunofluorescence, staining for IgG*



There are discontinuous areas with homogeneous staining for IgG. On higher magnifications IgG is located mainly in mesangial areas with some extension into the peripheral capillary walls as well.

Figure 4. *Electronmicroscopy*



A glomerular segment with variation in the thickness of the glomerular basement membrane is shown. The glomerular basement membrane appears structurally abnormal and high magnification shows the presence of 20 nm wide straight fibrils located within the glomerular basement membrane (arrow). These fibrils were also seen in the mesangium. There is podocyte swelling with focal foot process effacement. There also is mild endothelial swelling with some subendothelial lucency.

and the absence of diabetic retinopathy. Secondary focal glomerulosclerosis in a hyperfiltrating single kidney must be considered; however, in such cases patients develop proteinuria well before the onset of renal function deterioration.

Although a significant haematuria with red cell casts may be a clinical feature of diabetic nephropathy,[5] the active urinary sediment with dysmorphic erythrocytes and erythrocyte casts in this case suggested the presence of a second, unrelated form of glomerulonephritis.[6] In the diagnostic work-up positive autoimmune serology may help to narrow the differential diagnosis prior to renal biopsy. In our patient the serological findings were negative and the suspicion of a glomerular disease was ultimately confirmed by renal biopsy, which showed fibrillary glomerulonephritis. Performing a renal biopsy in a patient with a solitary kidney is not routinely done and deserves some comments. The presence of a single kidney is still a relative contraindication for performing a renal biopsy, even though the number of serious complications from renal biopsy has decreased in recent years with the use of ultrasound guidance and an automated biopsy gun. Therefore, we first attempted to induce remission with a short course of prednisone. Since the patient did not respond the necessity of a diagnostic procedure was discussed. Important aspects were the risks of a biopsy, the risks of prolonged and more intensive immunosuppressive therapy, and the likelihood of diagnosing a treatable disease such as membranous nephropathy, focal glomerulosclerosis or amyloidosis. In this diabetic patient the balance in our view was in favour of the renal biopsy.

Fibrillary glomerulonephritis is a glomerular disease that belongs to the class of the fibrillary glomerulopathies, a heterogeneous group of glomerular diseases characterised by the presence in the glomeruli of organised deposits with the structural appearance of fibrils or microtubules.[7-10]

The deposits in fibrillary glomerulonephritis are immunoglobulin derived, either polyclonal or monoclonal. These organised structures cannot be recognised by light microscopy, thus a diagnosis of fibrillary glomerulopathy can only be made with certainty if renal tissue is appropriately examined by electron microscopy. The light microscopic findings associated with fibrillary glomerulonephritis are quite diverse.[11] The most frequently encountered is a membranoproliferative pattern of injury (44%), less frequently observed are mesangial proliferative (21%), diffuse endocapillary proliferative (15%), membranous (7%) and sclerosing patterns of injury (13%). Most patients present with haematuria (60%), renal insufficiency (72%) and proteinuria (in all patients, with 52 % in the nephrotic range).

The literature has proposed various classifications or algorithms for the diagnosis of the fibrillary glomerulopathies.[9] Often, these schemes seem rather confusing, because they incorporate clinical and laboratory data. We provide an overview of the fibrillary glomerulopathies in *table 1*, and discern three major classes: deposition in the form of amyloid, the immunoglobulin derived nonamyloid fibrillary glomerulopathies and the nonimmunoglobulin derived fibrillary glomerulopathies. A diagnosis of fibrillary glomerulonephritis is thus made if Congo-Red negative immunoglobulin-derived deposits are present in the glomerulus in the absence of evidence of systemic diseases such as SLE, multiple myeloma or cryoglobulinaemia. Immunotactoid glomerulopathy is a special form of fibrillary glomerulonephritis, characterised

Table 1. *Overview of glomerular diseases characterised by the presence of organised glomerular deposits (fibrillary glomerulopathies)*[a]

	Congo red staining	IF (IgG)[b]	IF light chain restriction	Electronmicroscopy	Remarks
Amyloidosis					
AL amyloidosis	+	-	+ (not always)[c]	Nonbranching fibrils 8-10 nm	Localisation: mesangial, subendothelial, in the GBM
AA amyloidosis	+	-	-	Idem	Idem
Other	+	-	-	Idem	Idem
Immunoglobulin derived					
SLE	-	+	-	Organised deposits, with substructures with the appearance of curvilinear fibrils 8-15 nm	Deposits give impression of 'fingerprint', deposits can also be localised subepithelially; SLE is a clinical diagnosis based on ARA criteria
Cryoglobulinaemia	-	+	+ or -	Organised deposits with variable structural organisation, fibrils, tubules, grid-like, 6-62 nm	Typical is presence of cryoglobulin thrombi in the capillary lumina; cryoglobulinaemia is not always detected
Fibrillary gn	-	+	3-5% +	Nonbranching fibrils 12-24 nm	Localisation: see amyloid
Immunotactoid gn	-	+	60-70% +	Microtubules > 30 nm	Localisation: see amyloid
M-protein related to clinical evidence of systemic disease	-	+	+	Variable structures as described for cryoglobulins LCDD is characterised by electron-dense granular deposits	Often classified based on clinical diagnosis (CLL, MM). Difficult to differentiate from cryoglobulinaemia and immunotactoid gn. M-protein related glomerular disease better description?
Non-immunoglobulin derived					
Fibronectin gn	-	-	-	Mostly amorphous deposits, with sometimes irregular fibrils 10-12 nm	Fibronectin +
Collagen III gn	-	-	-	Striated bundles with periodicity of 60 nm, giving striped appearance	Typical collagen bundles easily recognised by electron microscopy. Collagen III +
Diabetes mellitus	-	-	-	10-120 nm	Localised in mesangium

[a]There is no uniform definition of fibrils in the literature. Most authors include diseases characterised by organised deposits that do not contain fibrils. [b]In M-protein related diseases the heavy chain is predominantly IgG, but it may be of another class (IgA, IgM) and in SLE there is usually a 'full house'. [c]Amyloid is composed of the variable part of the light chains, which does not contain the antigens recognised by the commercial antibodies. SLE = systemic lupus erythematosus; IF = immunofluorescence; LCDD = light chain deposition disease; gn = glomerulonephritis; CLL = chronic lymphatic leukaemia; MM = multiple myeloma; GBM = glomerular basement membrane; ARA = American Rheumatoid Association.

by the presence of microtubules that are arranged in parallel, with a hollow centre at high-power magnification, and a diameter of more than 30 nm. Overlap, however, does occur. Some authors consider fibrillary glomerulonephritis and immunotactoid glomerulopathy as a single entity with differences in the morphological spectrum, whereas others consider them to be a separate disease characterised by the size and organisation of the fibrils.

