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Editorial office 'The Netherlands Journal of Medicine'
Geeralien Derksen-Willemsen
Radboud University Nijmegen Medical Centre
Department of General Internal Medicine 541
PO Box 9101, 6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59, fax: +31 (0)24-354 17 34
E-mail: g.derksen@aig.umcn.nl

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EDITORIAL

Clinical guidelines to improve patient care

H. Wollersheim[1*], J. Burgers[2], R. Grol[1]

[1]Centre for Quality of Care Research, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, [2]Senior researcher, guidelines programme director, Dutch Institute for Quality Improvement in Health Care (CBO), Utrecht, the Netherlands, *corresponding author

ABSTRACT

The aim of clinical guidelines is to improve quality of care by translating new research findings into practice. There is evidence that the following characteristics contribute to their use: inclusion of specific recommendations, sufficient supporting evidence, a clear structure and an attractive lay out. In the process of formulating recommendations, implicit norms of the target users should be taken into account. Guidelines should be developed within a structured and coordinated programme by a credible central organisation. To promote their implementation, guidelines could be used as a template for local protocols, clinical pathways and interprofessional agreements.

INTRODUCTION

In this number of the journal, Jacobs *et al.* describe the use of a local clinical guideline on haemochromatosis.[1] They found the adherence of care providers to key recommendations insufficient and even consider certain elements of care provision undesirable. Naturally they asked themselves why this guideline failed to reach its goal. The aim of clinical guidelines is to improve patient care by providing recommendations about appropriate healthcare in specific clinical circumstances.[2] They should be based on the best evidence available, supplemented with clinical expertise and patient preferences. Guidelines are primarily developed to support care providers and patients, but may also be used by medical insurers in contracts and by governmental agencies in rationing healthcare policy.[3]

Guidelines are only one option for improving quality. They are especially useful in situations with uncertainty about appropriate practice, when evidence provides an answer.[4] In other situations integrated care pathways or the redesign of care processes may be more suitable. While guidelines can improve the quality of patient care, we will discuss how, and which limitations occur.[5]

BENEFITS

Clinical guidelines may improve patient care by providing easily accessible information regarding optimal care. They summarise research findings and make clinical decisions more transparent. By showing gaps in current knowledge, research activities can be prioritised. Ideally, the potential cost implications of applying the recommendations are discussed. Thereby they can increase the efficiency of care and in case of inappropriate use, reduce costs.[6]
By summarising the benefits and limitations of procedures and interventions, they contribute to patient safety. To empower patients, lay versions should inform patients about optimal care.[7] As clinical guideline development includes a systematic review of the recent scientific literature, an up-to-date guideline offers a sound basis for education. In contrast, textbooks contain material that is too general and often out of date.[8,9] As many guidelines cover topics that involve different disciplines they provide a foundation for multiprofessional agreements and collaboration.
Guidelines can be used as a reference for professional audit to evaluate the quality of care.

LIMITATIONS

If guidelines are applied inappropriately as in 'cookbook medicine', they may lead to misuse.
As a hypothetical standard patient is usually taken as a point of reference, the unique clinical presentation could be neglected. By doing so guidelines oversimplify complex clinical practice.[10] Inexperienced users could be encouraged to apply recommendations unthinkingly, even in situations in which departure from the recommendations is desirable.[11] Guidelines are produced on the basis of studies in selected populations in research settings. As a consequence their results often can not be reproduced in daily practice.[12] Because the development of a national guideline demands large resources,[13] their cost-effectiveness is sometimes questioned, despite positive examples.[14] In general, professionals strive for autonomy, which is threatened by the need to follow guidelines.[15] Accordingly, professionals fear an increase in their medico-legal exposure.[11]

DEVELOPMENT OF GUIDELINES

The implementation should be considered part of the development process. Selection of topics, composition of the guideline group, the work plan, search for evidence and involvement of clinical experts are all important in this. On the national level a representative and respected group of experts from relevant professional organisations reaches agreement on an area of healthcare. Consensus takes place on the basis of a systematic review and structured consensus. If there are marked differences between settings, translation to the local situation is mandatory. Following the instructive process with the focus on relevant local conditions is a major advantage for acceptance. A disadvantage is the time investment and the suboptimal results if a systematic review has not been performed.[16]

Topic selection

Of importance is the topic selection. The more relevant the topic for resolving the problems encountered, the more likely the guideline will be accepted. Some problems cannot be resolved by introducing guidelines,[17,18] as for example shortage or incorrect use of resources, malpractice resulting from inefficient procedures or topics related to patient preferences. Appropriate topics can be selected by the relevance and prevalence of the problem, controversy about optimal care, existence of proven solutions, barriers expected when implementing improvements and motivation and improvement skills of the care providers involved. Besides scientific also psychosocial, ethical, legal and financial aspects play a role in implementing guidelines. A systematic analysis prior to guideline development contributes to its successful application.[19]

Composition of the guideline group

Developing credible clinical guidelines requires a balanced working group including clinical and methodological expertise to promote broad consensus and to prevent bias from conflicts of interests.[19] It should also include representatives of patient groups.[17] Adequate staff support is needed to perform literature searches and a cost-effectiveness analysis.[20] A neutral chairman and formal group processes should be used to achieve consensus.

Work plan

Next, a work plan is formulated that describes the aims of the guideline, healthcare problems and settings covered, desired outcomes (mortality, morbidity, complications, hospital admissions, quality of life), target group involved (care providers and patient population), time schedule and division of the tasks.

Reviewing evidence

The literature search starts by identifying and reviewing existing guidelines and a systematic literature review, searching for scientific evidence, an assessment of its relevance and quality, and the involvement of clinical experts to formulate and prioritise recommendations. Guidelines on the same topic can be identified by searching the US National Guideline Clearinghouse (www.guideline.gov) and the resources of the Guidelines International Network (www.g-i-n.net). These databases together contain more than 2000 guidelines. To assess the quality of the guideline the 'Appraisal – Instrument for Guidelines Research and Evaluation' (AGREE) instrument can be used.[21] Its purpose is to provide a systematic framework for assessing key components of guideline quality including the process of development and reporting. The items cover the methodology as well as the clarity and applicability of the guideline.
Studies are best identified by systematic review. To identify high-quality systematic reviews the Cochrane Library with quarterly updates is an excellent source. If no existing review can be found a range of electronic databases (Medline, Cinahl, Embase, PubMed) should be searched. Further relevant individual studies are identified by asking experts and by hand-searching journals, reference lists of articles and abstract books. The relevance of the studies for the questions and patient group involved is evaluated on the basis of the abstract.[22] The next step is to evaluate the scientific strength of the published research.[23] Information about the advantages, disadvantages and costs of the studied interventions is examined. The evidence is categorised using predefined grading schemes for preventive, diagnostic, and therapeutic procedures. In *table 1* such a grading system is shown, as developed by the Dutch Institute for Quality Improvement in Healthcare (CBO).

Table 1 *Classification of the literature according to the strength of the evidence (CBO 2000)*

For articles concerning intervention (prevention or therapy)

A1 Systematic reviews of at least a few studies on the A2 level, of which the results of independent research studies are consistent

A2 Randomised comparative clinical research of good quality (randomised, double-blind, controlled trial of adequate scope and consistency)

B Randomised clinical trials of moderate quality or insufficient scope, or other comparative research (nonrandomised, cohort studies, patient-control studies)

C Noncomparative research

D Opinions of experts, such as the work group members

Involvement of clinical experts

Clinical experts should be involved because in almost half the clinical decisions there is no good scientific background.[24] When developing a clinical guideline for angina pectoris, only 21% of the recommendations could be based on randomised studies.[25] Even when there is consistent evidence for a given clinical practice, the optimal method of proceeding is seldom immediately clear. If evidence is found for certain care interventions, it is often necessary to determine whether the results can be extrapolated to other patient populations.

On the other hand, the use of experts causes problems. Some dominate the discussion with their individual preferences. By structuring the discussions, such problems can be avoided.[26]

If no evidence can be found an interview of experienced care providers can be performed as in the Rand-modified Delphi Procedure to quantitate 'expert opinion'.[27] A panel of experts judges the appropriateness of different treatments in a number of characteristic patients. The judgement of the appropriateness is determined by considering the advantages (effectiveness, rapidity and duration of the response) and disadvantages (invasiveness, side effects, complications) which are scored.

FORMULATION OF RECOMMENDATIONS

In formulating recommendations the scientific evidence and clinical expertise are brought together. The following issues should be considered to ensure implementation:

- Nature and strength of the scientific evidence; the balance between the advantages of a given intervention and its disadvantages.
- Generalisability and applicability to the target population.
- Cost-effectiveness of the proposed intervention.
- Achievability of the intervention in terms of required skills, instruments, time, available staff, patient's preferences and legal or financial limitations.
- Opinions, norms and values, and ethical considerations of the target users.

With a view to implementation, a work group cannot avoid the problem as to whether the healthcare system can afford the innovation. If a guideline recommends that a patient with a myocardial infarction must receive thrombolysis within 30 minutes of arrival at the hospital, then the entire care process must be directed to that aim.

In the interpretation of evidence by experts, normative and cultural opinions about the desired health benefit and the acceptable risks play a role. An analysis of guidelines for breast cancer revealed that in the USA regular breast self-examination is advised, while the French point to the insecurity that this can evoke.[28]

Levels of evidence

By including the level of evidence for each recommendation, the work group emphasises the degree to which application of the recommendations will lead to the intended results.[29] The addition of the results of reviews and the level of evidence creates a sense of thrust worthiness and makes the recommendations transparent, with a positive influence on the application in practice.

Ideally, all recommendations are formulated using a democratic voting procedure in which all relevant information (evidence, costs, preferences, organisational impact) has been considered.

An external review by a sample of concerned individuals (experts, patients, managers, insurers) should be part of the development process.

Promoting acceptability

To promote support, the draft has to be presented at an open meeting allowing the audience to express their comments and suggestions. If no consensus is reached, a voting system can be used.

To facilitate its applicability in daily care the guideline is piloted in practice. The results of the pilot and the consultation process are incorporated. Finally, the clinical guideline can be submitted for approval to an independent scientific council and to the professional organisations responsible.[30]

FORMAT OF THE GUIDELINE

The next step is designing an accessible and attractive format. Diagrams and algorithms may clarify the logic in the decision-making.[31] A summary of key recommendations provides a quick insight.

Clinical guidelines should be published in professional journals and posted to every possible user.

Electronic versions of the guideline and tools for application (e.g. patient leaflets, educational material, a practice summary on a plastic-laminated card) have to be developed.[32] For audit and performance review the guideline should include a set of clinical indicators.

EVALUATION OF THE GUIDELINE

The final step is the overall process of the evaluation of their application, their applicability and their effects. Relevant elements are:

- How well is the guideline known and applied? Are the recommendations understood and remembered? Are they used in quality improvement activities? If not, which are not and why?
- To what extent are they effective? Does their application lead to the objectives envisioned (better health, lower costs, better quality of life and more satisfied patients)?

UPDATING GUIDELINES

Clinical guidelines require updating if the majority of recommendations are out-of-date due to changes in research findings and new available diagnostic or therapeutic interventions. In general, guidelines should be reassessed for validity every three years.[33] In rapidly evolving fields, for example AIDS or colonic cancer, yearly review is necessary.

GUIDELINE QUALITY

High-quality guidelines can improve healthcare, but low-quality guidelines may harm patients.[5,8] The explosion of published guidelines may confront physicians with multiple conflicting guidelines on the same clinical subjects.[34] Many are of poor quality. Grilli *et al.* evaluated 431 guidelines developed by medical specialists. Only 5% met high-quality criteria and 54% did not meet any.[35] Recently an international appraisal instrument to assess guideline quality has been developed and validated: the AGREE instrument (see www.agree.collaboration.org. for details).[21]

CONCLUSION

To successfully introduce clinical guidelines, their development should consider the implementation from the very beginning.[36] This includes attention to the relevance of the topic, credibility (systematic development by rigorous transparent methodology), involvement of all relevant stakeholders and attention to the impact on resources, materials and facilities, accessibility and an attractive design and tools for application and monitoring in practice. To integrate guidelines into normal care processes they should be incorporated in local care protocols, disease management programmes and clinical pathways.

REFERENCES

1. Jacobs E, Swinkels D. Impact of the introduction of a guideline on the targeted detection of hereditary hemochromatosis. Neth J Med 2005;63:205-14.
2. Field MJ, Lohr KN. Guidelines for clinical practice. From development to use. Institute of Medicine. Washington DC: National Academy Press, 1992.
3. Norheim OF. Healthcare rationing - are additional criteria needed for assessing evidence based clinical practice guidelines? BMJ 1999;319:1426-9.
4. Woolf SH, Grol R, Hutchinson A, et al. Potential benefits, limitations, and harms of clinical guidelines. BMJ 1999;8:527-30.
5. Grimshaw JM, Russell IT. Achieving health gain through clinical guidelines; I. Developing scientifically valid guidelines. Qual Health Care 1993;2:243-8.
6. Shapiro DW, Lasker RD, Bindman AB, et al. Containing costs while improving quality of care: the role of profiling and practice guidelines. Ann Rev Public Health 1993;14:219-41.
7. Elwyn G. Shared decision making. Patient involvement in clinical practice [Thesis]. Nijmegen: Nijmegen University, 2001.
8. Haynes B, Haines A. Barriers and bridges to evidence based clinical practice. BMJ 1998;317:273-6.
9. Stuyt PMJ, de Vries Robee PF, van der Meer JWM. Why don't medical textbooks teach? The lack of logic in the differential diagnosis. Neth J Med 2003;61:383-7.
10. Tonelli MR. The philosophical limits of evidence-based medicine. Acad Med 1998;73:1234-40.
11. Hurwitz B. Legal and political considerations of clinical practice guidelines. BMJ 1999;318:661-4.
12. Knottnerus J, Dinant GJ. Medicine based evidence, a prerequisite for evidence based medicine. BMJ 1997;315:1109-10.
13. Burgers JS, Cluzeau FA, Hanna SE, Hunt C, Grol R, and the AGREE Collaboration. Characteristics of high quality guidelines: evaluation of 86 clinical guidelines developed in ten European countries and Canada. Int J Techn Assess Health Care 2003;9:148-57.
14. Mason J, Freemantle N, Nazareth I, Eccles M, Haines A, Drummond M, and the Evidence Based OutReach (EBOR) Trialists. The economics of influencing the behaviour of health professionals: findings of the EBOR trial. JAMA 2001;286:2988-92.
15. Tunis SR, Hayward RSA, Wilson MC, et al. Internists attitudes about clinical practice guidelines. Ann Intern Med 1994;120:957-63.
16. Varonen H, Mäkelä M. Practice guidelines in Finland: availability and quality. Qual Health Care 1997;6:75-9.
17. NHMRC. A guide to the development, implementation and evaluation of clinical practice guidelines. Canberra: Commonwealth of Australia, 1999.

18. Fink A, Kosecoff J, Chassin M, et al. Consensus methods: characteristics and guidelines for use. Am J Public Health 1984;74:979-83.
19. Shekelle P, Woolf S, Eccles M, et al. Developing guidelines. BMJ 1999;318:593-6.
20. Eccles M, Mason J, Freemantle N. Developing valid cost effectiveness guidelines: a methodological report from the North of England evidence based guideline development project. Qual Health Care 2000;9:127-32.
21. The AGREE Collaboration. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines. Qual Safe Health Care 2003;12:18-23.
22. Hayward RSA, Wilson MC, Tunis SR, et al. More informative abstracts of articles describing clinical practice guidelines. Ann Int Med 1993;118:731-7.
23. Grimshaw J, McAuley LM, Bero LA, et al. Systematic review of the effectiveness of quality improvement strategies and programmes. Qual Saf Health Care 2003;12:298-303.
24. Buchan H. Gaps between best evidence and practice: causes for concern. MJA 2004;180:S48-9.
25. Eccles M, Clapp Z, Grimshaw J, et al. North of England evidence based guidelines development project: methods of guideline development. BMJ 1996;312:760-2.
26. Sudlow M, Thompson R. Clinical guidelines: quantity without quality. Qual Health Care 1997;6:60-1.
28. Kahn KL, Kosecoff J, Chassin MR, et al. Measuring the clinical appropriateness of the use of a procedure. Can we do it? Med Care 1988;26:415-22.
29. Eisinger F, Geller G, Burke W, et al. Cultural basis for differences between US and French clinical recommendations for women at increased risk of breast and ovarian cancer. Lancet 1999;353:919-20.
30. CBO. Richtlijnontwikkeling binnen het kwaliteitsinstituut voor de gezondheidszorg. Utrecht: CBO, 2000.
31. Burgers JS, van Everdingen JE. Beyond the evidence in clinical guidelines. Lancet 2004;364:392-3.
32. Cretin S. Implementing guidelines: an overview. RAND-report. 1998.
32. McAlearny AS, Schweikhart SB, Medow MA. Doctors experience with handheld computers in clinical practice. BMJ 2004;328:1162-5.
33. Shekelle P, Eccles MP, Grimshaw JM, Woolf SH. When should clinical guidelines be updated. BMJ 2001;323:155-7.
34. Van Gelder IC. Differences in guidelines for the diagnostic evaluation and treatment of atrial fibrillation. Neth Heart J 2004;12:321-2.
35. Grilli R, Magrini N, Penna A, et al. Practice guidelines developed by specialty societies: the need for a critical appraisal. Lancet 2000;355:103-6.
36. Grol R, Grimshaw J. From best evidence to best practice: effective implementation of change in patient care. Lancet 2003;362:1225-30.

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REVIEW

Pulmonary hypertension: its diagnosis and management, a multidisciplinary approach

M.C. Vonk[1*], A.P.J. van Dijk[2], Y.F. Heijdra[3], H.F.M. van der Heijden[3], S.J.H.Bredie[4], F.H.J. van den Hoogen[1]

Departments of [1]Rheumatic Diseases, [2]Cardiology, [3]Pulmonary Diseases and [4]General Internal Medicine, Radboud University Nijmegen Medical Centre, the Netherlands, [*]corresponding author

ABSTRACT

Pulmonary hypertension is a devastating complication of various, but rare diseases and can also occur as an isolated entity. It causes morbidity and mortality in all patients. Ongoing research has provided some insight into the pathophysiology and clinical manifestations, and new therapeutic options have recently become available for some types of pulmonary hypertension. In order to provide optimal care for an individual patient it is mandatory to establish the type and severity of the pulmonary hypertension in each patient. The diagnostic protocol used in our hospital is presented along with a description of two case histories. An algorithm of the different therapeutic strategies now available is given as well as recommendations for follow-up.