The presence of a monoclonal protein (M-protein) in the kidney as detected by IF should be used as an important criterion for further classification of fibrillary glomerulopathies. It is well known that in up to 20% of patients with M-protein associated renal diseases (such as light chain deposition disease or AL amyloidosis) a M-protein is found in the kidney but not in the serum or in the urine. Since the characteristics of the glomerular fibrils are dependent on the physicochemical properties of the immunoglobulins it is not surprising that M-proteins more often form well-organised deposits with a periodical structure in contrast to polyclonal immune globulin derived fibrils which are more randomly arranged. Indeed, M-proteins are more often detected in immunotactoid glomerulopathy (66%) than in fibrillary glomerulonephritis (3%). Detection of a monoclonal protein in IF should lead to an intensive search for associated conditions, since the incidence of lymphoproliferative malignancies is markedly higher in patients with the immunotactoid as compared with the fibrillary variant.[12-14]

To date there is no proven effective therapy for fibrillary glomerulonephritis.[7,11] The results of treatment with prednisone or cytotoxic agents are disappointing. Intensified treatment of hypertension and proteinuria preferably with ACE inhibitors may improve the outcome. We advocate a trial of immunosuppressive treatment only in patients with evidence of M-protein related deposits.[15] Of the affected patients, 45% develop end-stage renal failure (ESRD) within 24 months.[11] The prognosis is determined by serum creatinine and the presence of tubulo-interstitial fibrosis at presentation. Patients with ESRD may be offered renal transplantation. Unfortunately, the disease often recurs after transplantation (50% recurrence rate), although the course of disease after transplantation is often more protracted.[16]

REFERENCES

1. Ismail N, Becker B, Strzelczyk P, Ritz E. Renal disease and hypertension in non-insulin-dependent diabetes mellitus. Kidney Int 1999;55:1-28.
2. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 1984;310:356-60.
3. Klein BE, Moss SE, Cruickshanks KJ. Ten-year incidence of gross proteinuria in people with diabetes. Diabetes 1995;44:916-23.
4. Gaede P, Vedel P, Parving HH, Pedersen O. Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. Lancet 1999;353:617-22.
5. Lopes de Faria JB, Moura LA, Lopes de Faria SR, Ramos OL, Pereira AB. Glomerular hematuria in diabetics. Clin Nephrol 1988;30:117-21.
6. Huussen J, Koene RAP, Hilbrands LB. The (fixed) urinary sediment, a simple and useful diagnostic tool in patients with haematuria. Neth J Med 2004;62:4-9.
7. Schwartz MM, Korbet SM, Lewis EJ. Immunotactoid glomerulopathy. J Am Soc Nephrol 2002;13:1390-7.
8. Alpers CE. Immunotactoid (microtubular) glomerulopathy: An entity distinct from fibirllary glomerulonephritis. Am J Kidney Dis 1992;19:185-91.
9. Korbet SM, Schwartz MM, Lewis EJ. The fibrillary glomerulopathies. Am J Kidney Dis 1994;23:751-65.
10. Van Ginneken EEM, Assmann KJM, Koolen MI, Jansen JLJ, Wetzels JFM. Fibrillary-immunotactoid glomerulopathy with renal deposits of IgAλ: a rare cause of glomerulonephritis. Clin Nephrol 1999;52:383-9.
11. Rosenstock JL, Markowitz GS, Valeri AM, et al. Fibrillary and immunotactoid glomerulonephritis: Distinct entities with different clinical and pathologic features. Kidney Int 2003;63:1450-61.
12. Bridoux F, Hugue V, Coldefy O, et al. Fibrillary glomerulonephritis and immunotactoid (microtubular) glomerulopathy are associated with distinct immunologic features. Kidney Int 2002;62:1764-75.
13. Moulin B, Ronco PM, Mongenout B, et al. Glomerulonephritis in chronic lymphocytic leukemia and related B-cell lymphomas. Kidney Int 1992;42:127-35.
14. Brady H. Fibrillary glomerulopathy. Kidney Int 1998;53:1421-9.
15. Fogo A, Quereshi N, Horn RG. Morphologic and clinical features of fibrillary glomerulonephritis versus immunotactoid glomerulopathy. Am J Kidney Dis 1993;22:367-77.
16. Pronovost PH, Brady HR, Gunning ME, Espinoza O, Rennke H.G. Clinical features, predictors of disease progression and results of renal transplantation in fibrillary/immunotactoid glomerulopathy. Nephrol Dial Transplant 1996;11:837-42.

Netherlands
The Journal of Medicine

CASE REPORT

Azathioprine-induced shock in a patient suffering from undifferentiated erosive oligoarthritis

G. Demirtaş-Ertan, A.T. Rowshani*, I.J.M. ten Berge

Divisions of Clinical Immunology and Rheumatology, and Nephrology, Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands,
*corresponding author: tel.: +31 (0)20-566 59 39 (page 58816), fax : +31 (0)20-691 49 04,
e-mail: T.Rowshani@AMC.UVA.NL

ABSTRACT

Shock due to a hypersensitivity response to azathioprine is unpredictable, occurs seldom and bears a potentially fatal outcome. Azathioprine is widely used in the treatment of autoimmune diseases and in solid organ transplantation. Here, we present a patient who suffered from undifferentiated erosive oligoarthritis and was treated with azathioprine. This patient developed anaphylactic shock which was interpreted as a side effect of azathioprine. Although rare, similar cases were described since 1980.

KEYWORDS

Azathioprine, azathioprine-induced shock, hypersensitivity, shock, undifferentiated oligoarthritis

INTRODUCTION

Azathioprine (AZA) is a frequently prescribed drug, used in the treatment of autoimmune and chronic inflammatory diseases. Moreover, it is used in the prevention of allograft rejection after solid organ transplantation. AZA is an imidazole derivative of 6-mercaptopurine (6-MP) and together with 6-MP and 6-thioguanine (6-TG) forms the three major thiopurines. The adverse effects of AZA can be subdivided into two types: allergic and nonallergic. The nonallergic adverse reactions are dose-dependent and are thought to be related to thiopurine metabolites and include myelosuppression and hepatotoxicity. The allergic-type reactions are rare, dose-independent and occur within weeks following the drug introduction. A broad spectrum of reactions can occur, ranging from pancreatitis, hepatitis, skin rash, fever, arthralgias, malaise, nausea, diarrhoea and abdominal pain to the development of anaphylactic reactions.[1,2] Only a few cases of AZA-induced shock have been reported previously.[3-14] AZA-induced shock is characterised by an unpredictable clinical course with a potentially fatal outcome. The 6-mercaptopurine component has been suggested to be responsible for the induction of toxic side effects, while the imidazole component more likely underlies the hypersensitivity reaction.[3,5-7,14,15]
Here, we present a patient in whom AZA induced a circulatory collapse with an atypical clinical presentation, probably triggered by patient's own rechallenge with this drug.

CASE REPORT

A 46-year-old Caucasian man with a two-year history of undifferentiated erosive oligoarthritis presented with a 72-hour history of persistent fever up to 40°C, nausea and vomiting. In the past, he had suffered from recurrent episodes of arthritis, localised in both ankles and in the left knee and had been treated with different courses of disease-modifying antirheumatic drugs including high-dose prednisone, methotrexate and salazopyrine without sufficient response. At presentation, he was on prednisone 15 mg/day orally. He had no known drug allergies. The fever was abrupt in onset and not accompanied by chills. Two weeks earlier, AZA had been introduced at a dose of 50 mg/day and then increased to 100 mg /day three days before presentation. After the first two doses of 100 mg, he began to feel unwell. He developed severe nausea and vomiting and stopped taking the AZA. However, one day before hospitalisation he rechallenged himself by taking

AZA again. Within a few hours he developed high fever without rigors, accompanied by general malaise, arthralgia, myalgia and nausea. At presentation, he was not acutely ill, had a regular pulse rate of 146 beats/min and a blood pressure of 160/85 mmHg. No signs of active arthritis were detected, nor was a rash observed. The remainder of the physical examination was unremarkable. Laboratory tests showed a C-reactive protein of 210 mg/l and a white blood cell count of 6.6 x 10^9/ml with 0.02 x 10^9/ml eosinophils. There were no signs of renal dysfunction or liver enzyme abnormalities. Serum amylase was normal. Urine examination did not show white blood cells or micro-organisms. Chest X-ray revealed a normal cardiac silhouette without infiltrate(s). The patient was admitted under the clinical diagnosis of drug fever due to AZA, which was immediately stopped. Within twelve hours, the patient's clinical condition and haemodynamic status changed dramatically. He developed dizziness, nausea and vague upper abdominal pain, located in the epigastric and right upper quadrants. Quite suddenly, a profound hypotensive shock with a decrease in the blood pressure to 80/50 mmHg occurred. Respiratory function was normal and pulmonary examination revealed no crackles or expiratory wheezes. No jugular venous distension was noted. Abdominal examination was normal without signs of peritonitis. At that moment, liver enzymes were elevated: aspartate aminotransferase 384 U/l, alanine aminotransferase 232 U/l and total bilirubin 21 umol/l, without a rise in serum amylase or lipase. No leucocytosis and no signs of haemorrhage were observed. Abdominal ultrasound showed gallbladder wall thickness and some pericholecystic fluid but no gallstones. The pancreas and common bile duct looked normal. No dilation of intra- and extra-hepatic bile ducts was seen.

Patient's clinical condition was then ascribed to an acute cholecystitis complicated by sepsis. Fluid resuscitation and antibiotic treatment consisting of amoxicillin-clavulanic acid and gentamycin, and supplemental intravenous hydrocortisone (3 x 50 mg) did not lead to a prompt clinical improvement. A percutaneous cholecystectomy was performed, but the gallbladder appeared not to be inflamed, perforated or gangrenous. In spite of this, within 24 hours of admission, the patient's haemodynamic status stabilised. When all blood, urine, throat and gall cultures appeared to remain sterile, the antibiotics were stopped. Within two days, the patient recovered completely. Retrospectively, this patient seemed to have suffered from AZA-induced hypersensitivity shock, probably triggered by his own rechallenge.

DISCUSSION

An uncommon but potentially fatal side effect of AZA treatment is the onset of a hypersensitivity reaction that can lead to shock with involvement of multiple organ systems. However, this occurs in only a minority of the patients receiving this drug. A search in the Medline database for publications back to 1980 revealed fourteen case reports referring to AZA-induced shock.[4-14] Detailed description of these patients is given in *table 1*. Severe hypotension requiring inotropic support and intravascular volume suppletion was described in almost all these cases.[4-6,8,9,14] Onset of symptoms was unpredictable, starting from one day to two months after institution of therapy. Rechallenge led to an acute response with occurrence of fever, nausea, and hypotension within hours.[6-14] As in our case, more indolent vague abdominal symptoms were also reported as the presenting symptoms of such episodes. Except for the use of AZA, no other cause for the circulatory shock was found in our patient. The diagnosis of AZA-induced shock in this case is supported by a close relationship in time between the onset of symptoms and institution of therapy as well as patient's own rechallenge.

Neither AZA, 6-MP nor 6-TG has intrinsic activity; they must undergo extensive metabolism to ultimately produce the 6-thioguaninenucleotides (6-TGNs), which are structurally similar to purine bases and in this way interfere with *de novo* synthesis of proteins and nucleic acids. Two key enzymes seem to be important in this regard: thiopurine (*S*)-methyltransferase (TPMT) and the dephosphorylating enzyme inosine triphosphate pyrophosphatase (ITPase). TPMT has been shown to exhibit genetic polymorphism with three known variant alleles highly associated with defective phenotype of TPMT in Caucasians. Low TPMT status results in overproduction of 6-TGNs and hence probable overdosing, and high TPMT status results in overproduction of 6-methylmercaptopurine and hence likely hepatotoxicity. An impaired activity of ITPase may lead to pancreatic toxicity, rash, neutropenia and gastrointestinal symptoms probably due to the accumulation of a metabolite, 6-thioinosine triphosphate. It has been hypothesised that 6-TG is less intensively metabolised by TPMT and that ITPase is probably not involved in its metabolism. Therefore, 6-thioguanine (6-TG), an agent leading more directly to the formation of 6-TGNs, may be an alternative in AZA or 6-MP intolerance. Theoretically, 6-TG may therefore be beneficial in patients with high TPMT status or in patients with impaired ITPase activity. Although the benefit of performing TPMT status measurement is still a matter of debate, some recommendations have been made. If a patient has a high TPMT status, then administration of full doses of thiopurines from the start is possible. Patients with normal TPMT activity may receive 2 to 2.5 or 1 to 1.5 mg/kg per day of AZA or 6-MP, respectively. Patients with intermediate TPMT activity should have an empiric dose reduction of 50%, while patients with low TPMT activity should only be treated with great caution and at very low

Table 1. *Well-described case reports with azathioprine-induced shock back to 1980*