KEYWORDS

Diagnostic protocol, pulmonary hypertension, systemic sclerosis, unexplained breathlessness

INTRODUCTION

Pulmonary hypertension is a life-threatening condition which can occur either as an isolated entity or as a complication of various diseases. If left untreated, it causes increasing breathlessness and eventually death in all cases. Pulmonary hypertension is defined as a mean pulmonary artery pressure of >25 mmHg at rest or >30 mmHg during exercise, measured during right heart catheterisation.[1]

The most common symptoms of pulmonary hypertension are breathlessness, fatigue and (near) syncope.[2] Since these symptoms are nonspecific, pulmonary hypertension is often overlooked or diagnosed only in advanced stages. Pulmonary hypertension is a well-known complication of connective tissue diseases such as systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD). Because of the physical limitations arising from these underlying diseases, patients with pulmonary hypertension as a complication of these diseases complain of shortness of breath at a later stage than the normal population. Deaths that have previously been assigned to heart attacks may in fact have been caused by pulmonary hypertension. Pulmonary hypertension usually occurs late in the course of collagen vascular diseases. Since the majority of these patients are seen at regular intervals by rheumatologists or internists, this presents us with an opportunity to screen for pulmonary hypertension at an earlier stage, thus making it possible to start a therapy that may prevent progression of pulmonary hypertension and premature death. Although pulmonary function testing and echocardiography are widely used as screening tests and can identify patients with advanced pulmonary hypertension, these tests cannot be considered to be adequate for the exclusion of pulmonary hypertension in breathless patients. Therefore, a clinical history of longstanding dyspnoea should potentially be regarded as a sign of pulmonary hypertension, irrespective of the findings of pulmonary function testing and echocardiography,[3] and should prompt a right heart catheterisation, the gold standard for the assessment of pulmonary hypertension.

In 2003, the World Health Organisation (WHO) proposed a new classification for pulmonary hypertension. The basis of this new classification (*table 1*) is the notion of common pathophysiological processes involving the pulmonary blood vessels in all forms of the disorder.[4] These pathophysiological processes include (i) endothelial dysfunction which results in exaggerated vasoconstriction and impaired vasodilatation, promoting vascular remodelling of all layers of the vessel wall;[5] (ii) proliferation of the adventitia limiting vascular elasticity; (iii) hypertrophy of the medial smooth muscle promoting vasoconstriction and (iv) occlusion of the vascular lumen due to intima proliferation and *in situ* thrombosis. The availability of new therapies that have been shown to slow down or prevent progression of pulmonary hypertension has caused a growing interest among physicians to diagnose pulmonary hypertension at an early stage. Since various specialities can be confronted with patients with pulmonary hypertension, we started a multidisciplinary pulmonary hypertension outpatient clinic (MPHO clinic), comprising a rheumatologist/internist, pulmonologist, cardiologist and a specialised nurse, in order to facilitate early and fast diagnosis according to a protocol, and to institute the best therapies currently available.

In this paper we describe two patients who presented to this MPHO clinic, to illustrate the different diagnostic procedures of our protocol and the instituted therapies.

Table 1 *Revised WHO clinical classification of pulmonary hypertension (Venice 2003)*[6]

Group 1: Pulmonary arterial hypertension
Idiopathic
Familial
Associated with
- Collagen vascular disease
- Congenital systemic to pulmonary shunts
- Portal hypertension
- HIV infection
- Drugs/toxins
- Other

Associated with significant venous or capillary involvement
- Pulmonary veno-occlusive disease
- Pulmonary capillary haemangiomatosis

Persistent pulmonary hypertension of the newborn

Group 2: Pulmonary hypertension with left heart disease
Left-sided atrial or ventricular heart disease
Left-sided valvular heart disease

Group 3: Pulmonary hypertension associated with lung diseases and/or hypoxaemia
Chronic obstructive lung disease
Interstitial lung disease
Sleep disorder breathing
Alveolar hypoventilation disorders
Chronic exposure to high altitude
Developmental abnormalities

Group 4: Pulmonary hypertension due to chronic thrombotic and/or embolic disease
Thromboembolic obstruction of proximal pulmonary arteries
Thromboembolic obstruction of distal pulmonary arteries
Nonthrombotic pulmonary embolism (tumour, parasites, foreign material)

Miscellaneous
Sarcoidosis, histiocytosis X, lymphangiomatosis, compression of pulmonary vessels (adenopathy, tumour, fibrosing mediastinitis)

CASE REPORT 1

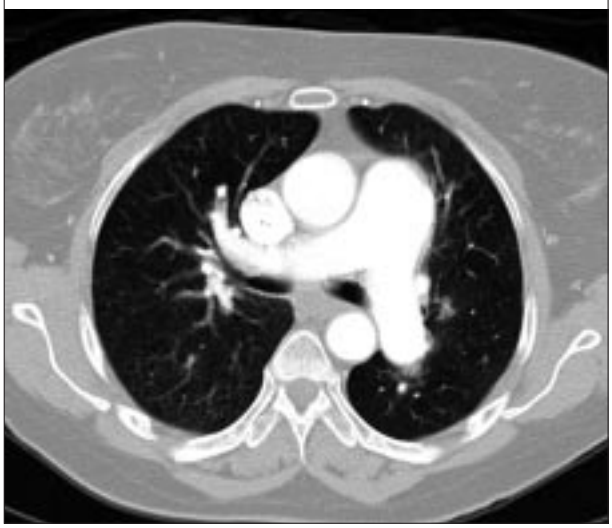
A 22-year-old Asian female was referred to our outpatient clinic. She had been diagnosed with SLE seven years before with symptoms of fever, Raynaud's phenomenon, pericarditis, pleuritis, ANA and anti-dsDNA positivity, and at a later stage also with arthritis of the knees. From the onset of her disease she had been treated with low-dose prednisone and azathioprine until three years ago when her SLE was considered to be in remission. On presentation she reported a two-year history of shortness of breath on exertion with an inability to exercise. Symptoms of right-sided heart failure were not present. On physical examination she was dyspnoeic while undressing, with an unremarkable internal and rheumatological examination. The electrocardiogram (ECG) revealed right ventricular hypertrophy. The results of the laboratory investigations were unremarkable except for ANA positivity with anti-SM autoantibodies and anti-dsDNA antibodies 10 IU/ml present. Pulmonary function testing showed no evidence of restrictive or obstructive lung disease, but a marked decrease in diffusion capacity was present: carbon monoxide transfer (DLCO) 60% of predicted. The echocardiogram showed pulmonary hypertension with normal left ventricular function. A right heart catheterisation revealed a pulmonary artery pressure of 79/37 mmHg with a mean of 54 mmHg. Right atrial pressure was 12 mmHg (normal), and the cardiac index was 2.9 l/min/m^2. The pulmonary vascular resistance was increased to 781 dynes.sec.cm^{-5}. Since pulmonary hypertension was established, a vasodilator test was performed. First the patient breathed 100% oxygen, and the measurement of the pulmonary artery pressure was repeated and found to be unchanged. Next prostacyclin up to 12 ng/kg/min was used to determine vasoreactivity of the pulmonary arteries, that is a significant decrease in pulmonary artery pressure, which was not present in this case. A ventilation/perfusion scan was negative for pulmonary embolism, as was the ultrasound of the abdomen for portal hypertension. A six-minute walk test (6-MWT) was performed. This is the distance a patient can walk in six minutes with encouragement, in a standard-

ised setting.[7] A healthy person has a 6-MWT of 650 to 750 meters. This patient had a 6-MWT of 505 m. She was diagnosed with pulmonary arterial hypertension, WHO group I, related to autoimmune disorder (*table 1*), NYHA functional class III. Treatment was started with bosentan, an oral dual endothelin receptor antagonist, 62.5 mg twice daily during the first four weeks, and 125 mg twice daily thereafter. Bosentan was well tolerated and after three months of treatment her NYHA functional class had improved from III to II and her 6-MWT was 537 m. The treatment was considered successful and continued. Until now, 18 months later, our patient is doing well and still on the same medication.

CASE REPORT 2

A 57-year-old Caucasian woman was referred for a second opinion. Her medical history was unremarkable until eight years ago, when a gradual progression of shortness of breath occurred. Because the echocardiogram was suggestive of pulmonary hypertension the patient was referred to the MPHO clinic for further evaluation. On physical examination she was dyspnoeic while undressing. Internal and rheumatological examinations were unremarkable; in particular there were no signs of right-sided heart failure present. Laboratory evaluation was normal. Pulmonary function testing revealed only a slight reduction in the diffusion capacity (DLCO was 79% of predicted), without signs of restrictive or obstructive lung disease. Blood gas analysis showed hypoxaemia, with PaO_2 of 5.9 kPa at room air. The echocardiogram performed in our hospital was also suggestive of pulmonary hypertension, which was confirmed with right heart catheterisation. Her pulmonary artery pressure was 104/55 mmHg, with a mean of 69 mmHg. The pulmonary vascular resistance and the cardiac index were 787 dynes.sec.cm^{-5} and 1.87 l/min/m^2 respectively. Vasoreactivity testing was negative. A ventilation/perfusion scan was negative for pulmonary embolism, as was the ultrasound of the abdomen for portal hypertension. The CT angiography of the lungs revealed dilated central pulmonary arteries and was otherwise unremarkable (*figure 1*). Her 6-MWT was 397 m. The diagnosis of idiopathic pulmonary arterial hypertension NYHA functional class III was made and treatment was started with bosentan. After three months she had improved to NYHA class II and her 6-MWT improved to 462 m.

Figure 1 *CT angiography of case 2, showing dilated central pulmonary arteries*



DIAGNOSIS OF PULMONARY HYPERTENSION: A DIAGNOSTIC PROTOCOL

Pulmonary hypertension should be suspected in any patient with unexplained shortness of breath. Next, two noninvasive tests can be performed to obtain further indications of pulmonary hypertension: pulmonary function testing and echocardiography. A decreased diffusion capacity with signs of mild restrictive lung disease is suggestive of pulmonary hypertension.[8] With transthoracic echocardiography an estimation of the systolic pulmonary artery pressure from the tricuspid regurgitation jet velocity and the jugular venous pressure can be made.[9] The diagnosis of pulmonary hypertension should be confirmed by right heart catheterisation, widely considered the gold standard for the diagnosis of pulmonary hypertension. During right heart catheterisation direct measurements of pulmonary artery pressure, right heart pressures, mixed venous oxygen saturation, cardiac output, pulmonary vascular resistance, and response to vasodilator drugs can be made. Also, right heart catheterisation is mandatory to measure the pulmonary wedge pressure to exclude left-sided heart disease as a cause of pulmonary hypertension. Once diagnosed, pulmonary hypertension should be classified according to the WHO classification and to the degree of functional disability, based on exercise performance, according to the New York Heart Association (NYHA) criteria (*table 2*), in order to establish the best treatment options.[1] The differential diagnosis of pulmonary hypertension can be extracted from the WHO classification. For the analysis of patients with pulmonary hypertension we designed a protocol that is performed in each patient with suspected pulmonary hypertension. These patients are seen at a special outpatient clinic, the MPHO clinic, where they are examined by a multidisciplinary team consisting of a rheumatologist/internist, pulmonologist, cardiologist and a specialised nurse. Prior to the diagnostic work-up, a careful history of each patient is taken, with special attention for the symptoms of dyspnoea, symptoms related

Table 2 *Modified New York Heart Association functional classification*[1]

Class I
Patients with pulmonary hypertension in whom there is no limitation to usual physical activity. Ordinary physical activity does not cause increased dyspnoea, fatigue, chest pain, or near syncope.

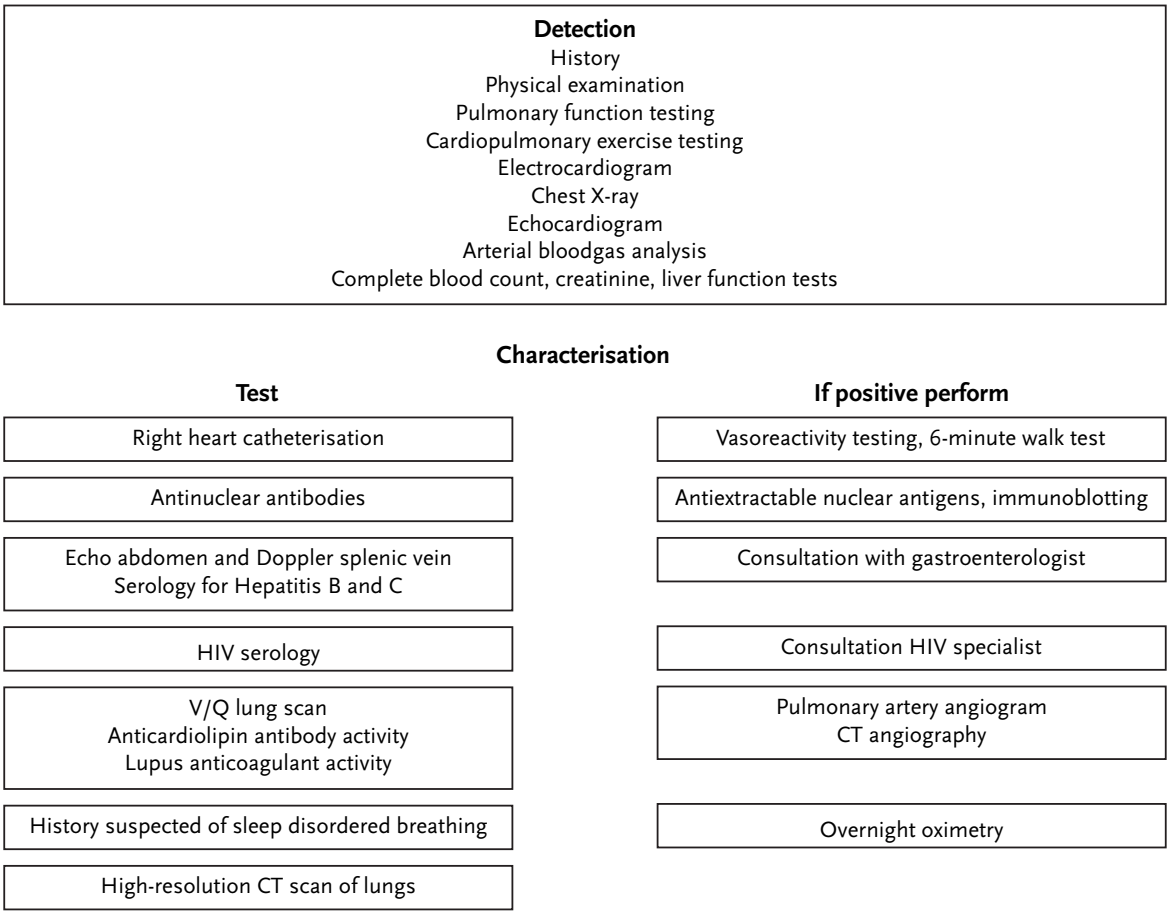
Class II
Patients with pulmonary hypertension who have a mild limitation of physical activity. They are comfortable at rest. There is no discomfort at rest, but normal physical activity causes increased dyspnoea, fatigue, chest pain, or near syncope.

Class III
Patients with pulmonary hypertension who have a marked limitation of physical activity. There is no discomfort at rest, but less than ordinary physical activity causes increased dyspnoea, fatigue, chest pain, or near syncope.

Class IV
Patients with pulmonary hypertension who are unable to perform any physical activity without symptoms and who may have signs of right ventricular failure. Dyspnoea and/or fatigue may be present at rest and symptoms are increased by almost any physical activity.

to autoimmune disorders, and prior drug and toxin use. A routine internal and rheumatological examination is performed, focussed on signs and symptoms of pulmonary hypertension and diseases known to be complicated by pulmonary hypertension. Furthermore, pulmonary function testing, including exercise tests, namely a 6-MWT and if possible a symptom limited exercise test on a cycle ergometer, chest X-ray, ECG, echocardiography and blood analysis are performed directly following the consultation. When the results of history and examinations are further indicative of pulmonary hypertension, right heart catheterisation is carried out, and if pulmonary hypertension is confirmed vasoreactivity tests are performed. The protocol used by our multidisciplinary team is schematically shown in *figure 2*. This approach consists of both essential tests, which are performed on each patient, and complimentary tests. After the results of the tests have become available the patients are discussed by the multidisciplinary team and the appropriate treatment is chosen and proposed to the patient during a follow-up visit. Patients with pulmonary hypertension are included in a prospective follow-up study.

Figure 2 *Diagram showing analysis of pulmonary hypertension*



TREATMENT STRATEGIES

General treatment

The general therapeutic options for pulmonary hypertension include oxygen therapy in patients with hypoxaemia, anticoagulant drugs if there are no contraindications and digoxin and diuretics in cases of right-sided heart failure. If the pulmonary vasoreactive tests are positive, the treatment of choice is high-dose calcium channel blockers. Vasoreactivity is considered to be present when the mean pulmonary artery pressure has decreased by at least 10 mmHg to 40 mmHg or less with normal or high cardiac output after intervention with pulmonary vasodilators, such as 100% oxygen, nitric oxide inhalation or intravenous prostacyclin.[1] Only 10 to 15% of the idiopathic pulmonary arterial hypertension patients meet these criteria.[10] The percentage of vasoreactive patients in the group with pulmonary arterial hypertension associated with autoimmune disorders is unknown, but believed to be even lower. Favourable clinical and prognostic effects of high-dose calcium channel blockers in vasoreactive patients have been shown in nonrandomised, noncontrolled studies.[11,12] The occurrence of side effects such as oedema, headache, tachycardia and hypotension limits the use of high-dose calcium channel blockers. If there is no vasoreactivity present or high-dose calcium channel blockers have to be discontinued because of side effects, the next treatment steps are based upon the WHO classification and functional NYHA classification.

Specific treatment

Specific treatment is available for some of the causes of pulmonary hypertension, for example pulmonary arterial hypertension, WHO group I. Bosentan, an oral endothelin antagonist, has been approved for pulmonary arterial hypertension, NYHA class III. The efficacy of bosentan has been established in two randomised placebo-controlled trials in which a significant improvement in the 6-MWT occurred after 12 weeks of treatment.[13,14]
Epoprostenol has been approved for the treatment of patients of the same WHO group I, in NYHA class III but also NYHA class IV. In several randomised controlled trials in idiopathic pulmonary hypertension the beneficial effects of epoprostenol were proven on survival, exercise tolerance, functional class and pulmonary vascular haemodynamics.[15-17] In pulmonary arterial hypertension (PAH) occurring in association with autoimmune disorders, a randomised controlled study showed a significant improvement in exercise capacity and haemodynamics in the treatment group.[18]
Recently, sildenafil, an oral phosphodiesterase inhibitor, has been shown to be effective in pulmonary arterial hypertension patients, both in patients with idiopathic PAH and in patients with PAH associated to autoimmune disorders, with an improvement in symptoms and exercise capacity[19-21] and a large placebo-controlled phase III trial is pending.
Other new drugs, such as treprostinil, a subcutaneously administrated prostaglandin, and sitaxsentan, a selective endothelin-A receptor antagonist, have given promising results in randomised clinical trials.[22]

When pulmonary arterial hypertension is diagnosed but patients are in NYHA class I or II, they are evaluated again after six months or sooner if there are signs of clinical deterioration. If a patient is in NYHA class III, the treatment of choice is bosentan. At the start of this therapy the patient is also informed about treatment possibilities when bosentan fails, including (heart) lung transplantation. The efficacy of bosentan is evaluated after three months and every three months thereafter. The treatment is considered effective if the 6-MWT is at least stable. If the treatment with bosentan fails, the next treatment option is continuously intravenously administered epoprostenol. This is also the treatment of choice for patients in NYHA class IV.
Other therapeutic options include combination therapies with epoprostenol and bosentan[23] and/or sildenafil.[24]
Atrial septostomy, a surgical procedure to create a right-to-left shunt that increases the cardiac output in cases of right-sided ventricular failure can also be considered.[25-27] Current indications for this procedure are limited and considered a bridging therapy to transplantation.
Treatment for the other causes of pulmonary hypertension, namely the WHO group II to V, is mainly supportive, and is focussed on the underlying disease. Physicians should pay special attention to pulmonary hypertension WHO group IV, chronic thrombotic and/or embolic diseases, because bilateral pulmonary thromboendarterectomy can be a curative treatment for these patients.[28]

CONCLUSION

Patients with pulmonary hypertension have a life-threatening disease. In order to provide adequate care for these patients it is necessary to perform a complete analysis to rule out the other differential diagnostic possibilities and to establish the type of pulmonary hypertension. We have described the protocol for pulmonary hypertension in our hospital, including treatment and follow-up strategies. Collaboration of a multidisciplinary team of rheumatologists/internists, pulmonologists, cardiologists and specialised nurses accounts for the most optimal diagnostic and therapeutic procedures.