Reference	Subject sex/age	Disease	Daily treatment dose (mg/day)	Clinical presentation	Time to onset of symptoms (days)	Azathioprine-induced shock after rechallenge
3	♂ 40	PA	100	Hypotension, fever, rash, diarrhoea	16	No rechallenge
4	♂ 49	AIH	50	Hypotension nausea, vomiting, liver enzyme abnormalities	14	No rechallenge
5	♀ 17	LCV	100	Fever	15	Yes
6	♀ 68	BP	150	Nausea, vomiting, diarrhoea	21	Yes
6	♀ 62	PN	50	Nausea	21	Yes
7	♂ 31	MS	150	Fever, rash	14	Yes
8	♀ 45	RA	100	Hypotension, fever, vomiting, liver enzyme abnormalities	6	Yes
9	♀ 30	SS	100	Fever	7	Yes
10	♀ 50	RA	50	Hypotension, fever	14	Yes
10	♀ 51	RA	75	Hypotension, vomiting	56	Yes
11	♂ 27	SLE	50	Nausea, vomiting	NR	Yes
12	♂ 56	PA	75	Fever	7	Yes
12	♂ 62	RA	75	Fever	8	Yes
13	♀ 32	MCTD	50	Hypotension, fever, chills, diarrhoea, liver enzyme abnormalities	14	Yes
Our case	♂ 46	EOA	100	Nausea, vomiting	14	Yes

PA = psoriatic arthritis; AIH = autoimmune hepatitis; LCV = leucocytoclastic vasculitis; BP = bullous pemphigoid; PN = polyarteritis nodosa; MS = multiple sclerosis; RA = rheumatoid arthritis; SS = systemic sclerosis, MCTD = mixed connective tissue disorder; EOA = erosive oligoarthritis; NR = not reported.

doses initially, approximately 10% of the standard dose.[2,16] We attempted to determine the TPMT status in our patient. Since this patient was switched to therapy with leflunomide with a good response, he refused to undergo TPMT status measurement.

The diagnosis of hypersensitivity reaction caused by AZA can usually only be made retrospectively since other possible causes of hypotension, such as sepsis or haemorrhage, should be excluded first. AZA-induced shock should be considered in the diagnostic work-up of unexplained circulatory collapse. Considering the rarity of this complication, a high clinical suspicion is needed to establish this diagnosis.

REFERENCES

1. Sanderson J, Ansari A, Marinaki T, Duley J. Thiopurine methyltransferase: should it be measured before commencing thiopurine drug therapy? Ann Clin Biochem 2004;41:294-302.
2. Al Hadithy AF, de Boer NK, Derijks LJ, Escher JC, Mulder CJ, Brouwers JR. Thiopurines in inflammatory bowel disease: pharmacogenetics, therapeutic drug monitoring and clinical recommendations. Dig Liver Dis 2005;37:282-97.
3. Sinico RA, Sabadini E, Borlandelli S, Cosci P, Di Toma L, Imbasciati E. Azathioprine hypersensitivity: report of two cases and review of the literature. J Nephrol 2003;16:272-6.
4. Fields CL, Robinson JW, Roy TM, Ossorio MA, Byrd RP Jr. Hypersensitivity reaction to azathioprine. South Med J 1998;91:471-4.
5. Brown G, Boldt C, Webb JG, Halperin L. Azathioprine-induced multisystem organ failure and cardiogenic shock. Pharmacotherapy 1997;17:815-8.
6. Knowles SR, Gupta AK, Shear NH, Sauder D. Azathioprine hypersensitivity-like reactions--a case report and a review of the literature. Clin Exp Dermatol 1995;20:353-6.
7. Jones JJ, Ashworth J. Azathioprine-induced shock in dermatology patients. J Am Acad Dermatol 1993:29:795-6.
8. Rosenthal E. Azathioprine shock. Postgrad Med J 1986;62:677-8.
9. Major GA, Moore PG. Profound circulatory collapse due to azathioprine. J R Soc Med 1985;78:1052-3.
10. Zaltzman M, Kallenbach J, Shapiro T, et al. Life-threatening hypotension associated with azathioprine therapy. A case report. S Afr Med J 1984;65:306.
11. Cunningham T, Barraclough D, Muirden K. Azathioprine-induced shock. Br Med J (Clin Res Ed) 1981;283:823-4.
12. Trotta F, Menegale G, Fiocchi O. Azathioprine-induced hypotension with oliguria. Arthritis Rheum 1982;25:1388-9.
13. Keystone EC, Schabas R. Hypotension with oliguria: a side-effect of azathioprine. Arthritis Rheum 1981;24:1453-4.
14. Sofat N, Houghton J, McHale J, Higgens CS. Azathioprine hypersensitivity. Ann Rheum Dis 2001;60:719-20.
15. Garey KW, Streetman DS, Rainish MC. Azathioprine hypersensitivity reaction in a patient with ulcerative colitis. Ann Pharmacother 1998;32:425-8.
16. Derijks LJ, Gilissen LP, Engels LG, et al. Pharmacokinetics of 6-mercaptopurine in patients with inflammatory bowel disease: implications for therapy. Ther Drug Monit 2004;26:311-8.

Netherlands
The Journal of Medicine

CASE REPORT

Successful treatment of liposomal amphotericin B refractory *Candida glabrata* fungaemia in a patient undergoing a stem cell transplantation

B.A.J. Veldman[1*], P.E. Verweij[2,4], N.M.A. Blijlevens[3,4]

Departments of [1]Internal Medicine, [2]Medical Microbiology and [3]Haematology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, [4]Nijmegen University Centre for Infectious Diseases, Nijmegen, the Netherlands, [*]corresponding author: e-mail: b.veldman@aig.umcn.nl

ABSTRACT

Blood stream infections caused by *Candida glabrata* are difficult to manage. We describe a patient who underwent an allogeneic peripheral stem cell transplantation for acute myeloid leukaemia. The patient developed *C. glabrata* fungaemia that was refractory to liposomal amphotericin B therapy. After changing the therapy to caspofungin, blood cultures became sterile within two days and the patient recovered clinically. The patient died shortly after due to graft-versus-host disease and at autopsy there was no evidence of residual or persistent *Candida* infection. Caspofungin was effective in liposomal amphotericin-B refractory *C. glabrata* fungaemia and proved to rapidly clear the infection. Treatment options for candidaemia are discussed.

KEYWORDS

Candida glabrata, candidaemia, caspofungin, fungaemia

Stem cell transplant (SCT) recipients are at increased risk of developing opportunistic infections. Although the risk for fungaemia is low because prophylaxis is usually used with fluconazole, invasive infections due to *Candida glabrata* remain difficult to manage. *C. glabrata* is becoming increasingly prevalent in immunocompromised patients, and causes significant morbidity and mortality.[1,2] We report a patient with acute myeloid leukaemia who developed a fungaemia with *C. glabrata*, refractory to treatment with liposomal amphotericin B.

CASE REPORT

A 60-year-old female, with acute myeloid leukaemia in complete remission, was admitted for scheduled allogeneic peripheral SCT, following a preparative myeloablative regimen consisting of idarubicin, busulphan and cyclophosphamide. A subclavian catheter was inserted uneventfully on the day of admission. She received ciprofloxacin and valaciclovir as antimicrobial prophylaxis. The patient was colonised with *C. glabrata*, as cultures from the oral cavity as well as faeces were repeatedly positive, but no antifungal prophylaxis was initiated. During prior chemotherapy, she had not received antifungal therapy.

After the conditioning regimen, it was suspected that she had developed enterocolitis or typhlitis as she had continuous, but not voluminous, diarrhoea. Cultures of the stools remained positive for *C. glabrata*.

Three days before the SCT the patient developed fever up to 39.3°C On physical examination she was haemodynamically stable, had grade II mucositis of the mouth, normal breathing sounds and no cardiac murmur.[3] The abdomen revealed no abnormalities. At the extremities there were no special findings, especially no petechiae. Her prophylactic antibiotic therapy (ciprofloxacin) was discontinued and ceftazidime was empirically initiated because of febrile neutropenia as blood cultures remained sterile.

Eight days after SCT her peripheral blood showed signs of bone marrow repopulation and on day 11 her leucocyte count was above 0.5 x 10^9/l. At that time she had persistent fever, oral mucositis but no diarrhoea. She had peripheral oedema and was tachypnoeic. Her oxygen saturation was 92%. Because of the lack of clinical improvement and persistent mucositis with colonisation of *C. glabrata*,

fluconazole 800 mg once daily was started on the ninth day after SCT pre-emptively. Three blood cultures, taken eight days after transplantation, became positive for *C. glabrata* on day 15. Twelve prior blood cultures were sterile.
The central venous catheter was removed and antifungal therapy was switched to liposomal amphotericin B (Ambisome, 3 mg/kg/day). Despite liposomal amphotericin B treatment for twelve days, six out of ten blood cultures remained positive for *C. glabrata*.
As the patient's clinical condition worsened, liposomal amphotericin B was discontinued and intravenous caspofungin was initiated: 70 mg loading dose and 50 mg once daily maintenance dose. Extensive imaging, including magnetic resonance imaging of the brain, ultrasound, echocardiography and computed tomography scanning, revealed no localisation of *C. glabrata*. From two days after starting caspofungin, blood cultures remained sterile. Positive cultures from faeces and the oral cavity indicated persisting colonisation with *C. glabrata*.
After an initial improvement 20 days after transplantation, the patient developed symptoms of severe generalised graft-versus-host disease (GvHD) of the skin, liver and lungs resulting in respiratory insufficiency. On day 36 she died of respiratory insufficiency. At autopsy pulmonary GvHD was found, which was determined to be the cause of death. Blood cultures and cultures taken from internal organs were sterile and histology showed no evidence of invasive candidiasis.

DISCUSSION

Despite the relative increase in blood stream infections with nonalbicans *Candida* species, colonisation with *C. glabrata* rarely leads to invasive infection in haematology patients. If invasion occurs, this is mostly due to one of the many factors that are associated with increased risk of development of invasive infections in general.[4] The damage to the epithelium of the intestinal tract (mucosal barrier injury),[5] due to cytotoxic therapy and total body irradiation, predisposes to translocation of gut micro flora to the blood.[4] *Candida* species adhere avidly to synthetic catheters, making intravenous catheters another source of (persistent) fungaemia. In these cases removal of the intravascular catheters eliminates the source of *Candida*. But whether fungaemia originates from skin/catheter colonisation or from gastrointestinal colonisation has been subject of considerable debate.[6] A recent review by Nucci and Anaissie suggests a central role for the gut as the primary source of *Candida*.[6] In another study a clear-cut sequence of colonisation from stools followed by skin was observed, indicating the gut as the primary source of *Candida* colonisation. Nucci and Colombo also failed to find an association between presence of a central venous catheter and the occurrence of fungaemia.[7] Identification of the source is essential for implementation of preventive strategies. Finally, the depth and duration of neutropenia correlates well with the frequency of fungaemia, indicating a central role for neutrophils in the host defence against disseminated candidiasis.[8] But also in non-neutropenic patients, persistence of candidaemia occurs frequently.[9,10] In our patient, persistent intestinal colonisation with *C. glabrata* together with an impaired mucosal barrier after myeloablative therapy, probably predisposed to the development of fungaemia.

C. glabrata often has increased minimum inhibitory concentrations (MIC) of many azoles compared with other *Candida* species, such as *C. albicans*. In a recent survey, up to 8% of over 1400 *C. glabrata* cultures proved resistant to fluconazole.[11,12] In addition, *C. glabrata* has 4 to 40 times higher MIC values of amphotericin B compared with *C. albicans*.[13] Besides, in *in-vitro* studies Canton *et al.* have shown that the minimum fungicidal concentration (MFC) of amphotericin B against various *Candida* species can be substantially higher than their MICs.[13]

Ostrosky-Zeichner *et al.* investigated the effect of voriconazole in over 50 patients with candidaemia or invasive candidiasis, not responding to prior antifungal therapy.[14] Treatment with voriconazole resulted in an overall response rate for all *Candida* species of 56% and for *C. glabrata* of 38%. Patients in whom previous azole therapy failed had a response rate of 58%. Therefore voriconazole can be used as salvage therapy in candidaemia.

In the present case, *C. glabrata* fungaemia persisted despite treatment with liposomal amphotericin B. Therefore the treatment was changed to caspofungin, a relatively new antifungal drug belonging to the class of echinocandins.[15] Caspofungin is thought to exert antifungal activity by blocking cell wall synthesis by inhibition of the synthesis of 1,3-β-D-glucan, which is essential for structural integrity and osmotic stability of the yeast.[15,16] Since the target is the cell wall, which is absent in human eukaryotic cells, the drug has few side effects.
Several clinical trials comparing caspofungin with amphotericin B in the treatment of invasive fungaemia have recently been performed. Mora-Duarte *et al.* found that caspofungin was as effective as amphotericin B in treating invasive fungaemia, and that treatment with caspofungin resulted in significantly less side effects.[10]

In non-neutropenic patients as well as neutropenic patients, the first choice of treatment of candidaemia is intravenous fluconazole.[17] This does not account for blood stream infections with fluconazole-resistant micro-organisms such as *C. krusei* or organisms with a reduced

susceptibility to fluconazole such as *C. glabrata*. Then treatment with caspofungin, voriconazole or possibly liposomal amphotericin B should be considered.

Although our patient had persistent fungaemia, blood and tissue cultures remained sterile after switching antifungal therapy to caspofungin. She eventually died from pulmonary insufficiency and at autopsy histological examination and cultures indicated full recovery from the fungaemia. Therefore her death does not seem to be attributable to the fungaemia.