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REFERENCES

1 Barst RJ, McGoon M, Torbicki A, et al. Diagnosis and differential assessment of pulmonary arterial hypertension. JACC 2004;43:S40-7.
2 Rich S, Dantzker DR, Ayres SM, et al. Primary Pulmonary Hypertension - A National Prospective-Study. Ann Intern Med 1987;107:216-23.
3 Mukerjee D, St George D, Knight C, et al. Echocardiography and pulmonary function as screening tests for pulmonary arterial hypertension in systemic sclerosis. Rheumatology 2004;43:461-6.
4 Gibbs JSR, Higenbottam TW, Black C, et al. Recommendations on the management of pulmonary hypertension in clinical practice. Heart 2001;86:I1-13.
5 Humbert M, Morrell NW, Archer SL, et al. Cellular and molecular pathobiology of pulmonary arterial hypertension. Am J Resp Crit Care Med 2002;165:341-4.
6 Rubin LJ. Diagnosis and management of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 2004;126:57-10.
7 Guyatt GH, Pugsley SO, Sullivan MJ, et al. Effect of Encouragement on Walking Test-Performance. Thorax 1984;39:818-22.
8 Denton CP, Black CM. Pulmonary hypertension in systemic sclerosis. Rheum Dis Clin North Am 2003;29:335-49.
9 Yock PG, Popp RL. Noninvasive Estimation of Right Ventricular Systolic Pressure by Doppler Ultrasound in Patients with Tricuspid Regurgitation. Circulation 1984;70:657-62.
10 Galie N, Seeger W, Naeije R, Simonneau G, Rubin LJ. Comparative analysis of clinical trials and evidence-based treatment algorithm in pulmonary arterial hypertension. JACC 2004;43:S81-8.
11 Raffy O, Azarian R, Brenot F, et al. Clinical significance of the pulmonary vasodilator response during short-term infusion of prostacyclin in primary pulmonary hypertension. Circulation 1996;93:484-8.
12 Nunes H, Humbert M, Sitbon O, et al. Prognostic factors for survival in human immunodeficiency virus-associated pulmonary arterial hypertension. Am J Resp Crit Care Med 2003;167:1433-9.
13 Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. Lancet 2001;358:1119-23.
14 Rubin LJ. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med 2002;346:1258.
15 Barst RJ, Rubin LJ, Mcgoon MD, Caldwell EJ, Long WA, Levy PS. Survival in Primary Pulmonary Hypertension with Long-Term Continuous Intravenous Prostacyclin. Ann Intern Med 1994;121:409-15.
16 Barst RJ, Rich S, Widlitz A, Horn EM, McLaughlin V, McFarlin J. Clinical efficacy of sitaxsentan, an endothelin-A receptor antagonist, in patients with pulmonary arterial hypertension - Open-label pilot study. Chest 2002;121:1860-8.
17 McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension - The impact of epoprostenol therapy. Circulation 2002;106:1477-82.
18 Badesch DB, Tapson VF, Mcgoon MD, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease - A randomized, controlled trial. Ann Intern Med 2000;132:425-34.
19 Bhatia S, Frantz RP, Severson CJ, Durst LA, Mcgoon MD. Immediate and long-term hemodynamic and clinical effects of Sildenafil in patients with pulmonary arterial hypertension receiving vasodilator therapy. Mayo Clin Proc 2003;78:1207-13.
20 Michelakis ED, Tymchak W, Noga M, et al. Long-term treatment with oral sildenafil is safe and improves functional capacity and hemodynamics in patients with pulmonary arterial hypertension. Circulation 2003;108:2066-9.
21 Prasad S, Wilkinson J, Gatzoulis MA. Sildenafil in primary pulmonary hypertension. N Engl J Med 2000;343:1342.
22 Barst RJ, Langleben D, Frost A, et al. Sitaxsentan therapy for pulmonary arterial hypertension. Am J Resp Crit Care Med 2004;169:441-7.
23 Humbert M, Sitbon O, Simonneau G. Novel therapeutic perspectives in pulmonary arterial hypertension. Eur Resp J 2003;22:193-4.
24 Sastry BKS, Narasimhan C, Reddy NK, Raju BS. Clinical efficacy of sildenafil in primary pulmonary hypertension - A randomized, placebo-controlled, double-blind, crossover study. JACC 2004;43:1149-53.
25 Moscucci M, Dairywala IT, Chetcuti S, et al. Balloon atrial septostomy in end-stage pulmonary hypertension guided by a novel intracardiac echocardiographic transducer. Cath Cardiovasc Interv 2001;52:530-4.
26 Rothman A, Sklansky MS, Lucas VW, et al. Atrial septostomy as a bridge to lung transplantation in patients with severe pulmonary hypertension. Am J Card 1999;84:682-6.
27 Sandoval J, Rothman A, Puildo T. Atrial septostomy for pulmonary hypertension. Clin Chest Med 2001;22:547-55.
28 Pengo V, Lensing AWA, Prins MH, et al. Incidence of chronic thromboembolic pulmonary hypertension after pulmonary embolism. N Engl J Med 2004;350:2257-64.

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ORIGINAL ARTICLE

The effect of arginine vasopressin on endothelin production in the human forearm vascular bed

C.T. Postma[1*], S.M.J. Maessen[1], Th. Thien[1], P. Smits[2**]**

[1]Department of Internal Medicine, Division of General Internal Medicine and [2]Department of Pharmacology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, e-mail: c.postma@aig.umcn.nl, *corresponding author

ABSTRACT

Objectives: To study whether arginine vasopressin (AVP) can stimulate endothelin production and/or release *in vivo*, in the human forearm vasculature.
Design: The effect of the infusion of AVP into the brachial artery on endothelin production across the human forearm vascular bed was studied in healthy male volunteers, and was compared with intra-arterial infusion of placebo. In another group the effects of AVP on endothelin production were studied after a prior infusion of L-N^G-monomethyl-arginine (L-NMMA), a nitric oxide-synthase inhibitor. In a fourth group the effect of L-NMMA alone, without AVP infusion, on endothelin production was studied.
Methods: We measured the effects of AVP, placebo, L-NMMA followed by AVP and L-NMMA followed by placebo on arterial and venous endothelin concentrations in the forearm of four groups, each consisting of five healthy male volunteers. Forearm blood flow was measured by strain gauge plethysmography. The endothelin production was calculated as forearm blood flow times (venous - arterial) endothelin concentration.
Results: The group infused with L-NMMA followed by infusion of 8 ng AVP/min per dl forearm volume showed a significant rise in endothelin production from 1.3 (1.8) to 5.0 (2.0) pg/min/dl at 15 minutes ($p<0.05$, ANOVA). This rise in endothelin production was also significantly different from the endothelin production at 15 minutes in the other three groups ($p<0.01$, ANOVA).
Conclusion: In healthy male volunteers intra-arterial infusion of AVP induced a rise in endothelin production in the forearm within 15 minutes, but only after prior infusion of L-NMMA. This observation suggests that the AVP-induced production of nitric oxide offsets AVP-mediated release of endothelin.

KEYWORDS

Arginine vasopressin, endothelin, forearm, human, L-N^G-monomethyl-arginine

INTRODUCTION

Endothelin is a potent vasoconstrictive factor mainly derived from endothelial cells. The production of endothelin is regulated *in vitro* and *in vivo* by a variety of hormones, other vasoactive substances and conditions of vascular stress.[1] In human disease states, endothelin plays a role in disorders related to the vascular system such as myocardial infarction and congestive heart failure, postischaemic renal failure and in preeclampsia.[1-5] There is as yet no convincing evidence that endothelin has a role in the aetiology of essential hypertension.[6,7] Although there has been a rapid increase in knowledge of the importance of endothelin in the pathophysiology of vascular diseases, much remains to be clarified. Studies of the dynamic regulation of endothelin *in vivo* in humans have been reported, but most of these studies were hampered by the fact that only endothelin concentrations were measured and not its production, and also that the

**Th. Thien and P. Smits were not involved in the handling and review process of this paper.

stimulus was mostly accomplished by physical means that can possibly lead to a cascade of endothelial reactions caused by mechanical factors.[8-10] To study endothelin regulation *in vivo* in humans, a humoral factor modulating this process would be more appropriate.

Many *in vitro* experiments have shown that endothelin production can be stimulated strongly by arginine vasopressin (AVP), and this vasoactive agent can be used *in vivo* in humans.[11-16] Stimulation of endothelin production by AVP is mediated by the V_1 receptor.[11,12] AVP also stimulates nitric oxide (NO). This stimulation is mediated by the V_2 receptor.[17] NO in turn inhibits endothelin production.[18-20] NO synthesis can be inhibited by L-N^G-monomethyl-arginine (L-NMMA), a NO-synthase inhibitor.

With these data as a basis we set up a study to investigate endothelin production responses to intra-arterial AVP administration alone and AVP together with L-NMMA. Recent studies have made it clear that storage granules containing endothelin-1 are present in endothelial cells that can be degranulated by a number of chemical and mechanical stimuli.[21-25] A confirmation of this mechanism was reported recently in DOCA-salt hypertensive rats.[26] *In vivo* studies in humans suggested a possible rise in endothelin production five minutes after a stimulus.[8,9] The time needed before endothelin production *in vivo* in humans increases is thus uncertain so we studied endothelin production reactions for a period of 180 minutes. We did so in the human forearm model in male volunteers only, as sex hormones can influence endothelin levels relative to the menstrual cycle.[27]

METHODS

Approval for the study protocol was given by the local ethics committee of the Radboud University Nijmegen Medical Centre. All subjects gave informed consent. Twenty healthy nonsmoking male volunteers participated in the study. After an overnight rest and breakfast in the early morning, the experiments were performed in the afternoon, starting at 13.00 hours. During the 24 hours preceding the experiment, subjects did not consume caffeine- or alcohol-containing drinks or food, nor did they take any medication. They were also not allowed vigorous exercise on the two days preceding the test. The subjects were in a supine position during the study in a quiet room with a constant temperature of 20°C. The brachial artery of the left arm was cannulated. A deep venous catheter was introduced in the antecubital region of the same arm. Experiments started after an equilibration period of 30 minutes. Forearm blood flow (FBF) of both arms was measured by venous occlusion mercury-in-silastic strain gauge plethysmography. To obtain the mean FBF it was measured six times and averaged. Blood pressure and heart rate were recorded intra-arterially (Hewlett-Packard, GmbH, Böblingen, FRG) (the average of six measurements). Arterial and venous blood samples were drawn at 0, 5, 15, 60, 120 and 180 minutes. We used a radioimmunoassay kit (Nichols Institute, Wijchen, the Netherlands) following C_{18} extraction for the quantitative determination of endothelin levels in plasma.[5] The antiserum showed 67% cross-reaction with endothelin-2 and 84% with endothelin-3. Endothelin production was calculated as FBF times (venous - arterial) endothelin concentration. AVP (Pitressin, Parke Davis, Berlin) 0.05 g/l was diluted with NaCl 0.9%. AVP was infused into the brachial artery for 180 minutes at a dose of 8 ng/min per dl of forearm volume, which was determined by water displacement. L-NMMA was dissolved in NaCl 0.9% and administered for 15 minutes in a dose of 30 mg intra-arterially, preceding the AVP or placebo infusions. NaCl 0.9% was used as placebo infusion. All drugs were infused by an automatic syringe infusion pump. The levels of AVP were calculated because it was not feasible to introduce a second arterial line to draw blood for arterial AVP levels. The AVP levels were calculated as follows: 8 ng/min/dl divided by the flow in ml/min/dl times 0.6 (1-haematocrit) gives the plasma AVP levels in ng/ml. The experiments were done in four groups of five subjects. The first group was infused with 8 ng/min/dl forearm volume (FAV) of AVP for 180 minutes. The second group was infused with L-NMMA for 15 minutes followed by the same dose of AVP for 180 minutes. The third group was infused with placebo only for 180 minutes. A fourth group was infused with L-NMMA for 15 minutes followed by infusion of placebo for 60 minutes.

Statistical analysis

ANOVA tests were used to compare the effects of AVP, L-NMMA and AVP, placebo and L-NMMA and placebo on endothelin production. AVP concentrations between groups were also compared by ANOVA. Changes in BP, FBF and the changes in venous and arterial endothelin concentrations were assessed by ANOVA and if appropriate Student's t-test. In case of non-Gaussian distribution signed-rank tests were used. Statistical significance was set at two-tailed $p<0.05$. The Bonferroni correction for multiple comparisons was used when appropriate.

RESULTS

No differences in clinical parameters were evident between the four groups of subjects (*table 1*). After the first set of experiments, including the infusion of AVP alone, the infusion of AVP after prior L-NMMA infusion and the placebo infusion, measurements of endothelin at 120 and 180 minutes were skipped in the group infused

Table 1 *Clinical data of the study subjects by infusion group (mean ± SD are given)*

Group	Age (years)	Body mass index (kg/m²)	Forearm volume (litre)
AVP	23.3 ± 3.0	22.2 ± 2.4	1.1 ± 0.2
L-NMMA + AVP	23.3 ± 2.9	22.8 ± 1.5	1.1 ± 0.1
Placebo	23.8 ± 2.9	23.4 ± 1.7	1.1 ± 0.1
L-NMMA + placebo	24.6 ± 3.5	22.0 ± 0.6	1.1 ± 0.3

AVP = arginine vasopressin; L-NMMA = L-N^G-mono-methyl-arginine.

with L-NMMA followed by placebo, since no changes in endothelin levels at these time points were detected. Thus, only the results until 60 minutes for all experiments are given. The computed plasma AVP levels were in the range of 0.7 to 6 ng/ml (*table 2*). The AVP levels between the AVP group and the AVP + L-NMMA group were not significantly different (*table 2*). Also within groups the AVP levels were not significantly different.
The FBF in the AVP group was 4.1 (0.6) ml/min/dl at baseline, 5.4 (2.0) ml/min/dl at 15 minutes and 3.8 (1.3) at 60 minutes (p>0.05) (*table 3*). The flow in the contralateral arm did not show any significant changes either, suggesting that local infusion had not resulted in systemic effects. The mean arterial pressure (MAP) was 72.6 (6.4) at baseline and 72.3 (8.4) mmHg at 60 minutes (*table 3*). Administration of 8 ng AVP/min/dl alone for 60 minutes revealed no significant effect on endothelin production (*table 4, figure 1*).

Table 2 *Arterial and venous endothelin levels and AVP levels (mean ± SD)*

Group	Baseline	5 minutes	15 minutes	60 minutes
Arterial/venous endothelin levels (pg/ml)				
- AVP	2.6(1.1)/2.7(1.0)	3.3(1.4)/3.9(0.8)	4.5(1.5)/4.1 (1.1)	4.0(1.4)/5.0 (2.0)
- L-NMMA + AVP	2.6(1.2)/3.1(1.0)	3.0(0.7)/3.7(0.9)	3.1(1.0)/4.4 (0.8)	4.2(1.5)/4.5 (1.3)
- Placebo	2.6(0.9)/2.6(0.4)	2.5(0.8)/3.0(0.7)	2.8(0.8)/2.7 (1.5)	2.8(0.7)/3.0 (0.5)
- L-NMMA + placebo	1.8(0.6)/2.4(0.9)	1.6(0.4)/2.5(0.5)	2.2(0.8)/2.4 (0.6)	2.2(0.4)/2.8 (1.1)
Computed AVP levels (ng/ml)				
- AVP	1.2 (0.19)	1.3 (0.37)	1.1 (0.30)	1.5 (0.37)
- L-NMMA + AVP	2.2 (0.57)	1.5 (0.33)	1.2 (0.28)	1.6 (0.55)

AVP = arginine vasopressin; L-NMMA = L-N^G-mono-methyl-arginine.

Table 3 *Forearm blood flow and mean arterial pressure (mean ± SD)*

Group	Baseline	5 minutes	15 minutes	60 minutes
Forearm blood flow, experimental arm (ml/min/dl)				
- AVP	4.1 (0.6)	4.7 (2.1)	5.4 (2.0)	3.8 (1.3)
- L-NMMA + AVP	2.1 (0.7)	3.6 (1.0)	4.6 (1.4)	3.6 (1.1)
- Placebo	1.8 (0.6)	1.6 (0.4)	1.5 (0.5)	1.3 (0.5)
- L-NMMA + placebo	1.7 (0.9)	1.5 (0.6)	1.6 (0.6)	1.7 (0.7)
Mean arterial pressure (mmHg)				
- AVP	72.6 (6.4)	76.8 (6.8)	74.4 (7.2)	72.3 (8.4)
- L-NMMA + AVP	87.0 (7.5)	81.9 (7.1)	76.5 (5.6)	75.8 (5.1)
- Placebo	78.3 (6.9)	77.7 (8.7)	80.5 (7.7)	83.0 (8.2)
- L-NMMA + placebo	87.1 (4.7)	87.1 (5.3)	87.6 (3.5)	89.6 (4.1)

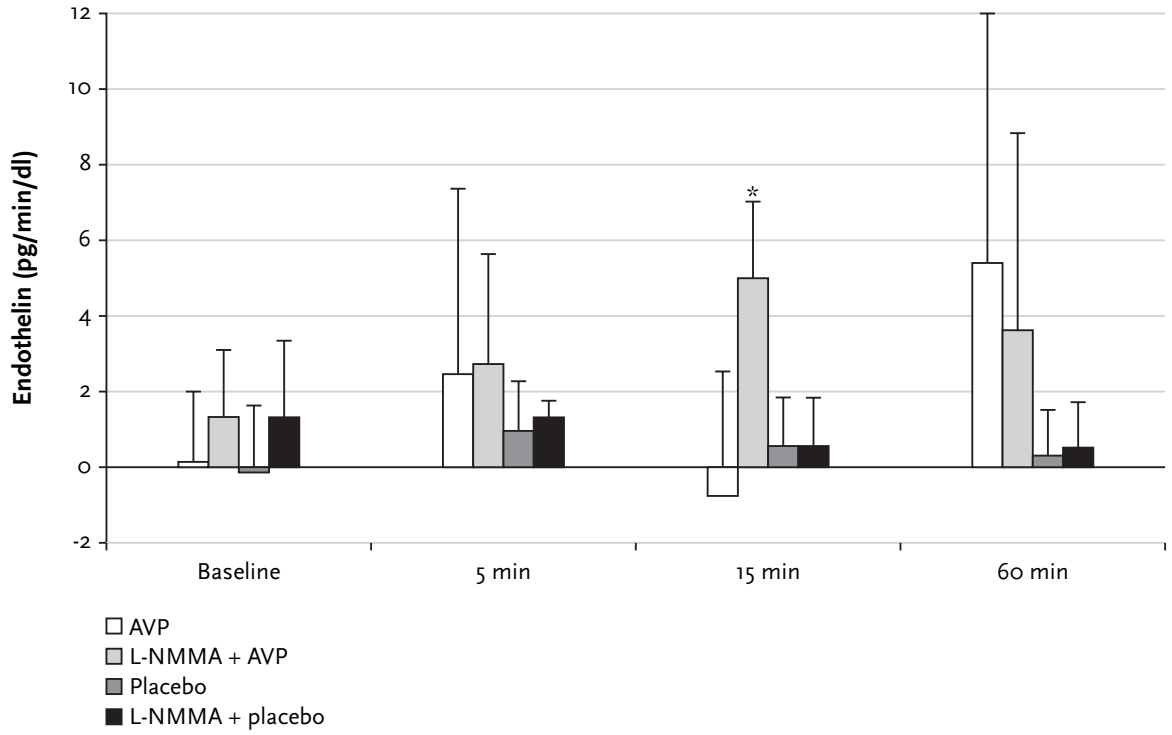
AVP = arginine vasopressin; L-NMMA = L-N^G-mono-methyl-arginine.