CONCLUSION

This case shows that in haematology patients with an impaired mucosal barrier, the gut is an important source of fungaemia. The change of antifungal therapy to caspofungin resulted in eradication of the yeast from the blood and tissues. With the availability of the echinocandins and the new azoles, in our view there is no place for (liposomal) amphotericin B in the initial treatment of candidaemia.

REFERENCES

1. Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different Candida species. Clin Infect Dis 1997;24(6):1122-8.
2. Bodey GP, Mardani M, Hanna HA, et al. The epidemiology of Candida glabrata and Candida albicans fungemia in immunocompromised patients with cancer. Am J Med 2002;112(5):380-5.
3. Donnelly JP, Muus P, Schattenberg A, de Witte T, Horrevorts A, de Pauw BE. A scheme for daily monitoring of oral mucositis in allogeneic BMT recipients. Bone Marrow Transplant 1992;9(6):409-13.
4. Ellis M. Preventing microbial translocation in haematological malignancy. Br J Haematol 2004;125(3):282-93.
5. Blijlevens NM, Donnelly JP, de Pauw BE. Mucosal barrier injury: biology, pathology, clinical counterparts and consequences of intensive treatment for haematological malignancy: an overview. Bone Marrow Transplant 2000;25(12):1269-78.
6. Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? Clin Infect Dis 2001;33(12):1959-67.
7. Nucci M, Colombo AL. Risk factors for breakthrough candidemia. Eur J Clin Microbiol Infect Dis 2002;21(3):209-11.
8. Wingard JR. Importance of Candida species other than C. albicans as pathogens in oncology patients. Clin Infect Dis 1995;20(1):115-25.
9. Rex JH, Bennett JE, Sugar AM, et al, Candidemia Study Group and the National Institute. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. N Engl J Med 1994;331(20):1325-30.
10. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med 2002;347(25):2020-9.
11. Pfaller MA, Diekema DJ, Messer SA, Hollis RJ, Jones RN. In vitro activities of caspofungin compared with those of fluconazole and itraconazole against 3,959 clinical isolates of Candida spp., including 157 fluconazole-resistant isolates. Antimicrob Agents Chemother 2003;47(3):1068-71.
12. Pfaller MA, Messer SA, Boyken L, et al. Caspofungin activity against clinical isolates of fluconazole-resistant Candida. J Clin Microbiol 2003;41(12):5729-31.
13. Canton E, Peman J, Viudes A, Quindos G, Gobernado M, Espinel-Ingroff A. Minimum fungicidal concentrations of amphotericin B for bloodstream Candida species. Diagn Microbiol Infect Dis 2003;45(3):203-6.
14. Ostrosky-Zeichner L, Oude Lashof AM, Kullberg BJ, Rex JH. Voriconazole salvage treatment of invasive candidiasis. Eur J Clin Microbiol Infect Dis 2003;22(11):651-5.
15. Denning DW. Echinocandins: a new class of antifungal. J Antimicrob Chemother 2002;49(6):889-91.
16. Bartizal K, Gill CJ, Abruzzo GK, et al. In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). Antimicrob Agents Chemother 1997;41(11):2326-32.
17. Wout JW, Kuijper EJ, Verweij PE, Kullberg BJ. Nieuwe ontwikkelingen in de antifungale therapie: fluconazole, itraconazole, voriconazole, caspofungin. Ned Tijdschr Geneeskd 2004;148(34):1679-84.

Netherlands
The Journal of Medicine

PHOTO QUIZ

A patient with swollen ears and ECG abnormalities

H.W.M. van Laarhoven[1*], M.J. Veerkamp[2], M. Pruszczynski[3], M. van Deuren[1]

Departments of [1]Internal Medicine, [2]Rheumatology and [3]Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, [*]corresponding author: tel.: +31 (0)24-361 88 19, fax: +31 (0)24-354 17 34, e-mail: h.vanlaarhoven@onco.umcn.nl

KEYWORDS

Atrioventricular dissociation, polychondritis

CASE REPORT

A 30-year-old male presented to the Emergency Room complaining of feeling generally unwell with nausea and vomiting. He had been well until the morning of presentation, except for some symptoms of dyspnoea and a mild, stabbing pain in the left of his chest on deep inspiration. He had recently been investigated by the otolaryngologist because of hearing problems, and had been under the care of the rheumatologist for a year because of joint pain, red eyes and swollen painful red ears. He was taking non-steroidal anti-inflammatory drugs (NSAIDs) because of his joint pains and bronchodilatatory inhalation because of asthma. On examination he was afebrile and not in distress, blood pressure 110/60 mmHg, pulse rate irregular at 50 to 60 beats/min. On both ears the antehelix was nearly absent (*figure 1*). Further general physical examination revealed no abnormalities. D-dimers were <500 ng/ml and troponin-I <0.20 μg/l, CRP was <5 (all normal). The chest X-ray was normal and the ECG is shown in *figure 2*.

WHAT IS YOUR DIAGNOSIS?

See page 132 for the answer to this photo quiz.

Figure 1. *Disappearing antehelix*

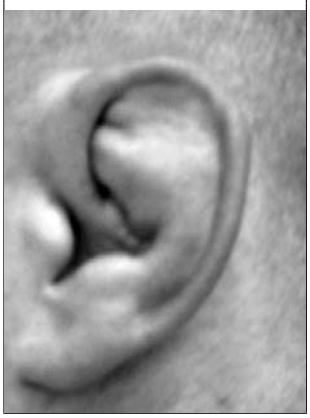
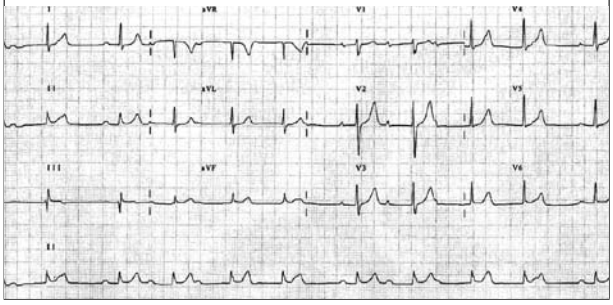


Figure 2. *ECG on admission*



LETTER TO THE EDITOR

Helicobacter pylori, obesity and gastro-oesophageal reflux disease: is there a relation?

In a recent issue of this Journal, Loffeld[1] concluded that there is a definite relation between body mass index (BMI) and the occurrence of gastro-oesophageal reflux disease (GERD). He also discussed the relation between obesity, GERD, and Helicobacter pylori. The exact relation and the consequences are not yet entirely clear.

As Loffeld describes, the most important pathophysiological mechanism causing reflux is long-lasting spontaneous relaxation of the lower oesophageal sphincter (LOS) or low pressure in the LOS. A hiatal hernia is an additional risk factor. Finally, increased intra-abdominal pressure plays an important role in the mechanism of reflux. Since these factors are generally accepted to be present in patients with obesity, these patients are expected to be at risk to develop GERD.