Table 4 *Endothelin production (pg/min/dl) (mean ± SD) in the four groups in 60 minutes*

Group	Baseline	5 minutes	15 minutes	60 minutes
AVP	0.0 ± 1.8	2.4 ± 4.9	- 0.8 ± 4.1	5.4 ± 6.6
L-NMMA + AVP	1.3 ± 1.8	2.7 ± 2.9	5.0 ± 2.0*	3.6 ± 5.2
Placebo	-0.2 ± 1.6	0.9 ± 0.7	0.5 ± 1.2	0.3 ± 1.2
L-NMMA + placebo	1.3 ± 2.0	1.3 ± 0.4	0.5 ± 1.1	0.5 ± 1.2

AVP = arginine vasopressin; L-NMMA = L-N^G-mono-methyl-arginine. *Indicates significant difference compared with values in the same group p<0.05 and compared with all other groups p<0.01 (ANOVA repeated measures).

Figure 1 *Endothelin production in human forearm vasculature*



***Indicates significant difference to other time points of the same group and to same time point of all other groups, p<0.05.**

The group infused with L-NMMA preceding the infusion of AVP showed a significant rise in endothelin production from baseline 1.3 (1.8) to 5.0 (2.0) pg/min/dl at 15 minutes (p<0.05) (*table 4, figure 1*). This peak in endothelin production was also significantly different from the endothelin production, at this point in all other groups (p<0.01) (*table 4*). The FBF decreased from 4.0 (1.7) at the start of the infusion of L-NMMA to 2.1 (0.7) ml/min /dl 15 minutes thereafter. Subsequently, the FBF increased from 2.1 (0.7) at baseline to 3.6 (1.0) and 4.6 (1.4) ml/min/dl at 5 and 15 minutes, respectively. After this it stabilised at 3.6 (1.1) ml/min/dl at 60 minutes (*table 3*). In the contralateral arm there were no significant changes in flow either.

The arteriovenous difference in endothelin levels showed a nonsignificant rise from 0.5 (0.8) pg/ml at baseline to 1.3 (0.6) pg/ml at 15 minutes (*table 3*). The MAP did not change significantly in this group (*table 3*).

There were no significant changes in endothelin production in the group that was infused with L-NMMA for 15 minutes followed by placebo for 60 minutes (*table 4*). The FBF decreased, but not significantly, from 3.3 (2.2) to 1.7 (0.9) after infusion of L-NMMA, and no change was seen after the subsequent placebo infusion. The contralateral arm did not show any significant changes either.

The MAP showed no changes in this group (*table 3*). The single infusion of placebo for 60 minutes did not result in significant changes in endothelin production (*table 4*).

The FBF remained unchanged at both arms. The MAP showed a nonsignificant increase from 78.3 (6.9) at baseline to 83.0 (8.2) mmHg at 60 minutes (*table 3*).

DISCUSSION

In the present study we found that intra-arterial infusion of AVP after preceding infusion of L-NMMA can cause a rise in endothelin production in the forearm of healthy male volunteers.

The effect of AVP on endothelin production has been studied extensively *in vitro*. It has been established that AVP is involved in the mechanism of endothelin-1 immunoreactivity release through activation of protein kinase C and mobilisation of intracellular Ca^{2+} resulting from the common receptor-mediated phosphoinositol breakdown in endothelial cells.[11,12] In *in vitro* experiments, both in animal and human cell cultures, endothelin production could be stimulated with an AVP concentration of 10^{-10} M. Dose-dependent increases in endothelin production could be established with increasing concentrations of AVP up to 10^{-6} M and 10^{-5} M.[13-15]

The optimal AVP concentration to stimulate endothelin production, as deducted from these *in vitro* experiments, is between 10^{-5} M and 10^{-8} M. In order to obtain a concentration of 10^{-8} M to 10^{-5} M in the human forearm it would have been necessary to supply 11 μg to 11 mg AVP/min/dl FAV. In the present study we used an AVP dose of 8 ng /min/dl because from previous experiments we knew the safety and efficacy of this dose in human experiments.[28] The computed plasma levels that were reached by this dose were between 0.7 and 6.0 ng/ml. With this dose we found a significant increase in endothelin production, albeit only after a preceding infusion of 30 mg L-NMMA. As we measured no significant rise after L-NMMA alone, nor after placebo infusion alone, it is reasonable to assume that AVP decisively contributed to the rise in endothelin production. The levels of AVP did not show a decline during the study but the levels stayed relatively stable. This observation suggests that AVP induces the endothelial release of nitric oxide which in turn inhibits the AVP-induced release of endothelin. Through blockade of NO synthesis, by L-NMMA, the AVP-induced release of endothelin is apparently unmasked. If higher concentrations of AVP had been used, L-NMMA might not have been necessary to demonstrate the AVP-induced increases in endothelin production. However, the use of high dosages of AVP in humans is restricted by the adverse effects of this substance. On the other hand the concentration of endothelin measured by means of the same radioimmunoassay kits in our study after AVP and L-NMMA infusion was of the same magnitude as that established in patients with advanced congestive heart failure, a condition well known for leading to a considerable rise in endothelin levels.[29] Also from this respect higher doses of AVP might not be without adverse effects on the circulation.

The rapid rise in endothelin concentrations that we detected *in vivo* underscores the results of previous studies that provided evidence for the release of endothelin via the regulated secretory pathway because such a relatively rapid rise is hardly in agreement with *de novo* synthesis but rather with release from endothelial storage granules.[25] There might also be factors that hamper the adequate detection of a rise in endothelin output such as a high local clearance of endothelin, by which it is possible that a high percentage of the locally raised endothelin production is metabolised before it reaches the plasma. Another contributing factor could be that about 75% of endothelin is possibly released via the albuminally.[25,30,31] As we measured a significant rise in endothelin levels in the plasma, the total rise in endothelin release must have been very high as the plasma levels we detected were as high as those in subjects with a chronic endothelin production stimulating condition such as congestive heart failure.[29]

As the production or overflow as we defined it includes flow, flow could have an impact on the production if the flow changes had been large. But at the moments the highest endothelin productions were measured FBF was not always higher but sometimes even lower compared with the measurements at other time points. After the infusion of L-NMMA, the flow in the experimental arm in the groups in which this was done declined, but not significantly so. This seemingly contradictory effect is probably caused by the fact that no pulse cuff was adjusted to exclude the circulation of the hand. Because of this, no estimation of the effects of L-NMMA or AVP on FBF could be made. We deliberately did not use a pulse cuff as flow changes after L-NMMA or AVP were not the goal of this study. FBF was only measured to calculate endothelin production. Therefore, we also made no statistical comparisons between groups regarding flow data. Within groups the FBF can be considered relatively stable during the experiments and metabolic effects of flow changes can be regarded as of no importance to the outcome.

We did not register a rise in endothelin production after four to five minutes, as has been found before.[8,9] But in those studies only endothelin concentration was measured so flow changes may have confounded the outcome. In our study the measurement of FBF was directly aimed at the correction for this factor.

In the present study the peak of endothelin production was observed after 15 minutes of AVP infusion that had been preceded by L-NMMA infusion for 15 minutes. This might indicate that the AVP effect on endothelin might dissolve quickly despite ongoing stimulation. There was an indication towards a higher output of endothelin during the entire period of AVP infusion in both groups compared

with placebo, but these trends were not significant. So, whether this decline in the stimulatory effect is real or a result of the small groups we studied must be examined further.

Further experiments in this *in vivo* human model are warranted to unravel more about the endothelin physiology in the endothelium of the human forearm.

CONCLUSION

In healthy male subjects AVP induced a rise in endothelin production in the forearm within 15 minutes, but only after prior infusion of L-NMMA. This observation suggests that the AVP-induced production of nitric oxide offsets AVP-mediated release of endothelin.

REFERENCES

1. Levin ER. Endothelins. New Engl J Med 1995;333:356-63.
2. Omland T, Lie RT, Aakvaag A, Aarsland T, Dickstein K. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. Circulation 1994;89:1573-9.
3. Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. Circulation 1994;89:1580-6.
4. Terpening CM. Mediators of chronic heart failure: How drugs work. Ann Pharmacother 2001;35:1066-74.
5. Kraayenbrink AA, Dekker GA, van Kamp GJ, van Geijn HP. Endothelial vasoactive mediators in preeclampsia. Am J Obstet Gynecol 1993:160-5.
6. Vanhoutte PM. Is endothelin involved in the pathogenesis of hypertension? Hypertension 1993;21:747-51.
7. Hirai Y, Adachi H, Fujiura Y, Hiratsukas A, Enomoto M, Imaizumi T. Plasma endothelin-1 level is related to renal function and smoking status but not to blood pressure: an epidemiological study. J Hypertens 2004;22:713-8.
8. Fyhrquist F, Saijonmaa O, Metsärinne K, Tikkanen I, Rosenlöf K, Tikkanen T. Raised plasma endothelin-1 concentration following cold pressor test. Biochem Biophys Res Commun 1990;169:217-21.
9. Stewart DJ, Cernacek P, Costello KB, Rouleau JL. Elevated endothelin-1 in heart failure and loss of normal response to postural change. Circulation 1992;85:510-7.
10. Kaufman H, Oribe E, Oliver J. Plasma endothelin during upright tilt: relevance for orthostatic hypotension? Lancet 1991;338:1542-5.
11. Emori T, Hirata Y, Ohta K, Shichiri M, Marumo F. Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. Biochem Biophys Res Commun 1989;160:93-100.
12. Emori T, Hirata Y, Ohta K, et al. Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. Hypertension 1991;18:165-70.
13. Sakamoto H, Sasaki S, Nakamura Y, Fushimi K, Marumo F. Regulation of endothelin-1 production in cultured rat mesangial cells. Kidney Int 1992;41:350-5.
14. Spatz M, Stanimirovic D, Bacic F, Uematsu S, McCarron RM. Vasoconstrictive peptides induce endothelin-1 and prostanoids in human cerebromicrovascular endothelium. Am J Physiol 1994;266:C654-60.
15. Tomobe Y, Yanagisawa M, Fujimori A, Masaki T, Goto K. Arginine-vasopressin increases the release of endothelin-1 into perfusate of rat mesenteric artery. Biochem Biophys Res Commun 1993;191:654-61.
16. Imai T, Hirata Y, Emori YanagisawaM, Masaki T, Marumo F. Induction of endothelin-1 gene by angiotensin and vasopressin in endothelial cells. Hypertension 1992;19:753-7.
17. Tagawa T, Imaizumi T, Endo T, et al. Vasodilatory effect of arginine vasopressin is mediated by nitric oxide in human forearm vessels. J Clin Invest 1993;92:1483-90.
18. Kourembanas S, McQuillan LP, Leung GK, Faller DV. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. J Clin Invest 1993;92:99-104.
19. Ohkita M, Takaoka M, Shiota Y, Nojiri R, Matsumara Y. Nitric oxide inhibits endothelin-1 production through the suppression of nuclear factor kappa B. Clin Sci 2002;103(suppl 48):S68-71.
20. Warner TD. Relationships between the endothelin and nitric oxide pathways. Clin Exp Pharmacol Physiol 1999;26:247-52.
21. Nakamura S, Naruse M, Naruse K, Demura H, Uemura H. Immunocytochemical localization of endothelin in cultured bovine endothelial cells. Histochemistry 1990;94:475-7.
22. Hemsén A. Biochemical and functional characterization of endothelin peptides with special reference to vascular effects. Acta Physiol Scandinavica 1991;suppl 602:1-61.
23. Doi Y, Ozaka T, Fukushige H, Furukawa H, Yoshizuka M, Fukimoto S. Increase in number of Weibel-Palade bodies and endothelin-1 release from endothelial cells in the cadmium-treated rat thoracic aortas. Virchows Arch 1996;428:367-73.
24. Ozaka T, Doi Y, Kayashima K, Fujimoto S. Weibel Palade bodies as a storage site of calcitonin gene-related peptide and endothelin-1 in blood vessels of the rat carotid body. Anat Rec 1997;247:388-94.
25. Russel FD, Davenport AP. Secretory pathways in endothelin synthesis. Br J Pharmacol 1999;126:391-8.
26. Yu M, Gopalakrishnan V, Wilson TW, McNeill JR. Endothelin antagonist reduces hemodynamic responses to vasopressin in DOCA-salt hypertension. Am J Physiol Heart Circ Physiol 2001;281:H2511-7.
27. Polderman KH, Stehouwer CDA, van Kamp GJ, Dekker GA, Verheugt FWA, Gooren LJG. Influence of sex hormones on plasma endothelin levels. Ann Int Med 1993;118:429-32.
28. Van Lieberg AF, Knoers NVAM, Monnens LAH, Smits P. Effects of AVP and DD-AVP on forearm vasculature of healthy subjects and patients with V2 receptor defect. J Hypertens 1995;13;1695-700.
29. Tuinenburg AE, van Veldhuisen DJ, Boomsma F, van den Berg MP, de Kam PJ, Crijns HJGM. Comparison of plasma neurohormones in congestive heart failure patients with atrial fibrillation versus patients with sinus rhythm. Am J Cardiol 1998;81:1207-10.
30. Lundberg JM, Ahlborg G, Hemsen A, et al. Evidence for release of endothelin-1 in pigs and humans. J Cardiov Pharmac 1991;17(suppl 7):S350-3.
31. Yoshimoto S, Ishiraki Y, Sasaki T, Murota S-I. Effect of carbon dioxide and oxygen on endothelin production by cultured porcine cerebral endothelial cells. Stroke 1991;22:378-83.

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ORIGINAL ARTICLE

Impact of the introduction of a guideline on the targeted detection of hereditary haemochromatosis

E.M.G. Jacobs[1,2*], C.F.M. Meulendijks[3], L. Elving[2], G.J van der Wilt[3], D.W. Swinkels[1]

Departments of [1]Clinical Chemistry (564), [2]Internal Medicine and [3]Medical Technology Assessment, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, tel.: +31 (0)24-361 76 38, fax: +31 (0)24-354 17 43, e-mail: e.jacobs@aig.umcn.nl, *corresponding author

ABSTRACT

Background: In 1998 a clinical guideline for the targeted, accurate and early detection and treatment of *HFE*-related hereditary haemochromatosis (HH), which comprises a test for the causative *HFE*-gene mutations, was introduced in our outpatient department.
Methods: The impact of this guideline was evaluated retrospectively. Data were acquired from medical records of patients with discharge diagnosis codes suggestive of HH (n=878 patients), obtained from a period before (n=422) and after guideline introduction (n=456).
Results: Combined measurements of serum transferrin saturation and serum ferritin rose from 12.2% (n=53) to 29.5% (n=138, p<0.001), leaving 70% of the patients eligible for HH not tested for iron parameters. The *HFE*-gene mutation detection test was correctly used in 11 (40.7%) of 27 tested patients and improperly interpreted in six (22.2%) of these 27 patients. Five new HH patients were diagnosed before and 13 after introduction. Seven of these 13 patients appeared to be incorrectly diagnosed, due to misinterpretation of laboratory results. Diagnostic costs of case detection for each accurately diagnosed patient were € 2380 before and € 2600 after introduction of the guideline.
Conclusion: Evaluation of the introduction of a practical guideline for targeted HH detection reveals a low compliance with the guideline, resulting in both a small percentage of patients tested for HH and overdiagnosis of HH. Therefore, the introduction of the guideline should be combined with a more appropriate implementation strategy which includes education on its most critical points, i.e. the indication and interpretation of the iron parameters and the *HFE* genotype.

KEYWORDS

Genetic testing, guideline adherence, haemochromatosis, practice guideline, serum iron measurements

INTRODUCTION

The medical and scientific interest in *HFE*-related hereditary haemochromatosis (HH), iron overload disease, quickly expanded after the discovery of the causative C282Y and H63D mutations in the haemochromatosis (*HFE*) gene in 1996.[1] The C282Y mutation is now the most common autosomal recessive mutation in people of northern European descent, with an estimated prevalence of the genetic susceptibility for HH by homozygous C282Y mutation of one in 200 to 250 persons.[2,3] Symptoms that can be attributed to iron overload are fatigue, arthralgia and cardiac rhythm disorders.[4-6] Furthermore, diabetes mellitus, elevated liver enzymes, liver cirrhosis, hepatocellular carcinoma and cardiac failure can be considered as signs of HH,[5,7,8] the last three being the most common cause of death in untreated HH patients.[6,7]
The first step in the diagnosis of HH consists of the recognition that these symptoms and signs in combination with persistent elevated serum transferrin saturations and elevated serum ferritin concentrations may be attributed to HH, especially when these laboratory values remain unexplained.[4,5,9,10] The diagnosis of HH is confirmed by the presence of homozygosity for the C282Y mutation, by compound heterozygosity for the C282Y and H63D mutation in the *HFE* gene and by iron overload shown in a liver biopsy, on exclusion of secondary causes of iron

tissue accumulation such as ineffective erythropoiesis, haemolysis, concomitant liver pathology and recurrent blood transfusions.[2,5,10] Treatment consists of extraction of the excessive amount of iron from the body by phlebotomy.[4,5,11] When these phlebotomies are initiated before the development of irreversible symptoms and damage, HH patients have a normal life expectancy.[11,12] Therefore, it is crucial that patients with HH are detected early in the course of the disease by measurement of their (elevated) serum iron parameters. However, these parameters are often not evaluated, as HH patients frequently present at the age of 50 to 60 years with nonspecific symptoms, which are often ascribed to age-related and common disorders.[5,13] This nonspecific presentation of the disorder reduces recognition of the disease and leads to high medical consumption and associated medical and nonmedical costs.[14,15] To enhance the awareness among physicians of HH in patients with these nonspecific symptoms and to improve the quality and effectiveness of the diagnostic pathway of HH, including the new *HFE*-mutation analysis, a guideline for case detection and treatment of *HFE*-related HH was developed in our university hospital in 1998 by a multidisciplinary haemochromatosis study group.
In the present study we aimed to evaluate retrospectively i) physicians' compliance with the diagnostic procedures, ii) the number of detected HH patients, iii) the correctness of the HH diagnoses, and iv) costs per detected patient, during a two-year period before and after introduction of the clinical guideline.

METHODS

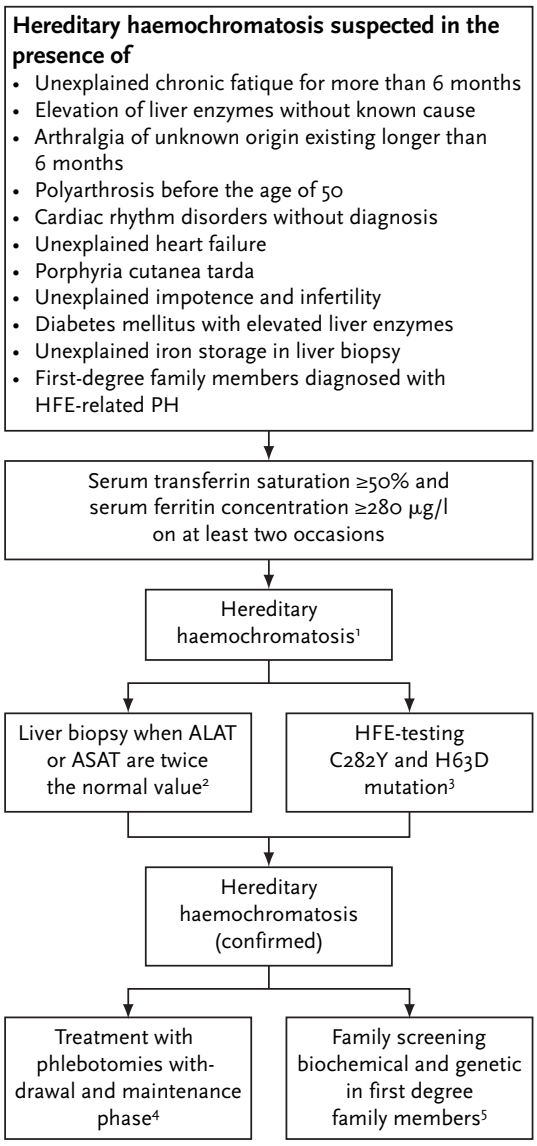
The multidisciplinary guideline was introduced in 1998 and contained recommendations to screen for HH when a patient presented with signs or symptoms as described in *figure 1*. The guideline was developed in our university hospital by a multidisciplinary haemochromatosis study group. This group consisted of physicians from the departments of general internal medicine, haematology, rheumatology, clinical genetics, gastroenterology and clinical chemistry. International evidence-based studies and expert opinion were translated into a guideline suitable for the local situation.[8,11,16-20] The guideline was introduced and explained during sessions held in the outpatient department of internal medicine of our university medical hospital. After its introduction, the guideline was available on the intranet of our hospital server.
According to the guideline, HH was diagnosed in symptomatic patients when the serum transferrin saturation was above 50% on at least two different occasions (one of which after overnight fasting), in combination with a serum ferritin concentration at least twice the upper limit of the normal value of 280 μg/l. For the diagnosis of HH it was recommended to exclude other factors that are known to influence the iron parameters, such as blood transfusions, iron supplementation, haemolytic anaemia, (alcoholic) hepatitis, non-HH-related liver disease and acute or chronic infections. Thus, a correctly diagnosed HH patient was defined as a patient with an elevation of both serum iron parameters in the absence of concomitant factors that influence iron parameters.
After detection of the biochemical iron overload, the guideline recommended testing for the C282Y and H63D mutation in the *HFE* gene to determine whether the patient had an *HFE*-related form of HH (*figure 1*). Genetic testing was also recommended for first-degree relatives of a symptomatic *HFE*-related HH proband. Liver biopsy was advised when a persisted elevation of both iron parameters was combined with a serum alanine aminotransferase (ALAT) or aspartate aminotransferase (ASAT) concentration more than twice the normal value, to either exclude concomitant liver pathology or to obtain a prognosis for the condition of the iron overloaded liver. When either *HFE* genotype (C282Y homozygosity and C282Y/H63D compound heterozygosity) or liver biopsy confirmed the diagnosis of HH, family screening and treatment was recommended (*figure 1*). The latter consisted of phlebotomy therapy in two phases. The first phase of weekly phlebotomies was meant to withdraw iron from the overloaded tissues, the second phase, of two to eight phlebotomies a year, to maintain a low body iron level. For first-degree relatives who appeared to be C282Y homozygous or C282Y/H63D compound heterozygous and had not yet developed iron overload, a phlebotomy schedule consisting of two blood donations a year, similar to that used for regular blood donors, was recommended (*figure 1*).

We retrospectively compared the diagnostic procedures for patients with features suggestive of HH who visited the outpatient department of internal medicine between a period before (January 1995 to December 1996) and after (May 2000 to April 2002) introduction of the guideline. The choice of the latter period allowed sufficient time for uptake of the novel procedures from the 1998 guideline, whereas the first period was chosen before the discovery of the *HFE*-gene mutation.[1]

Patients

Patients were selected for evaluation by using discharge diagnosis codes (classification system ICD-9-CM-codes).[21] Some patients received more than one diagnosis code. The discharge codes included were 'unexplained chronic fatigue for more than six months', 'elevated liver enzymes or liver cirrhosis without explanation', 'unexplained arthralgia', 'diabetes mellitus', 'hereditary haemochromatosis' or 'iron metabolism disorders' and 'porphyria cutanea tarda'.

Figure 1 *Flowchart guideline for targeted detection of hereditary haemochromatosis (HH) as introduced in 1998*



[1]Provided concomitant factors which influence the iron parameters, such as blood transfusions, iron supplementation, haemolytic anaemia, non-HH related liver disease and acute or chronic infection, could be excluded. Whenever doubt with respect to liver pathology, liver biopsy was recommended. [2]To exclude concomitant liver pathology or to obtain a prognosis for the condition of the iron overloaded liver. [3]For confirmation of the diagnosis *HFE*-related HH. C282Y homozygosity and C282Y/H63D compound heterozygosity confirm the diagnosis *HFE*-related HH. *HFE* testing was also advised when the patient had first-degree relatives with known *HFE*-related HH. [4]Two blood donations a year with control of the iron parameters once every three years, was recommended for C282Y homozygous and C282Y/H63D compound heterozygous first-degree family relatives, of a *HFE*-related proband clinically expressing HH, who had not yet developed iron overload. [5]Genetic HH screening was recommended only when the proband was diagnosed with *HFE*-related HH. PH = primary haemochromatosis, ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase.

Inclusion of diabetes mellitus patients was restricted to patients with concomitant elevation of liver enzymes (more than twice the upper limit of the reference value). Excluded were diabetes mellitus type 1 patients under the age of 35, patients suffering from chronic viral hepatitis, chronic alcohol abuse at the time of the study, cholestatic pathology and HH patients diagnosed with iron overload HH elsewhere. By means of an inventory form the following data were extracted from the medical records: serum transferrin saturation, serum ferritin concentration, *HFE*-mutation analysis, liver biopsy, HH diagnosis and the presence of co-factors that might result in falsely elevated iron parameters (such as blood transfusions, iron supplementation, haemolytic anaemia, (alcoholic) hepatitis, acute or chronic infections, hepatic injury and end-stage liver disease).

Compliance and statistics

Compliance of the physicians with the guideline was calculated by (number of guideline items followed)/(items followed + items not followed) x 100%. These items consisted of serum transferrin saturation, serum ferritin concentration, *HFE*-gene testing and liver biopsy. For the period before guideline introduction the same items, except the *HFE*-gene testing, were scored. Differences in diagnosis codes, gender, age and compliance scored before and after guideline introduction were tested for significance using a χ^2 test.

Costs

The impact of the guideline introduction on resource utilisation was assessed, taking into account direct medical costs only. The costs for diagnosing HH were approximated from laboratory costs (serum transferrin saturation, serum ferritin and *HFE*-mutation detection) and the costs for ultrasound-guided liver biopsy, with one-day hospital stay. For unit cost prices, national rates were used as proxies of actual resource utilisation, except for hospitalisation, for which a standard cost price was used.[22] Volumes of tests used were derived from chart review. Costs per case of correctly diagnosed HH patients were calculated and expressed in Euros.

RESULTS

Patient selection and characteristics

During the two observation periods, a total of 9096 individual patients visited the outpatient department of internal medicine, providing us with 902 discharge diagnosis codes consistent with the possible presence of HH representing 878 patients, 422 patients from the period before and 456 patients from the period after guideline introduction (*table 1*). In addition, 16 patients could not be

included as their medical records were missing; four from the period before and 12 from the period after guideline introduction (*table 1*).
Of the patients, 352 (40%) were male; 177 (41.9%) in the group before and 175 (38.4%) after guideline introduction (*table 2*). Overall, 561 (63.8%) of the patients were 50 years of age or older (*table 2*).

Diagnostic accuracy

Serum iron parameters

In the period before guideline introduction, serum transferrin saturation was measured in 29.7% (n=129) of all diagnosis codes. After introduction of the guideline, this percentage rose to 36.8% (n=172, p<0.05) (*table 3*). The serum ferritin measurements were performed in 17.3% (n=75) of the patients before and in 71.8% (n=336, p<0.001) after introduction. This significant rise in serum ferritin measurements was observed for patients from all diagnosis codes, except for those with 'liver cirrhosis of unknown origin'. There was a pronounced rise in serum ferritin measurements for the diagnosis codes of 'chronic fatigue of unknown origin' (from 10.9% (n=32) before to 74.7% (n=245, p<0.001) after), 'diabetes mellitus with elevated liver enzymes' (from 18.0 % (n=11) before to 44.9%

Table 1 *Discharge diagnosis codes included in the study for both the periods before and after introduction of the guideline*

Diagnosis codes	Number of diagnosis codes				
	Before introduction		After introduction		
	n	%[1]	n	%[1]	
Arthralgia, of unknown origin, >6 months	16	3.7	18	3.8	ns[2]
Chronic fatigue, of unknown origin, >6 months	294	67.7	328	70.1	ns[2]
Diabetes mellitus with elevated liver enzymes[3]	61	14.1	49	10.5	ns[2]
Haemochromatosis or disturbed iron metabolism	6	1.4	13	2.8	ns[2]
Liver enzyme elevation of unknown origin[4]	27	6.2	52	11.1	p<0.05[2]
Liver cirrhosis of unknown origin	26	6.0	8	1.7	p<0.001[2]
Porphyria cutanea tarda	4	0.9	0	0.0	n.d.
Medical records not available[5]	4	0.9	12	2.6	ns[2]
Total number of included diagnosis codes	*434*		*468*		
Total number of included patients[6]	*422*		*456*		

[1]100% = total of diagnosis codes included in that period. [2]Significance of difference in number of patients included between the periods before and after implementation of the guideline. [3]All patients diagnosed with diabetes mellitus type 2 or diabetes mellitus type 1 after the age of 35 years. Liver enzymes were elevated when they were more than twice the normal values. [4]Cholestatic diseases, viral hepatitis and chronic alcohol abuse at time of diagnosis were excluded. [5]Diagnosis codes of patients' medical records that were not available: before introduction three codes 'diabetes of unknown origin' and one code 'chronic fatigue', after introduction 12 codes 'adult onset diabetes mellitus of unknown origin'. [6]One patient could have more than one diagnosis code. ns = non significant; n.d. = not determined.

Table 2 *Gender and age (>50 years) of the patients included*

	Before implementation		After implementation		Total		
	n	%	n	%	n	%	
Gender							
All	422	48.1[3]	56	51.9[3]	878	100.0[3]	
Male	177	41.9[1]	175	38.4[2]	352	40.0[3]	ns[4]
Female	245	58.1[1]	281	61.6[2]	526	60.0[3]	ns[4]
Age >50 years							
All	268	63.5[1]	293	64.1[2]	561	63.8[3]	
Male	113	26.8[1]	97	21.2[2]	210	23.9[3]	ns[4]
Female	155	36.7[1]	196	42.9[2]	351	39.9[3]	ns[4]

[1]100% = total included patients in the group before implementation (n=422). [2]100% = total included patients in the group after implementation (n=456). [3]100% = total included patients of the two periods together (n=878). [4]Significance of difference between the periods before and after implementation of the guideline. n = number of patients.

(n=22, p<0.01) after) and 'elevated liver enzymes of unknown origin' (from 29.6% (n=8) before to 73.1% (n=38, p<0.01) after). The hallmark test for the diagnosis of HH, i.e. the combination of serum transferrin saturation and serum ferritin measurement, also increased with guideline introduction from 12.2% (n=53) in the period before to 29.5% (n=138, p<0.001) in the period after guideline introduction. This rise in combined measurement of serum transferrin saturation and serum ferritin concentration was significant for all diagnosis codes, except for the small groups of patients diagnosed with 'arthralgia of unknown origin' and 'liver cirrhosis of unknown origin'. For all the discharge codes the absolute number of serum transferrin saturation measurements was comparable with the absolute number of serum ferritin measurements after guideline introduction. Only the 'chronic fatigue of unknown origin' defined group showed a striking difference between the two measurements: 87 serum transferrin saturation measurements *vs* 245 serum ferritin measurements in the period after guideline introduction.

HFE-mutation analysis

HFE-gene mutation analyses were performed in 27 patients after protocol introduction (*table 4*). According to the guideline, *HFE* testing was recommended for only 11 (40.7%) of these 27 patients (numbers 1-11); nine of them had a combination of elevated serum transferrin saturation and elevated serum ferritin concentration and two of them were screened within the framework of family screening. In six of these 11 patients the clinical diagnosis HH could be confirmed on follow-up, since both iron parameters remained elevated and no other explanation to account for these elevated levels was found (*table 4*). One of them had a non-*HFE*-related form of HH, confirmed by the amount of iron withdrawn by phlebotomy to obtain normal serum iron parameters (number 6). In three of these 11 patients the HH diagnosis could not be confirmed (numbers 7-9): one patient's liver biopsy contained no iron, one patient's serum transferrin saturation returned to normal levels when measured on a second occasion and one patient had normal transferrin saturation levels, which alternated with high transferrin saturation levels upon blood transfusion. The physicians were correct not to diagnose HH in these three patients. The two patients who were *HFE*-gene tested in the context of family screening (numbers 10 and 11) were falsely diagnosed as iron overloaded and treated as HH patients. The guideline recommended follow-up of these patients and to phlebotomise them only twice a year. The remaining 16 (of the 27) *HFE*-tested patients should not have been tested following the guideline, since only one of the two serum iron parameters was elevated. Moreover, five of these 16 patients (numbers 12-16) were incorrectly diagnosed with HH by the physicians, for some of them most likely based on their *HFE*-gene genotype only.
Three patients were not tested for *HFE*-gene mutations in the period after guideline introduction despite their combination of elevated serum iron parameters. In two of these three patients serum iron parameters appeared to be temporarily influenced by blood transfusions. The remaining patient underwent a liver biopsy to exclude liver pathology. This liver biopsy revealed no iron.

Liver biopsy

Liver biopsies were taken for 60 diagnosis codes, representing 53 patients, 26 (49.1%) before and 27 (50.9%) after guideline introduction. In 49 of these 53 patients, the decision to perform a liver biopsy was based on a suspicion of concomitant liver disease. In four of these 53 patients, liver biopsy was performed in the absence of elevated liver enzymes or (probable) liver disease. Three of these four patients underwent liver biopsy before guideline introduction and the availability of the *HFE*-gene test. All three patients had elevated serum transferrin saturations (>50%) and serum ferritin levels (>280 μg/l). The presence of an increased amount of iron in their liver biopsy (diagnosed by an independent pathologist)

Table 3 *Diagnostic test use for serum iron parameters before and after guideline introduction*

Serum iron parameters	Diagnosis codes				
	Before implementation		After implementation		
	n	%	n	%	
Serum transferrin saturation	129	29.7	172	36.8	p<0.05[1]
Serum ferritin concentration	75	17.3	336	71.8	p<0.001[1]
Combination of serum TS and serum ferritin concentration	53	12.2	138	29.5	p<0.001[1]

Diagnostic test use is expressed as the percentage of the total number of diagnosis codes included in that period. Data are obtained from the medical records. Both the serum transferrin saturation and the serum ferritin concentration were scored no more than once per diagnosis code. [1]Significance of difference in increase in serum iron parameter(s) between the periods before and after implementation of the guideline. TS = transferrin concentration.

confirmed the diagnosis HH. The remaining fourth patient underwent his biopsy after guideline introduction in the presence of an elevated serum transferrin saturation and in the absence of an increased serum ferritin level. The liver biopsy revealed no increased amount of iron and HH was correctly excluded.

Number of detected patients

The introduction of the guideline led to an increase in diagnoses of HH, from five patients (1.2%) before to 13 patients (2.9%) after introduction of the guideline (*table 4*). This increase, however, was not statistically significant.

Phlebotomy treatment was started for all 18 patients. The physicians' diagnoses of iron overload appeared to be incorrect for seven of the 13 patients, as at least one of the serum iron parameters was not elevated (patients 10-16, *table 4*). Three of these patients were at risk of developing iron overload based on their C282Y homozygosity, but had not yet developed iron overload as their ferritin levels were normal (patients 10-12). All three were females and aged 41, 45 and 55 years, respectively. There was no over-diagnosis of HH before guideline introduction.