We studied the association between BMI and hiatal hernia or GERD in patients with morbid obesity. We retrospectively analysed the preoperative data of 198 morbidly obese patients (BMI >40 kg/m², or BMI >35 kg/m² in combination with relevant comorbidity) treated by gastric banding from March 1995 to December 2000. Data of the extensive preoperative protocol were analysed for BMI, symptoms of GERD, use of PPI or H2 blockers, and result of gastroscopy. Endoscopy was performed in 170 patients (157 females, 13 males; age 37 years, range 20 to 69 years; BMI 44.9 kg/m², range 35.6 to 60.9 kg/m²). GERD symptoms were reported in 50 patients (29.4%), eight of them were treated by PPI or H2 blockers. Hiatal hernias (HH) were seen in 81 patients (47.6%) and symptomatic in 30 (37.0%). Of patients without HH, 27.6% reported symptoms of GERD. Endoscopic signs of reflux oesophagitis were present in 61.7% of patients with HH, vs 12.4% in those without ($p<0.001$). BMI in patients with and without GERD symptoms was not different (44.9±5.2 kg/m² vs 45.0±5.8 kg/m²).

We concluded that in morbidly obese patients GERD symptoms occur independently of BMI, but are related to the presence of HH. Nevertheless, based on our findings, overweight can not be excluded as a risk factor for GERD, since we did not compare our morbidly obese population with the general population. However, it seems that being obese and getting more obese does not increase the risk of developing GERD. Treatment of GERD in morbidly obese patients is medical.

However, treatment of obesity and especially surgical treatment of morbid obesity is relevant. The number of patients with obesity is growing and will give rise to serious health problems, such as diabetes, hyperlipidaemia, hypertension, and obstructive sleep apnoea.[2-4] Recent follow-up studies have demonstrated that bariatric surgery resulted in long-term weight loss, and an improved lifestyle. Furthermore a substantial majority of patients with diabetes, hyperlipidaemia, hypertension, and obstructive sleep apnoea experienced complete resolution or improvement.[3-5]

R.M. Kiewiet, A.C.M. van Vliet*
Department of Internal Medicine, Albert Schweitzer Hospital, PO Box 444, 3300 AK Dordrecht, the Netherlands, tel.: +31 (0)78-654 11 11, fax: +31 (0)78-652 33 77, e-mail A.C.M.vanVliet@asz.nl, *corresponding author

REFERENCES

1. Loffeld RJ. Helicobacter pylori, obesity and gastro-oesophageal reflux disease. Is there a relation? A personal view. Neth J Med 2005;63(9):344-7.
2. Van Houwelingen KG, van Uffelen R, van Vliet ACM. The sleep apnoea syndromes. Review article. Eur Heart J 1999;20:858-66.
3. Buchwald H, Avidor Y, Braunwald E, et al. Bariatric surgery. A systematic review and meta-analysis. JAMA 2004;292:1724-37.
4. Sjöström L, Lindroos A-K, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683-93.
5. Pontiroli AE, Folli F, Paganelli P, et al. Laparoscopic gastric banding prevents type 2 diabetes and arterial hypertension and induces their remission in morbid obesity. A 4-year case-controlled study. Diabetes Care 2005;28:2703-9.

ANSWER TO PHOTO QUIZ (ON PAGE 130)

A PATIENT WITH SWOLLEN EARS AND ECG ABNORMALITIES

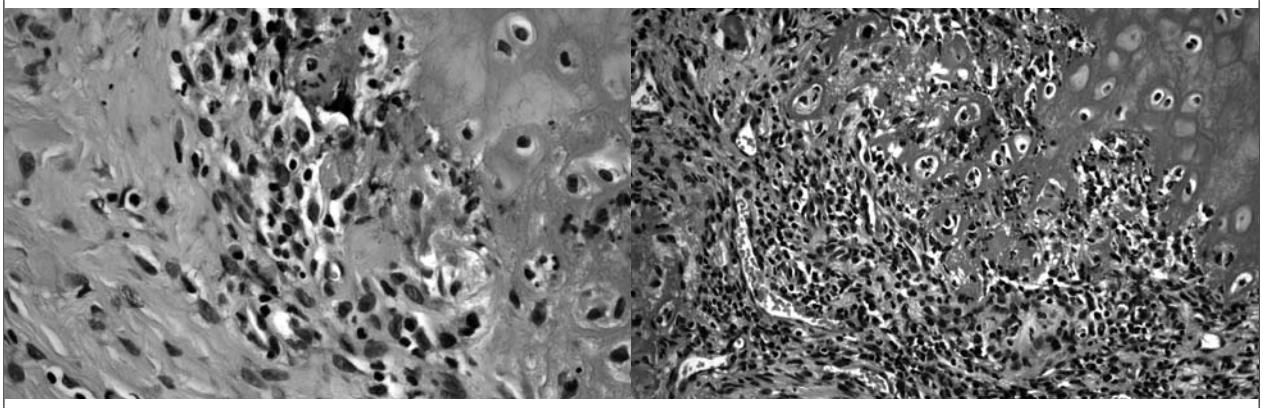
DIAGNOSIS

The patient presented with general malaise. Because of dyspnoea and chest pain, an ECG was performed. This showed a complete atrioventricular block. The abnormalities of the ears observed in this patient, together with a history of scleritis/uveitis, hearing problems and joint pain are compatible with the diagnosis relapsing polychondritis. This diagnosis had been confirmed a few months earlier, by a biopsy of the auricle that showed chronic perichondritis with resorption of cartilage and fibrosis (*figure 3*). Relapsing polychondritis is characterised by recurrent inflammation and destruction of the cartilage.[1] Subacute onset of pain, redness and swelling of the ear, due to auricular chondritis, is the most frequent presenting symptom.[2] In addition, inflammation of the eyes, nose and laryngotracheal cartilage may occur. Involvement of the external auditory canal or Eustachian tube or serous otitis media may result in conductive hearing loss. According to the original McAdam's criteria the presence of three or more of the following clinical features is required to define relapsing polychondritis:[3]

- Bilateral auricular chondritis.
- Nonerosive, seronegative inflammatory polyarthritis.
- Nasal chondritis.
- Ocular inflammation (conjunctivitis, keratitis, scleritis/episcleritis, uveitis).
- Chondritis of the respiratory tract.
- Cochlear and/or vestibular dysfunction (conductive hearing loss, tinnitus and/or vertigo).