In total, we found one case of a missed HH diagnosis (patient 33, *table 4*). In this patient, included in the group

Table 4 *Characteristics of patients in whom HFE-gene analysis was performed or who were diagnosed with hereditary haemochromatosis according to the physicians or the guideline*

		Serum iron parameters		HFE gene mutations		Liver biopsy	HH diagnosis	
Patient	Evaluation period[1]	Transferrin saturation ≥50%[2]	Ferritin ≥280 μg/l[2]	C282Y	H63D		Physician[3]	Guideline[3]
1	After	+	+	Heterozygous	Heterozygous	n.d.	+	+
2-5	After	+	+	Homozygous	Negative	n.d.	+	+
6	After	+	+	Negative	Negative	n.d.	+	+[4]
7	After	+	+	Heterozygous	Negative	n.d.	_	_[5]
8	After	+	+	Negative	Negative	Micro nodular cirrhosis, Perls negative	_	_
9	After	+	+	Negative	Heterozygous	n.d.	_	_[5]
10-11	After	+	_	Homozygous	Negative	n.d.	+	_[6]
12	After	+	_	Homozygous	Negative	Perls negative	+	_
13	After	_	+	Heterozygous	Negative	n.d.	+	_
14	After	_	_	Heterozygous	Negative	n.d.	+	_
15	After	_	+	Negative	Homozygous	n.d.	+	_
16	After	_	+	Negative	Negative	n.d.	+	_
17-18	After	_	+	Negative	Heterozygous	Steatosis, Perls negative	_	_
19	After	_	+	Negative	Negative	Steatohepatitis, Perls negative	_	_
20	After	+	_	Negative	Heterozygous	Perls negative	_	_
21	After	_	+	Negative	n.d.	n.d.	_	_
22-23	After	_	+	Negative	Negative	n.d.	_	_
24	After	_	_	Heterozygous	Heterozygous	n.d.	_	_
25	After	_	_	Negative	Heterozygous	n.d.	_	_
26	After	_	+	Negative	Negative	n.d.	_	_
27	After	_	_	Negative	Negative	n.d.	_	_
28-29	Before	+	+	n.a	n.a	n.d.	+	+[7]
30	Before	+	+	n.a	n.a	Perls positive, hepatocellular carcinoma	+	+[7]
31	Before	+	+	n.a	n.a	Perls positive	+	+[7]
32	Before	+	+	n.a	n.a	Perls positive, cirrhosis	+	+[7]
33	Before	n.a	n.a	n.a	n.a	Autopsy liver: Perls positive, cirrhosis	_	+

Hereditary haemochromatosis (HH) diagnoses according to the physician: diagnoses of iron overload based on clinical grounds and treatment started for HH. HH diagnoses according to the guideline: HH diagnoses that should have been given according to the guideline. [1]Before = period before guideline implementation; after = period after guideline implementation. [2]- = Transferrin saturation <50% or serum ferritin <280 μg/l; + = transferrin saturation ≥50% or serum ferritin ≥280 μg/l. [3]- = No HH diagnosed; + HH diagnosed. [4]Non-*HFE* related HH. [5]Serum transferrin saturation that normalised when measured on a second occasion. [6]Patient was a first-degree relative of an *HFE*-gene-related HH patient. [7]HH diagnoses confirmed with either liver biopsy or number of phlebotomies. n.d. = not determined; n.a. = not available; Perls = Perls' staining, Prussian blue reaction used to detect iron in a liver biopsy.

before guideline introduction, the diagnosis of HH was only made postmortem, on autopsy. During life, the diagnosis of liver cirrhosis of unknown origin had been made. No iron parameters had been measured.
For nine patients with a combination of an elevated serum transferrin saturation and serum ferritin, HH was not diagnosed. Three of these patients were included in the period before and six patients were included in the period after guideline introduction. One patient from the first period was diagnosed with porphyria cutanea tarda and transferred to another hospital before further diagnosis and treatment could take place. For all the remaining eight patients the diagnosis of HH was correctly excluded either based on clinical evidence (blood transfusions recently given or spontaneous normalisation of iron parameters), or by a liver biopsy containing no increased amount of iron.

Costs

The total cost associated with the detection of new HH patients before introduction of the guidelines amounted to € 11,900. After the introduction, these costs rose to € 15,600. When these costs were ascribed to patients correctly diagnosed with iron overload proven HH, this resulted in € 2380 per correctly diagnosed patient before and € 2600 per correctly diagnosed patient after introduction of the guideline.

DISCUSSION

The introduction of the guideline for targeted *HFE*-related HH detection in the outpatient department of general internal medicine of our university hospital in 1998 led to an increased number of patients with symptoms consistent with HH, who were tested for serum iron parameters (serum transferrin saturation and ferritin). The number of HH diagnoses rose when compared with a period before guideline introduction. This rise, however, was not statistically significant. A shortcoming of the introduction of the guideline was the increase in the number of patients falsely diagnosed with HH.
The increase in both serum transferrin saturation and serum ferritin measurements in the period after introduction of the guideline was likely to result from the guideline introduction. This increase might have been positively influenced by more recently (after 1998) introduced guidelines in the department of internal medicine, i.e. on 'arthralgia' and on 'liver cirrhosis', which incorporated the recommendations of the HH guideline of 1998. It should, however, be noted that despite these increased numbers of iron parameters measured after guideline introduction, still approximately 70% of the patients with symptoms and signs consistent with HH were not tested for these parameters.
There was a remarkable difference in the magnitude of the raise in serum transferrin saturation and in serum ferritin measurements in the diagnosis code group 'chronic fatigue of unknown origin' after guideline introduction. This could be explained by the implementation of a guideline on 'chronic fatigue' in the outpatient department in 1999, which recommended only the measurement of serum ferritin, not combined with serum transferrin saturation, to detect HH among patients with symptoms suggestive of chronic fatigue.
Guideline compliance was also evaluated by the use of liver biopsies in the diagnosis of HH. According to the guideline, liver biopsies should be used to exclude additional liver pathology or to obtain a prognosis for the condition of the iron overloaded liver. Before the discovery of the *HFE* gene, liver biopsy was the gold standard for the confirmation of the diagnosis of hereditary iron overload. The compliance for the use of liver biopsies after the introduction of the guideline was good. Only one patient underwent a liver biopsy without elevation of both serum iron parameters or a possible liver disease.
The current study did not provide solid information on compliance with family screening for HH. However, the few notations made on this subject in the medical files suggested that physicians advised the proband to inform his or her family of the necessity of clinical, biochemical and/or genetic screening for HH.
Medical costs due to diagnostic procedures for each accurately diagnosed patient were similar before and after guideline introduction. However, these costs do not include costs due to incorrect diagnoses, i.e. patients in whom HH was missed or patients who were incorrectly diagnosed as having HH, nor do they include costs for treatment.
Compliance with the therapeutic aspects of the guideline was not thoroughly evaluated in the present study. However, it appeared that three homozygous C282Y patients of the 13 subjects diagnosed with HH were phlebotomised despite the absence of iron overload. For non-iron overloaded homozygous C282Y relatives of HH patients as well as for C282Y/H63D compound heterozygous relatives, the guideline recommended performing two blood donations twice a year as prevention. However, treatment of these non-iron overloaded patients is controversial and various treatment protocols have been proposed. Shortly after the discovery of the *HFE* gene, therapeutic protocols for these patients, such as the protocol described here, were based on the assumption of a high penetrance of the *HFE*-gene mutation and advised: i) performing phlebotomies several times a year to prevent iron accumulation, in order to maintain the serum ferritin level around 50 μg/l[5] and ii) twice yearly blood donation, with control of the iron parameters once every three years

(present guideline).[23] Since evidence is accumulating that the phenotypic penetrance of homozygosity for the C282Y mutation is low, it is currently advised to only start treatment when iron overload is proven and control for clinical and biochemical manifestations of HH every ten to twenty years.[24]

A drawback of the guideline introduction was the incorrect diagnosis of iron overload for several patients after guideline introduction (n=7). This was mainly due to erroneous use and interpretation of the *HFE* genotype (n=6). It appeared that *HFE* testing was more often used than strictly indicated and that once the *HFE* gene was genotyped, it dominated the results of the serum iron parameters and the liver biopsy. This dominant use and overestimation of the value of the *HFE* genotype in the diagnostic process of HH might be attributed to misinterpretation of the huge amount of international literature since 1996 which suggested the clinical relevance of the C282Y mutation due to the high clinical penetrance.[3,25-28] Only recently, evidence has accumulated that this penetrance of the homozygosity for the *HFE*-gene C282Y mutation might be very low.[9,29-32] But also, the fact that the diagnostic strategy of the present guideline lacks solid scientific evidence on its most crucial points (similar to the strategies throughout literature)[4,10,11,33,34] and is mainly based on professional expertise, might have decreased compliance. While awaiting the calculation of the cost-effectivity of both population and cascade screening, most HH patients in the Netherlands are still detected by case detection, i.e. early detection of patients with HH who seek medical attention for symptoms suggestive of HH. According to a recent report by Cadet *et al.* this strategy of targeted HH detection has been proven to be cost-effective.[35] Also the present study shows the potential cost-effectivity of targeted case detection in comparison with population screening as approximately one in 80 patients (1:84 (5 in 422) patients before, to 1:76 (6 in 457) patients after guideline introduction) have biochemical proven iron overload in comparison with one in 280 to one in 400 patients in the general population of northern European origin.[36,37] A disadvantage of this targeted approach, however, is the potential diagnostic delay in the course of the disease.

There were some limitations inherent to the study. First of all it was a retrospective study. This implicated that we had to interpret the thoughts of the physicians on the differential diagnosis of their patients' symptoms by looking at the diagnostic investigations performed on each patient. For example, we cannot be sure that every serum ferritin or serum transferrin saturation was performed in the light of HH diagnostics. Moreover, elevated iron levels might have been missed and with this also potential patients with HH, with a risk for organ damage and early death.[2,26] It is not possible to give solid numbers for those patients not recognised as having HH in the current study. As 87.8% (n=381) of the diagnosis codes eligible for HH before and 70.5% (n=330) of these diagnosis codes after guideline introduction were not evaluated for serum iron parameters, there could have been a fair number of missed HH diagnoses. However, for all the patients who were tested for both their serum iron parameters, we conclude that no eligible HH patients were incorrectly judged as being healthy. This also implicates that when HH was not diagnosed, despite the elevation of both serum iron parameters, this was done on correct clinical grounds, taking into account concomitant treatment or diseases, as the guideline recommended. The second limitation was the lack of control group. Therefore, we cannot exclude that the rise in diagnostic procedures is explained by the increase in the number of physicians who adhered to a more 'defensive' kind of medicine by adding test and/or the general trend in time for an increased use of iron parameters (i.e. ferritin) in the last decade.

We conclude that due to a relatively low compliance to the guidelines: i) approximately 70% of the patients with symptoms and signs consistent for HH were not tested for serum iron parameters and consequently patients with HH might have been missed and on the other hand ii) indication and interpretation of the genetic and iron parameters were misunderstood with as a result overdiagnosis of HH. The reasons why physicians do not follow clinical practice guidelines have been described by several groups.[38-40] One of them, Cabana *et al.*, clearly reviewed and summarised the literature on this subject in 1999. This resulted in the recognition of a variety of barriers to guideline adherence, which include: i) knowledge (awareness, familiarity), ii) attitude (agreement, self-efficacy, outcome expectancy, ability to overcome the inertia of previous practice) and iii) external barriers to performing recommendations. We believe that in general these barriers all attributed partly to the less optimal compliance of the 'haemochromatosis' guideline. We expect the most critical points of misuse and interpretation of iron parameters and genetic tests, observed in the present study, can be removed by a more professional evidence-based development and dissemination of the guideline that is combined by an appropriate education strategy on its most decisive aspects. In fact this approach has been adopted by a multidisciplinary team of medical professionals in the Netherlands, which recently started to develop an evidence-based guideline under the auspices of the Medical Scientific Board of the Dutch Institute for Healthcare CBO, in close cooperation with the Order of Medical Specialists. These guidelines will be evidence based and formulated following strict regulations (www.cbo.nl). Attention will also be paid to applicability in daily routine and the implementation strategy. Also, this team may learn from the shortcomings from the present study. It is expected that implementation

of this guideline into medical practice throughout the Netherlands in around early 2007 will increase compliance with the guideline, also on the decisive points.

We summarise that the introduction of a guideline for a targeted approach to HH screening increased the number of diagnostic procedures appropriate for HH investigation. The number of detected HH patients increased non-significantly, at comparable costs per case detected, with a drawback of falsely positive HH diagnoses. The HH overdiagnoses reflected the difficulties in indication and interpretation of both serum iron parameters and *HFE* genotypes. Therefore, the implementation strategy of the guideline should be improved to increase awareness and guarantee compliance with the indication and interpretation of both the iron and genetic parameters.

NOTE

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REFERENCES

1 Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13(4):399-408.
2 Bacon BR, Powell LW, Adams PC, Kresina TF, Hoofnagle JH. Molecular medicine and hemochromatosis: at the crossroads. Gastroenterology 1999;116(1):193-207.
3 Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 2001;154(3):193-206.
4 Bacon BR. Hemochromatosis: diagnosis and management. Gastroenterology 2001;120(3):718-25.
5 Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. J Hepatol 2000;33(3):485-504.
6 Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110(4):1107-19.
7 Yang Q, McDonnell SM, Khoury MJ, Cono J, Parrish RG. Hemochromatosis-associated mortality in the United States from 1979 to 1992: an analysis of Multiple-Cause Mortality Data. Ann Intern Med 1998;129(11):946-53.
8 Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 1997;25(1):162-6.
9 Distante S, Berg JP, Lande K, Haug E, Bell H. HFE gene mutation (C282Y) and phenotypic expression among a hospitalised population in a high prevalence area of haemochromatosis. Gut 2000;47(4):575-9.
10 Pietrangelo A. Hereditary hemochromatosis - a new look at an old disease. N Engl J Med 2004;350(23):2383-97.
11 Barton JC, McDonnell SM, Adams PC, et al. Management of hemochromatosis. Hemochromatosis Management Working Group. Ann Intern Med 1998;129(11):932-9.
12 Wojcik JP, Speechley MR, Kertesz AE, Chakrabarti S, Adams PC. Natural history of C282Y homozygotes for hemochromatosis. Can J Gastroenterol 2002;16(5):297-302.
13 Emery J, Hayflick S. The challenge of integrating genetic medicine into primary care. BMJ 2001;322(7293):1027-30.
14 Phatak PD, Guzman G, Woll JE, Robeson A, Phelps CE. Cost-effectiveness of screening for hereditary hemochromatosis. Arch Intern Med 1994;154(7):769-76.
15 Adams PC, Gregor JC, Kertesz AE, Valberg LS. Screening blood donors for hereditary hemochromatosis: decision analysis model based on a 30-year database. Gastroenterology 1995;109(1):177-88.
16 Adams PC, Kertesz AE, Valberg LS. Clinical presentation of hemochromatosis: a changing scene. Am J Med 1991;90(4):445-9.
17 Brissot P, Moirand R, Guyader D, Loreal O, Turlin B, Deugnier Y. Hemochromatosis after the gene discovery: revisiting the diagnostic strategy. J Hepatol 1998;28(suppl 1):14-8.
18 Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP. Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N Engl J Med 1996;335(24):1799-1805.
19 Guyader D, Jacquelinet C, Moirand R, et al. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. Gastroenterology 1998;115(4):929-36.
20 Powell LW, Summers KM, Board PG, Axelsen E, Webb S, Halliday JW. Expression of hemochromatosis in homozygous subjects. Implications for early diagnosis and prevention. Gastroenterology 1990;98(6):1625-32.
21 Brother E. Commission on Professional and Hospital Activities, International Classification of Diseases, 9th rev. ed. Clinical modification. 1978.
22 CTG tariffs 2003, Bijlage tariefbeschikking 5699-3000-03-02. 2003.
23 Swinkels DW, Marx JJ. [Diagnosis and treatment of primary hemochromatosis]. Ned Tijdschr Geneeskd 1999;143(27):1404-8.
24 Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103(8):2914-9.
25 Beutler E. Genetic irony beyond haemochromatosis: clinical effects of HLA-H mutations. Lancet 1997;349(9048):296-7.
26 Adams PC. Population screening for hemochromatosis. Hepatology 1999;29(4):1324-7.
27 Adams PC, Kertesz AE, McLaren CE, Barr R, Bamford A, Chakrabarti S. Population screening for hemochromatosis: a comparison of unbound iron-binding capacity, transferrin saturation, and C282Y genotyping in 5,211 voluntary blood donors. Hepatology 2000;31(5):1160-4.
28 Beutler E, Felitti V, Gelbart T, Ho N. The effect of HFE genotypes on measurements of iron overload in patients attending a health appraisal clinic. Ann Intern Med 2000;133(5):329-37.
29 Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G —>A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359(9302):211-8.

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30 Cox T, Rochette J, Camaschella C, Walkera A, Robson K. Clinical haemochromatosis in HFE mutation carriers. Lancet 2002;360:412.

31 Mura C, Le Gac G, Scotet V, Raguenes O, Mercier AY, Ferec C. Variation of iron loading expression in C282Y homozygous haemochromatosis probands and sib pairs. J Med Genet 2001;38(9):632-6.

32 Sham RL, Raubertas RF, Braggins C, Cappuccio J, Gallagher M, Phatak PD. Asymptomatic hemochromatosis subjects: genotypic and phenotypic profiles. Blood 2000;96(12):3707-11.

33 Powell LW. Diagnosis of hemochromatosis. Semin Gastrointest Dis 2002;13(2):80-8.

34 Powell LW, Dixon JL, Hewett DG. Role of early case detection by screening relatives of patients with HFE-associated hereditary haemochromatosis. Best Pract Res Clin Haematol 2005;18(2):221-34.

35 Cadet E, Capron D, Perez AS, et al. A targeted approach significantly increases the identification rate of patients with undiagnosed haemochromatosis. J Intern Med 2003;253(2):217-24.

36 Olsson KS, Eriksson K, Ritter B, Heedman PA. Screening for iron overload using transferrin saturation. Acta Med Scand 1984;215(2):105-12.

37 Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of haemochromatosis amongst asymptomatic Australians. Br J Haematol 1990;74(4):525-30.

38 Cabana MD, Rand CS, Powe NR, et al. Why don't physicians follow clinical practice guidelines? A framework for improvement. JAMA 1999;282(15):1458-65.

39 Van Wijk MA, van der Lei J, Mosseveld M, Bohnen AM, van Bemmel JH. Compliance of general practitioners with a guideline-based decision support system for ordering blood tests. Clin Chem 2002;48(1):55-60.

40 Maue SK, Segal R, Kimberlin CL, Lipowski EE. Predicting physician guideline compliance: an assessment of motivators and perceived barriers. Am J Manag Care 2004;10(6):383-91.

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ORIGINAL ARTICLE

Cross-sectional relationship between glycaemic control, hyperglycaemic symptoms and quality of life in type 2 diabetes (ZODIAC-2)

N. Kleefstra[1*], L.J. Ubink-Veltmaat[1,2], S.T. Houweling[1,2], K.H. Groenier[2], B. Meyboom-de Jong[2], H.J.G. Bilo[1]

[1]Department of Internal Medicine, Isala Clinics, Weezenlanden location, Zwolle, the Netherlands, tel.: +31 (0)38-424 25 18, fax: +31 (0)38-424 33 67, email: kleefstra@langerhans.com, [2]Department of General Practice, University of Groningen, Groningen, the Netherlands, *corresponding author

ABSTRACT

Background: To describe the relationship between glycaemic control, hyperglycaemic symptoms and quality of life (HRQOL) in type 2 diabetic patients.
Methods: In a shared-care diabetes project HRQOL was assessed. A total of 1664 patients with type 2 diabetes were identified in 32 primary healthcare practices. Of these patients, 1149 were included. HRQOL was measured using a generic questionnaire (Rand-36), completed by 1006 of the 1149 participants.
Results: The number of hyperglycaemic symptoms was higher in women (1.88) compared with men (1.64), without differences in mean haemoglobin A_{1c} (HbA_{1c}) (7.5%). Univariate analyses showed negative relationships between all dimensions of the Rand-36 and hyperglycaemic symptoms ($p<0.001$), but between only one dimension and HbA_{1c} ($p=0.005$). Multivariate analyses showed no association between any of the dimensions of the Rand-36 and HbA_{1c}, but the relationship between hyperglycaemic symptoms persisted in all dimensions ($p<0.001$). Notwithstanding these results, the presence of hyperglycaemic symptoms was related to higher HbA_{1c}.
Conclusion: In type 2 diabetic patients, as assessed by a generic questionnaire, there is an evident relationship between hyperglycaemic symptoms and HRQOL and not between HbA_{1c} and HRQOL. Subjective hyperglycaemic symptoms are, independent of HbA_{1c}, important for HRQOL in type 2 diabetic patients, and should therefore not be neglected in the management of diabetes.