A histologically compatible biopsy (ear, nose, respiratory tract) is recommended unless the diagnosis is clinically obvious. Cardiovascular manifestations are described in 24 to 52% of the patients,[4] heart valvular disease being the most common cardiovascular abnormality. However, in 4 to 6% of the patients a first to third degree atrioventricular block develops,[4] most probably due to fibrosis of the cardiac conducting system.[5] Steroids or other immunosuppressive drugs may be effective in acute inflammatory heart block, but in the long term a permanent pacemaker is often required.[4] Regular cardiological follow-up is mandatory in patients with relapsing polychondritis. The case shows that serious cardiac complications can develop, even when disease activity on other more easily recognised sites has disappeared.

Figure 3. *Microscopic picture of the excision from elastic auricular cartilage*



In the perichondrium a mixed inflammatory infiltrate is seen with lymphocytes, some neutrophilic and eosinophilic granulocytes. Cellular infiltrate penetrates through the perichondrium into the cartilage, which is eroded and displays loss of basophilia. Destroyed cartilage is replaced by connective tissue. The picture is consistent with perichondritis with extension of the inflammatory process into the cartilage. This lesion in combination with clinical signs and symptoms may lead to the diagnosis of relapsing polychondritis.

REFERENCES

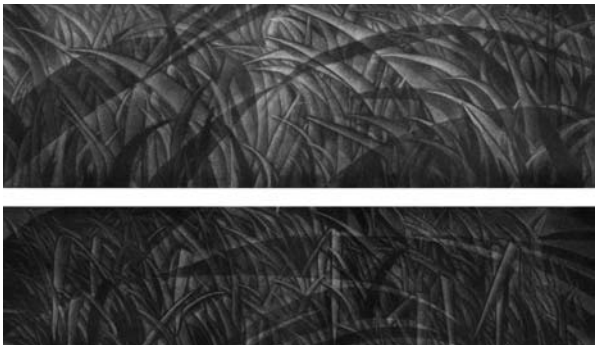
1. Kent PD, Michet CJ Jr, Luthra HS. Relapsing polychondritis. Curr Opin Rheumatol 2004;16:56-61.
2. Van Wermeskerken GK, van den Bergh WM, Frijns CJ. Diagnostic image (171). A man with a painful red ear. Relapsing polychondritis. Ned Tijdschr Geneeskd 2004;148:22.
3. McAdam LP, O'Hanlan MA, Bluestone R, Pearson CM. Relapsing polychondritis: prospective study of 23 patients and a review of the literature. Medicine (Baltimore) 1976;55:193-215.
4. Del Rosso A, Petix NR, Pratesi M, Bini A. Cardiovascular involvement in relapsing polychondritis. Semin Arthritis Rheum 1997;26:840-4.
5. Bowness P, Hawley IC, Morris T, Dearden A, Walport MJ. Complete heart block and severe aortic incompetence in relapsing polychondritis: clinicopathologic findings. Arthritis Rheum 1991;34:97-100.

Netherlands
The Journal of Medicine

ABOUT THE COVER

'Untitled'

Maja Ilic



Maja Ilic is a painter/engraver who uses mezzotint and dry needle techniques.
Maja explains her work as follows: *In my works, which I see as my children, I try to approach the soul and the infinity of nature and make it visual. I see nature and themes from nature as symbols that help me understand the modalities and infinities of my daily life. Most of my works have a Latin title. Latin is the basis of many languages, just as nature is the basis of our life. We are surrounded by nature and living in nature, but this nature and these surroundings are unpredictable and surprising (ex improvisio), and endless (ad infinitum), similar to the way someone exists (modalitas). I try to express my impressions of the world, but leave it to you to discover, in a little bit of colour in the shadow of my black and white mezzotint, your own picture of this world and this life. I hope that you will be able to find your own never-ending existence, as an Alice in wonderland. That is the goal of my work.*
Maja, a well-known artist, has worked with many local and international organisations. Her work has been selected for many exhibitions in India, the former Yugoslavia, France, Spain, USA, UK and the Netherlands. She is the winner of many prices; her work was chosen as Peace Prize in Tilburg in 1992 and in 2002 her work was selected and published in the book 'The world art collection'.

An original of this print is available at a price of € 200 and can be ordered from Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: Galerie-unita@planet.nl or www.galerie-unita.com.

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.

Manuscripts
Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

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

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process, number the lines in the margin and the pages.
Subheadings should not exceed 55 characters, including spaces.
Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for generic names of substances and materials.
A *Covering letter* should accompany the manuscript, identifying the person (with the address, telephone number, fax number and e-mail address) responsible for negotiations concerning the manuscript. The letter should make it clear that the final manuscript has been seen and approved by all authors. Conflicts of interest, commercial affiliations, consultations, stock or equity interests should be specified. In the letter one to three sentences should be dedicated to what this study adds. All authors should sign the letter.

Divide the manuscript into the following sections: Title page, Abstract, Keywords, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

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

The *Abstract*, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: Background, Methods, Results and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

Keywords: Include three to five keywords.

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

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

The *Results* should be presented precisely, without discussion.

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

Acknowledgement: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.

References should be numbered consecutively as they appear in the text (after the punctuation and in square brackets). Type the reference list with double spacing on a separate sheet. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med 1991;324:424-8).

Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

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

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

The use of bibliographic software programmes that are designed to generate reference lists such as Reference Manager© or Endnote© is highly encouraged. Authors can use the predefined output 'Vancouver' style from these programmes.

Tables should be typed with double spacing each on a separate sheet, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction. Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Indian ink drawings or sharp, strongly contrasting photographic prints on glossy paper are also acceptable. Lettering should be complete, of professional quality, and of a size appropriate to that of the illustration of drawing, with the necessary reduction in size taken into account. Figures should be no larger than 12.5 x 18 cm. Submit half-tone illustrations as black-and-white prints on glossy paper, with as much contrast as possible. Identify each figure on the back with a typed label, which shows the number of the figure, the name of the leading author, the title of the manuscript and the top of the figure. Colour figures are occasionally possible and will be charged to the authors.

Legends for figures should be typed, with double spacing, on a separate sheet.

Case reports

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

Letters to the editor

The editorial board will consider letters to the editor referring to articles previously published in the Journal. Letters should be no more than 500 words.

Books for reviewing

The editorial board will consider review papers of books.

Submission

Starting February 2006 all submissions to the *Netherlands Journal of Medicine* should be submitted online through Manuscript Central at http://mc.manuscriptcentral.com/nethjmed. Authors should create an account and follow the instructions. If you are unable to submit through Manuscript Central contact the editorial office at g.derksen@aig.umcn.nl or tel.: +31 (0)24-361 04 59.

Reviewing process

After external and editorial review of the manuscript the authors will be informed about acceptance, rejection or revision. We require revision as stated in our letter.

Proofs

Proofs will be sent to the authors to be carefully checked for printer's errors. Changes or additions to the edited manuscript cannot be allowed at this stage. Corrected proofs should be returned to the editorial office within two days of receipt.

Offprints

These are not available. The first author receives a sample copy of the Journal with the published article.