KEYWORDS

Cross-sectional studies, diabetes mellitus type 2, hyperglycaemia, primary health care, quality of life

INTRODUCTION

Over the past century, changes in human behaviour and lifestyle have led to a dramatic increase in the prevalence and incidence of diabetes mellitus type 2 worldwide. To effect a significant reduction in the resulting premature morbidity and mortality, an integrated approach to prevent diabetes mellitus type 2 and its complications is called for.[1]

Targets in diabetic treatment are to improve glycaemic symptoms and to reduce the risk of diabetes-related complications and as such improve patients' quality of life (HRQOL). To achieve treatment goals, physicians and patients must cooperate intensively; patients' compliance is essential. Patients can be expected to be more compliant when their HRQOL improves as a consequence. However, studies concerning the relationship between HRQOL and glycaemic control prove confusing. In patients with type 2 diabetes, an intensive regime to improve glycaemic control seemed to have no effect on the patient's HRQOL.[2] This was puzzling, since improved glycaemic control usually leads to a decrease in hyperglycaemic symptoms, which again is expected to improve HRQOL. The latter part is supported by Goddijn *et al.*[3] who found, in a longitudinal study with a small number of patients, that a reduction in hyperglycaemic symptoms leads to an improved HRQOL.

To optimise the treatment of diabetes mellitus, further study into the manner in which diabetes-related symptoms, glycaemic regulation and HRQOL are related is required. This study describes the cross-sectional relationship between glycaemic control, hyperglycaemic symptoms and HRQOL in a large cohort of patients with type 2 diabetes.

MATERIALS AND METHODS

Study population

In the region around Zwolle (the Netherlands), a large shared-care diabetes project was initiated in 1998, the Zwolle Outpatient Diabetes project Integrated Available Care (ZODIAC).
In this project, general practitioners (GPs) are supported by hospital-based diabetic specialist nurses (DSNs) in their care of type 2 diabetic patients.

In part of the project, all subjects with type 2 diabetes treated exclusively by their GPs in 32 practices are seen annually by DSNs. In the first year of the project, 1664 subjects with type 2 diabetes mellitus were identified in these practices. Patients treated by an internist (n=338) were excluded. Fifty-seven patients were excluded by their general practitioners because of a very short life expectancy or expected insufficient cognitive abilities to fill in the questionnaire. A total of 1269 patients were invited of whom 1149 (90.5%) participated.

With the invitation to visit the DSN, the patients received an HRQOL questionnaire (the Rand 36-Item Health Survey; Rand-36),[4] which they were requested to complete and hand in together with a list of their current medication. Their visit to the DSN included the following: (i) their medical history was taken and added to the history provided by the GP; (ii) height, weight and blood pressure were measured; (iii) the feet were examined thoroughly (data on the loss of sensibility were used in this study); and (iv) after the visit, blood and urine were collected. A cross-sectional analysis of the first-year data was made.

Measures

Medical history included year of onset diabetes, medication (general and diabetic), smoking, coronary heart disease and cerebrovascular disease. Coronary heart disease was considered to be present when the patient had a confirmed history of myocardial infarction, percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG). Cerebrovascular disease was considered to be present when there was a confirmed history of stroke or transient ischaemic attack (TIA). Neuropathy was defined as the inability to feel the 5.07 Semmes Weinstein monofilament at any one of three sites on each foot, which is a reasonably sensitive and specific assessment for diagnosing diabetic polyneuropathy.[5] The BMI was calculated from weight and length (kg/m^2).
The major endpoint for metabolic control was the haemoglobin A_{1c} (HbA_{1c}) level. Other laboratory measures were serum creatinine, total cholesterol, high-density lipoproteins (HDL), triglycerides, and albumin/creatinine ratio in urine. Creatinine clearance was calculated using the Cockcroft and Gault formula.[6]

The study group chose the Rand-36 to measure HRQOL based on previous experiences with this questionnaire. It is a reliable and valid generic measure, also in the Dutch population.[7,8] The Rand-36 contains nine health dimensions: physical functioning, social functioning, role limitations (physical), role limitations (emotional), mental health, vitality, pain, general health perception and health change. The nine dimensions were calculated in accordance with the Rand-36 manual,[4] except for 'social functioning' since one of the two items was measured on a six- instead of a five-point Likert scale. We used the formula as used by Van der Zee *et al.*[7] ((question 10j * 5 + question 6 * 6) - 11) * 100/49. To interpret the overall direction and importance of the HRQOL effects,[9] two summary measures were calculated: the Physical Component Summary and Mental Component Summary.[3] The range of the scales is from 0 to 100, a higher score meaning better HRQOL.

With the Rand-36, the patients received a list of glycaemic symptoms and were asked to indicate whether they had had any of six hyperglycaemic symptoms (fatigue, weight loss without clear cause, itching, thirst, increased fluid intake and increased urine production), or four hypoglycaemic symptoms (excessive sweating, shaky sensation, dizziness, and sudden appetite which disappeared when food was taken) in the preceding month.

Analysis

Due to skewed distribution of the Rand-36 scales, non-parametric statistics were used in the univariate analysis. To compare continuous variables with dichotomic or categorical variables, the Mann-Whitney test or Kruskal-Wallis test was used, respectively. The Spearman rank correlation was used to compare continuous variables. To compare normally distributed variables with dichotomic or continuous variables, (independent samples) the t-test or Pearson correlation was used, respectively. Multivariate analyses were performed using the parameter estimates of the general linear model (GLM). The assumption of the GLM that the distribution of the residual scores should be normal was checked by inspecting the distribution of the residuals using normal probability plots. The Rand-36 scores were used as dependent variables, the noncontinuous measures as fixed variables and the continuous measures

as covariates. The fixed or continuous variables were included for analysis whenever there was at least one significant univariate association with the Rand-36. SPSS 10.0 for windows was used for data analysis.

RESULTS

General characteristics

In *table 1* characteristics of the study population are summarised. Women (n=662) represent the majority (57.6%) of the study population. Mean age was 68.7 years, ranging from 21 to 97. Median diabetes duration was 5.3 years. Insulin was used by 195 (17.0%) patients. Mean HbA_{1c} (7.5) was in the intermediate range (7 to 8.5) according to the Dutch National Guidelines (upper limit of normal 6.0%).[10] No difference in HbA_{1c} was found between the sexes. The median number of hyperglycaemic symptoms, known from 823 subjects, was 1 for both men and women.

Table 1 *Characteristics of study population 1998 (n=1149)*

Male	487 (42.4)
Age (years)	68.7 ± 11.5 (21-97)
Diabetes duration (years)‡	5.3 (2.5,10.4) (0-58)
Diabetes therapy	
- Diet only	148 (12.9)
- Oral medication	806 (70.1)
- Insulin	169 (14.7)
- Insulin + oral medication	26 (2.3)
Metabolic regulation	
- HbA_{1c} (%)	7.5 ± 1.3 (4.8-13.1)
Macrovascular complications	
- CHD	233 (20.3)
- Stroke/TIA	145 (12.6)
Microvascular complications	
- Albuminuria*	460 (41.7)
- Neuropathy	333 (29.2)
Cockcroft (ml/min)	72.1 ± 28 (15-215)
Blood pressure (mmHg)	
- Systolic	155 ± 25 (95-240)
- Diastolic	84 ± 11 (45-135)
Body mass index (kg/m²)	28.9 ± 4.8 (16-48)
Cholesterol/HDL	5.3 ± 1.6 (1.9-13.6)
Triglycerides (mmol/l)	2.6 ± 1.6 (0.5-15.2)
Smokers	207 (18.2)
No. hyperglycaemic symptoms (n=823)‡	1 (0,3) (0-6)
No. hypoglycaemic symptoms (n=820)‡	1 (0,2) (0-4)

Data are means ± SD (range) or n (% of known data). ‡Data are median (P_{25},P_{75}) (range). *Albuminuria ≥3 mg/mmol creatinine.

Determinants of quality of life

Univariate analysis

In the total study population of 1149 subjects, 1006 (87.6%) completed the Rand-36. No differences in HbA_{1c} were found between patients who completed the Rand-36 and those who did not. Univariate analysis was undertaken for all measures. *Table 2* shows the results of the Rand-36 in relation to HbA_{1c}. One dimension (health change) correlates significantly with HbA_{1c} (r=-0.093, p=0.005). *Table 3* shows the results of the Rand-36 in relation to the number of hyperglycaemic symptoms. For all scales the correlation coefficients are at least -0.310 (p< 0.001), the dimension 'vitality' having the strongest correlation (r=-0.546).

All measures show at least one significant association with the Rand-36, except for smoking, blood pressure and cholesterol/HDL ratio (data not shown). When compared with males, females showed lower scores on the Rand-36 (on eight scales). Patients with neuropathy showed lower scores than patients without neuropathy (eight scales). Increasing age was also related to lower scores (six scales). An increase in the number of hypoglycaemic symptoms was associated with lower scores on all Rand-36 scales, with lower correlation coefficients (range (r)=-0.238 to 0.468) as compared with the number of hyperglycaemic symptoms. Coronary heart disease and duration of diabetes both were related significantly to two scales.

Table 2 *Results RAND-36 divided for HbA_{1c}*

HbA_{1c} category	Good	Inter-mediate	Poor	Spearman correlation*
Range	<7%	7-8.5%	>8.5%	
Total completed questionnaires	441	473	239	
Physical functioning	57.2	55.7	53.2	-.029
Social functioning	80.8	76.6	78.8	-.039
Role limitations (physical problem)	64.4	62.2	65.5	.024
Role limitations (emotional problem)	73.5	73.7	73.5	.021
Mental health	74.8	72.6	73.8	.003
Pain	71.7	69.7	70.7	-.009
Vitality	60.6	60.8	59.1	-.008
General health perception	61.9	61.5	60.6	-.006
Health change	48.5	47.0	42.4	-.093‡
Physical component summary	66.7	64.9	65.3	-.014
Mental component summary	70.6	69.6	70.0	.003

***Spearman correlation was measured for continuous variable. ‡p=0.005.**

Table 3 *Results RAND-36 divided for number of hyperglycaemic symptoms*

Number of symptoms	0	1-2	3-6	Spearman correlation
Total completed questionnaires	211	368	237	
Physical functioning	74.5	53.9	41.7	-.386‡
Social functioning	91.4	77.1	67.9	-.374‡
Role limitations (physical problem)	88.0	61.4	44.8	-.404‡
Role limitations (emotional problem)	91.5	72.3	58.7	-.328‡
Mental health	84.6	72.7	64.4	-.391‡
Pain	87.3	69.8	55.8	-.396‡
Vitality	79.2	58.7	45.6	-.546‡
General health perception	70.2	60.8	53.8	-.379‡
Health change	54.9	45.7	39.5	-.310‡
Physical component summary	82.2	64.3	52.3	-.500‡
Mental component summary	83.9	69.0	58.3	-.499‡

‡p<0.001.

Multivariate analysis

Table 4 shows the influence that each parameter had on the different Rand-36 dimensions after correction for all other included parameters by using the 95% confidence interval and the proportion variance explained (eta squared). The negative influence of the number of hyperglycaemic symptoms remained significant on all Rand-36 scales (all p<0.001). Six other parameters also showed an independent significant influence on some of the Rand-36 scales: increasing age (five scales), BMI (four scales), coronary heart disease (five scales), cerebrovascular disease (three scales), neuropathy (two scales) and being female was negatively associated with seven scales. In multivariate analyses, the remaining parameters lost their influence on the Rand-36 scales (data not shown).

When the number of hypoglycaemic symptoms is added as a parameter to the multivariate analyses, it is significant on all scales (data not shown), as is the number of hyperglycaemic symptoms. When the eta squared values of the number of hypoglycaemic and hyperglycaemic symptoms

Table 4 *Clinical parameter estimates of Rand-36 dimensions*

		Physical functioning	Social functioning	Role limitations (physical problem)	Role limitations (emotional problem)	Mental health	Pain	Vitality	General health	Health change	Physical component summary	Mental component summary
Age	LB	-1.14	-.45	-.92	-.74	-.09	-.33	-.20	-.07	-.34	-.46	-.27
	UB	-.79	-.12	-.38	-.22	.17	.03	.08	.14	-.09	-.19	-.00
	eta	.133‡	.014†	.030‡	.018‡	.000	.003	.001	.001	.014†	.030‡	.006*
BMI	LB	-1.63	-.82	-1.36	-.85	-.32	-0.99	-.49	-.34	-.46	-.85	-.46
	UB	-.84	-.08	-.12	.36	.30	-.16	.17	.14	.13	-.22	.15
	eta	.045‡	.007*	.007*	.001	.000	.009†	.001	.001	.002	.015†	.001
CDV	LB	9.39	3.20	-.28	-.81	-3.99	-4.05	.73	-2.33	-.53	1.39	-.63
	UB	21.18	14.49	18.70	17.67	5.09	8.25	10.45	4.84	8.18	10.90	8.47
	eta	.032‡	.012†	.005	.004	.000	.001	.007*	.001	.004	.009*	.004
Neuro-	LB	4.44	-2.44	-6.28	-3.58	-.97	.82	-2.47	-1.02	-.60	-.13	-.90
pathy	UB	12.60	5.37	6.50	8.88	5.35	9.39	4.25	3.93	5.42	6.31	5.28
	eta	.021‡	.001	.000	.000	.002	.007*	.000	.002	.003	.005	.003
CHD	LB	6.48	-.70	-3.78	-7.29	-2.07	.08	.19	1.08	-3.45	1.28	-1.39
	UB	16.06	8.52	11.41	7.52	5.40	10.16	8.13	6.89	3.65	8.98	6.02
	eta	.027‡	.004	.001	.000	.001	.005*	.005*	.009†	.000	.009†	.002
No.	LB	-8.13	-6.43	-11.99	-9.50	-5.62	-7.90	-8.74	-4.52	-4.39	-7.59	-6.76
hypergly-	UB	-5.81	-4.19	-8.37	-6.00	-3.82	-5.47	-6.82	-3.13	-2.67	-5.77	-5.03
caemic	eta	.152‡	.101‡	.142‡	.095‡	.123‡	,130‡	.247‡	.129‡	.076‡	.223‡	.203‡
symptoms												
Male/	LB	5.17	.79	1.44	4.26	3.11	4.34	.80	-1.86	-1.49	3.15	2.36
female	UB	12.81	8.13	13.36	15.81	9.07	12.35	7.12	2.77	4.16	9.17	8.11
	eta	.027‡	.007*	.008*	.016†	.021‡	.021‡	.008*	.000	.001	.022‡	.018‡

*p<0.05, †p<0.01, ‡p<0.001. CDV = cerebrovascular disease; CHD = coronary heart disease. The first and second numbers represent the lower (LB) and upper bound (UB) respectively of the 95% confidence interval of the regression coefficient (B). A negative number means the parameter has a negative relationship with the Rand-36 dimension. The third number represents the partial eta squared (it describes the proportion of total variability of the Rand-36 dimension attributable to a parameter).

are compared, the former scores higher on two scales: mental health (eta squared 0.137 *vs* 0.051) and pain (eta squared 0.085 *vs* 0.066).

Symptoms *vs* HbA_{1c}

Table 5 shows the quintiles of HbA_{1c} divided for the number of hyperglycaemic and hypoglycaemic symptoms. These data show that the number of hypoglycaemic symptoms is not related to HbA_{1c}, not even within the lowest quintile. However, the number of hyperglycaemic symptoms correlates significantly with HbA_{1c} (p=0.033). The average number of hyperglycaemic symptoms was 2.11 for patients in the highest quintile, and 1.62 to 1.71 for patients in the lower four quintiles. *Table 6* shows the mean HbA_{1c} divided for presence or absence of separate hyperglycaemic symptoms. HbA_{1c} was significantly higher in the group of persons with the presence of the symptoms thirst (p=0.002) and increased fluid intake (p=0.011).

Table 5 *Quintiles of HbA_{1c} divided for number of hyperglycaemic and hypoglycaemic symptoms*

HbA_{1c}	Hyperglycaemic symptoms (n)†	Hypoglycaemic symptoms (n)‡
4.8-6.4 (243)	1.71 ± 1.57	1.15 ± 1.17
6.5-7.0 (231)	1.71 ± 1.54	1.19 ± 1.13
7.1-7.6 (208)	1.71 ± 1.47	1.27 ± 1.18
7.7-8.5 (228)	1.62 ± 1.65	1.16 ± 1.13
8.6-13.1 (236)	2.11 ± 1.56	1.24 ± 1.17

†Correlation with HbA_{1c}: 0.064 (p=0.033). Correlation was measured for continuous variable. ‡Correlation with HbA_{1c}: 0.023 (p=0.261). Correlation was measured for continuous variable.

Table 6 *HbA_{1c} divided for each type of hyperglycaemic symptom*

Symptoms	Yes	No	P
Fatigue	7.48 ± 1.2 (441)	7.50 ± 1.3 (348)	.937
Weight loss	7.51 ± 1.3 (64)	7.27 ± 1.3 (717)	.109
Itching	7.58 ± 1.3 (221)	7.44 ± 1.2 (561)	.251
Thirst	7.73 ± 1.3 (196)	7.39 ± 1.2 (585)	.002
Increased fluid intake	7.76 ± 1.5 (191)	7.39 ± 1.2 (595)	.011
Increased urine production	7.55 ± 1.3 (332)	7.43 ± 1.2 (451)	.243

Data are means ±SD (number of patients).

DISCUSSION

This study shows a negative relationship between HRQOL and glycaemic symptoms as well as a positive relationship between hyperglycaemic symptoms and HbA_{1c}. However, no association between HRQOL and HbA_{1c} was found. Moreover, we found a negative association of HRQOL with female gender, age, BMI, CHD, cerebrovascular disease and neuropathy. Independent of each other, hypoglycaemic as well as hyperglycaemic symptoms have a strong influence on HRQOL. The influence on HRQOL by hyperglycaemic symptoms is stronger and associated with HbA_{1c}, whereas hypoglycaemic symptoms are not.

Goddijn *et al.*[3] concluded in a longitudinal study with 94 patients that in type 2 diabetic patients reduction of hyperglycaemic symptoms improved HRQOL. Earlier studies using the Rand-36, SF-20 or SF-36 were unable to find a relationship between HRQOL and HbA_{1c}.[11-14] We found only health change to be univariately and negatively associated with HbA_{1c} as did Johnson *et al.* in the Pima Indians.[15] This also applies to the longitudinal studies in which the above-mentioned questionnaires were used.[3,16] However, when other questionnaires were used, the association between HbA_{1c} and HRQOL showed mixed results, such as a significant negative association[12,14,17,18] or no clear relation.[2,14,19-22] The relation with glucose levels was also unambiguous.[23,24]

The negative relationships between HRQOL and complications are what we expected:[25,26] having a complication relates to worse HRQOL. And although care providers work hard to improve the risk profile for complications by monitoring the HbA_{1c}, blood pressure and lipid profile, other (modifiable) factors should also be addressed. In order to improve 'quality of care', some of the efforts should also be directed to factors such as BMI and hyperglycaemic symptoms, since our results show that they relate directly to HRQOL. At the moment a large part of diabetes management is directed by objective measures such as HbA_{1c}, which are not directly related to HRQOL. Hyperglycaemic symptoms receive far less attention. As Hanestad *et al.*[27] already postulated, this means that important aspects of the patient's life are neglected in diabetes care.

The absence of a relationship between HRQOL and HbA_{1c} is hard to explain, especially when considering the relationship between HRQOL and hyperglycaemic symptoms and HbA_{1c} and hyperglycaemic symptoms. One would expect that if one of these three parameters is high, it would relate positively to both other parameters. It may be debatable whether the symptoms chosen (as used by Goddijn *et al.*[3]) are truly hyperglycaemic.

According to Van der Does *et al.*,[22] fatigue is the dimension most directly related to the present glycaemic status. However, fatigue is a general and frequently occurring symptom present in both patients with diabetes and with depression. People with diabetes suffer more often from depression compared with people without diabetes,[28-30] and depression appears to be a good effect indicator of HRQOL,[31,32] in particular among female patients.[30] The presence of depression has not been assessed and may have been of influence on our results.[33] Except for the symptom fatigue, the presence of all other hyperglycaemic symptoms was associated with a higher HbA_{1c}. Polyuria, polydipsia and unexplained weight loss are classic symptoms of diabetes mellitus.[34] Moreover, most hyperglycaemic symptoms were found in patients with the highest HbA_{1c}, supporting the fact that they are indeed hyperglycaemic symptoms.

The limitations of our study are that the symptoms that were chosen were not taken from a validated symptom list, and that the number of missing values for the hypoglycaemic and hyperglycaemic symptoms was quite substantial. This may present an inaccurate representation of the symptoms within the study population. For the longitudinal follow-up of our population, validated symptom score lists have been introduced. And although the Rand-36 is closely related to the SF-36, the Rand-36 has not been as extensively validated as the SF-36.[35]

Improving glycaemic control decreases the risk of complications. Whether it increases HRQOL directly will have to be further studied longitudinally now that our cross-sectional results suggest that this is not the case. As such, physicians and DSNs have to be more aware of the importance of motivating treatment adherence, as stated earlier by Goddijn *et al.*[3] If the association between hyperglycaemic symptoms and HRQOL in the absence of a relation between HbA_{1c} and HRQOL is confirmed in a longitudinal design, caregivers will have to consider these symptoms as a separate treatment objective. This cross-sectional study of a large cohort is a basis for future longitudinal analyses.

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REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetic epidemic. Nature 2001;414:782-7.
2. U.K. Prospective Diabetes Study Group. Quality of life in type 2 diabetic patients is affected by complications but not by intensive policies to improve blood glucose or blood pressure control (UKPDS 37). Diabetes Care 1999;22:1125-36.
3. Goddijn PPM, Bilo HJG, Feskens EJM, Groenier KH, van der Zee KI, Meyboom-de Jong B. Longitudinal study on glycaemic control and quality of life in patients with Type 2 diabetes mellitus referred for intensified control. Diabet Med 1999;16:23-30.
4. Van der Zee KI, Sanderman R. Het meten van de algemene gezondheidstoestand met de RAND-36. Een handleiding. Noordelijk Centrum voor Gezondheidsvraagstukken. University of Groningen, 1993.
5. Valk GD, de Sonnaville JJJ, van Houtum WH, et al. The assessment of diabetic polyneuropathy in daily clinical practice: reproducibility and validity of Semmes Weinstein monofilaments examination and clinical neurological examination. Muscle Nerve 1997;20:116-8.
6. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
7. Van der Zee KI, Sanderman R, Heyink JW, de Haes H. Psychometric qualities of the RAND 36-item health survey 1.0: a multidimensional measure of general health status. Int J Behav Med 1996;3:104-22.
8. Van der Zee KI, Sanderman R, Heyink J. A comparison of two multidimensional measures of health status: the Nottingham Health Profile and the RAND 36-Item Health Survey 1.0. Qual Life Res 1996;5:165-74.
9. Testa MA, Simonson DC. Assessment of quality-of-life outcomes. N Engl J Med 1996;334:835-40.
10. Rutten GEHM, Verhoeven S, Heine RJ, et al. NHG-standaard diabetes mellitus type 2 (eerste herziening). Huisarts Wet 1999;42:67-84.
11. Hanninen J, Takala J, Keinanen-Kiukaanniemi S. Quality of life in NIDDM patients assessed with the SF-20 questionnaire. Diabetes Res Clin Pract 1998;42:17-27.
12. Anderson RM, Fitzgerald JT, Wisdom K, Davis WK, Hiss RG: A comparison of global versus disease-specific quality-of-life measures in patients with NIDDM. Diabetes Care 1997;20:299-305.
13. Ahroni JH, Boyko EJ, Davignon DR, Pecoraro RE. The health and functional status of veterans with diabetes. Diabetes Care 1994;17:318-21.
14. Trief PM, Grant W, Elbert K, Weinstock RS. Family environment, glycemic control, and the psychosocial adaptation of adults with diabetes. Diabetes Care 1998;21:241-5.
15. Johnson JA, Nowatzki TE, Coons SJ. Health-related quality of life of diabetic Pima Indians. Med Care 1996;34:97-102.
16. Weinberger M, Kirkman MS, Samsa GP, et al. The relationship between glycemic control and health-related quality of life in patients with non-insulin-dependent diabetes mellitus. Med Care 1994;32:1173-81.
17. Bardsley MJ, Astell S, McCallum A, Home PD. The performance of three measures of health status in an outpatient diabetes population. Diabet Med 1993;10:619-26.
18. Testa MA, Simonson DC, Turner RR. Valuing quality of life and improvements in glycemic control in people with type 2 diabetes. Diabetes Care 1998;21(suppl 3):C44-52.

19. Wandell PE, Brorsson B, Aberg H. Quality of life among diabetic patients in Swedish primary health care and in the general population: comparison between 1992 and 1995. Qual Life Res 1998;7:751-60.
20. Petterson T, Lee P, Hollis S, Young B, Newton P, Dornan T. Well-being and treatment satisfaction in older people with diabetes. Diabetes Care 1998;21:930-5.
21. Van der Does FEE, de Neeling JND, Snoek FJ, et al. Symptoms and Well-Being in Relation to Glycemic Control in Type II Diabetes. Diabetes Care 1996;19:204-10.
22. Van der Does FEE, de Neeling JND, Snoek FJ, et al. Randomized study of two different target levels of glycemic control within the acceptable range in type 2 diabetes. Effects on well-being at 1 year. Diabetes Care 1998;21:2085-93.
23. Mitchel BD, Stern MP, Haffner SM, Hazuda HP, Patterson JK. Functional impairment in Mexican Americans and non-Hispanic whites with diabetes. J Clin Epidemiol 1990;43:319-27.
24. Reenders K, van den Hoogen HJM, van Weel C. Functionele toestand, complicaties en comorbiditeit bij 249 NIDDM-patiënten. Huisarts Wet 1992;35:386-90.
25. Rubin RR, Peyrot M. Quality of life and diabetes. Diabetes Metab Res Rev 1999;15:205-18.
26. Hirsch A, Bartholomae C, Volmer T. Dimensions of quality of life in people with non-insulin-dependent diabetes. Qual Life Res 2000;9:207-18.
27. Hanestad BR, Graue M. To maintain quality of life and satisfactory metabolic control in type 2 diabetes patients: one and the same? Qual Life Res 1995;4:436-7.
28. Bourdel-Marchasson I, Dubroca B, Manciet G, Decamps A, Emeriau JP, Dartigues JF. Prevalence of diabetes and effect on quality of life in older French living in the community: the PAQUID Epidemiological Survey. J Am Geriatr Soc 1997;45:295-301.
29. Ormel J, Kempen GIJM, Deeg DJH, Brilman EI, Van Sonderen E, Relyveld J. Functioning, Well-being, and Health Perception in Late Middle-Aged and Older People: Comparing the Effects of Depressive Symptoms and Chronic Medical Conditions. JAGS 1998;46:39-48.
30. Peyrot M, Rubin RR. Levels and risks of depression and anxiety symptomatology among diabetic adults. Diabetes Care 1997;20:585-90.
31. Jacobson AM, de Groot M, Samson JA. The effects of psychiatric disorders and symptoms on quality of life in patients with type I and type II diabetes mellitus. Qual Life Res 1997;6:11-20.
32. Kohen D, Burgess AP, Catalan J, Lant A. The role of anxiety and depression in quality of life and symptom reporting in people with diabetes mellitus. Qual Life Res 1998;7:197-204.
33. U.S. Preventive Services Task Force. Screening for depression: Recommendations and rationale. Ann Intern Med 2002;136:760-4.
34. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.
35. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.

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CASE REPORT

Severe early onset osteopenia and osteoporosis caused by antiepileptic drugs

K. Beerhorst[1], F.C. Huvers[2], W.O. Renier[3*]

[1]Department of Neurology, Canisius Wilhelmina Hospital, Nijmegen, the Netherlands, Departments of [2]General Internal Medicine, and [3]Neurology and Child Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, tel.: +31 (0)24-361 33 94, fax: +31 (0)24-354 11 22, e-mail: w.renier@neuro.umcn.nl, *corresponding author

ABSTRACT

We describe two adult patients with epilepsy who received long-term antiepileptic drug therapy, a woman aged 39 years and a man aged 38 years, in whom severe osteopenia and osteoporosis, respectively, were diagnosed. Both had had epilepsy since childhood, both were seizure free and off medication for several years before the epilepsy started again. The female patient first sustained a complicated pelvis fracture after minor trauma. Next, both patients had infractions of several thoracic vertebrae after a generalised tonic-clonic seizure. Dual-energy X-ray absorptiometry for measurement of the bone mineral density revealed osteopenia in both. Bone biopsy was only performed in the male patient and showed moderate osteoporosis. Taking into consideration the young age for osteopenia and osteoporosis and the absence of other underlying causes, the long-term anticonvulsant therapy is the most likely cause of the development of osteopenia and osteoporosis in these patients.

Reviewing recent literature data, advice from healthcare organisations and medical guidelines, the authors were surprised by the lack of protocols and preventive measures for patients with epilepsy who have been on antiepileptic drug therapy for many years. With this article we stress the urgent need to develop protocols and guidelines for preventive interventions.

KEYWORDS

Antiepileptic drugs, epilepsy, osteopenia, osteoporosis

INTRODUCTION

Epilepsy is a chronic neurological disease with an estimated prevalence of five to seven patients per 1000 inhabitants in the Netherlands.[1] Seizures are successfully treated with antiepileptic drugs (AEDs) in about 70% of the patients. Usage of AEDs for 15 years is not uncommon, despite the fact that long-term AED therapy is a known risk factor for bone loss and fractures[2] and is associated with abnormalities in calcium metabolism, including hypocalcaemia, elevated levels of serum alkaline phosphatase and serum parathyroid hormone, reduced serum levels of biologically active vitamin D metabolites, radiological evidence of rickets and histological evidence of osteomalacia.[3-8] Patients aged >50 years and treated for ≥5 years with AED are estimated to have a doubled risk of osteoporotic fractures.[9] Of the female patients, 50% had a hip fracture unrelated to seizure activity.[10]

AEDs such as carbamazepine (CBZ), phenobarbital, and phenytoin accelerate hepatic microsomal metabolism of vitamin D to polar metabolites other than (25-OH) vitamin D (25-OHD) and increase the metabolism of 25-OHD into biologically inactive products.[11] Vitamin D and calcium play an essential role in diseases affecting bone metabolism. Hypovitaminosis D or hypocalcaemia can cause skeletal abnormalities, varying from osteopenia and osteoporosis to manifest osteomalacia.[12] Vitamin D deficiency is frequently cited as the cause of bone loss in patients with epilepsy.[2] Inadequate mineralisation of newly formed bone matrix as a consequence of this vitamin D deficiency can result in osteomalacia and rickets in adults and children, respectively.[10,13,14] Moreover, valproic acid (VPA) may have a direct effect on osteoblasts and osteoclasts resulting in an increased bone turnover and degradation.[15]

The first reports about negative effects of AEDs on bone metabolism were published in the 1960s to 1970s. In these reports particularly the first-generation AEDs, phenobarbital and phenytoin (both enzyme-inducing AEDs), were studied.[3,6,16] However, recent investigations show that the second-generation AEDs, CBZ and VPA, also have negative effects on bone metabolism.[2,8,10,15,17-19] Less is known about the latest or third-generation AEDs because they have not been on the market so long.
To illustrate these negative effects of long-term AED therapy on bone metabolism we describe two cases of adult patients with epilepsy since childhood and with an unexpected early onset osteopenia and osteoporosis after long-term (first- and second-generation) AED therapy. AED therapy was even interrupted for several years in both cases.

CASE REPORT 1

A 39-year-old Caucasian woman had suffered from epilepsy since childhood. The first generalised tonic-clonic seizure occurred at the age of 10 years. Several single- and multi-antiepileptic drug formulas were initiated with adequate consecutive serum drug levels. First-generation AEDs were replaced by second-generation AEDs because of increasing seizure frequency and/or side effects. Overall, the patient had been taking phenobarbital for nine years including six months in combination with ethosuximide, one year with VPA and two years with CBZ, VPA monotherapy for almost three years, oxcarbazepine monotherapy for eight years and lamotrigine monotherapy for three years. She had never taken phenytoin. For six years (from the age of 12 until 15 and from 25 to 28 years) she was seizure free without AEDs.
At the age of 5 and 7 years, the patient sustained a cruris fracture after trauma. She had never had any restriction in her mobility and was on a normal and varied diet with an average intake of dairy products. At the age of 35 years, she sustained a complicated pelvis fracture after a fall from a low chair. Physical and neurological examination revealed no other abnormalities. The patient weighed 72 kg with a length of 1.70 m (body mass index [BMI] 24.9 kg/m^2). She smoked ten cigarettes a day and had a moderate intake of alcohol. Her menstrual cycle was regular without any sign of premature ovarian failure. For several years she had lived in a country with a tropical climate and had had extensive sun exposure.
Laboratory examinations revealed normal values for serum calcium of 2.32 mmol/l, (25-OH) vitamin D 62 nmol/l and (1,25-(OH)$_2$) vitamin D$_3$ 48 pmol/l, thyroid-stimulating hormone (TSH) 2.38 mU/l, parathyroid hormone (PTH) 2.0 pmol/l and a 24-hour urine calcium excretion of 6.1 mmol. Kidney and liver function were normal.

Bone mineral density (BMD) measured with dual X-ray absorptiometry (DEXA) scanning is expressed in standard deviations from the average peak BMD in healthy young persons of the same gender (T-score) and from the mean BMD for persons of the same age and gender (Z-score).[10] Both scores represent fracture risk (*table 1*).

Table 1 *WHO criteria for defining bone density*[28]

Condition	Description
Normal	BMD value within 1.0 SD of the young adult reference mean (T ≥-1.0)
Ostepenia	BMD value of >1 SD below the young adult mean but <2.5 SD below this value (-1.0 >T >-2.5)
Osteoporosis	BMD value of ≥2.5 SD below the adult mean value (T ≤-2.5)
Established osteoporosis	BMD value of ≥2.5 SD below the adult mean value (T ≤-2.5) in the presence of one or more fragility fractures

WHO = World Health Organization; BMD = bone mineral density; SD = standard deviation.

DEXA scanning of the lumbar spine and of the femoral neck in the patient showed T-scores and Z-scores of -1.6 and -1.6 SD measured at the lumbar spine and -2.1 and -1.9 SD measured at the femoral neck, compatible with osteopenia.
After initial surgical treatment the patient was subsequently treated with both alendronine acid and calcitriol for one year, during which bone mass increased according to the altered T- and Z-scores which were -1.5 and -1.4 SD at the lumbar spine and -2.1 and -1.8 SD at the femoral neck. Since the patient wanted to become pregnant after recovery from the pelvic fractures, only the calcitriol was continued. After an uncomplicated pregnancy and delivery of a healthy girl, breast feeding was started and only calcitriol treatment has been continued until the present day.
At the age of 36 years the patient had a generalised tonic-clonic seizure. Postictally she complained of severe back pain. An X-ray of the spine revealed an infraction of two thoracic vertebrae in a still osteopenic skeleton.

CASE REPORT 2

A 38-year-old Caucasian male had suffered from epilepsy since childhood. The patient's history revealed no other diseases, nor bone fractures. He was on a varied diet and both body weight and body development have been normal. He had always refrained from smoking and alcoholic beverages and had practiced sports all his life.

He had his first seizure at the age of 7 years, several weeks after a paramyxovirus infection (measles). An EEG showed generalised epileptic paroxysms and AED treatment was started. Overall, the patient had been on phenobarbital for two years, phenytoin for nine years, CBZ for 12 years and VPA for seven years, in single or combined therapies. Standard doses of AEDs were used and drug levels had always been within therapeutic ranges. He was seizure free for a period of 24 years. During the last ten years of this period, he was not on any AEDs.

At the age of 31 years, the patient experienced severe back pain after a generalised tonic-clonic seizure. At physical examination, his body weight was 85 kg and length 1.90 m (BMI 23.5 kg/m^2). Except for pain over the thoracic and lumbar vertebrae no other abnormalities were found on physical and neurological examination.

Laboratory investigations revealed normal serum values for calcium of 2.33 mmol/l, phosphate 1.18 mmol/l, alkaline phosphatase 93 U/l, testosterone 21 nmol/l, TSH 0.43 mU/l, osteocalcine 3.2 μmol/l, (25-OH) and vitamin D 37 nmol/l. No Bence-Jones paraproteins were detectable in the urine. Urinary excretion of calcium and hydroxyproline were slightly elevated: 7 to 9 mmol/24 h (normal <5 mmol/24 h) and 0.31-0.33 mmol/24 h (normal 0.25 mmol/24 h), respectively. Hepatic and renal function were normal.

An EEG showed epileptic activity over the left temporal side. A CT scan of the brain was normal. X-rays of the thoracic and lumbar spine showed severe osteopenia and traumatic anterior flattening of vertebrae Th7 to Th10 with traumatic infractions.

BMD was measured with a DEXA scan: T-score -1.6 SD at the lumbar spine, T-score -1.3 SD at the femur neck, compatible with osteopenia. Bone biopsy showed moderate osteoporosis with slightly elevated bone turnover activity in the trabecular area.

Treatment with alendronine acid, a bisphosphonate, was started in addition to VPA. Two years after continuous alendronine acid treatment, a control DEXA scan showed an increase in the BMD of the lumbar spine with a T-score of -1.1 SD and at the femoral neck a T-score of -1.2 SD.

DISCUSSION

Both cases illustrate the negative effects of long-term AED therapy during childhood and adolescence on bone structure in adulthood. In both cases AED therapy was started in childhood. Both patients also had several years of seizure freedom in which they were off AED medication. The female subject was treated for nine years with first-generation AEDs during childhood and adolescence and for six years with second-generation AEDs. The male subject was treated for nine years with first-generation AEDs during childhood and adolescence and for 19.5 years with second-generation AEDs.

Other causes of osteopenia and osteoporosis could reasonably be excluded by the normal serum concentrations of calcium, vitamin D, presence of normal renal and hepatic function, absence of malabsorption or endocrine diseases and the otherwise normal physical development of the subjects.

The first cases of the association between bone disorders and AED therapy were reported around the 1960s to 1970s.[3,6,16] Prevalence varies between 4 to 70%, depending on the population studied.[3] The long-term negative effects of phenobarbital and phenytoin on bone structure are well known. Both AEDs are enzyme-inducing AEDs. They were used by our patients for several years during childhood. These enzyme-inducing AEDs accelerate hepatic microsomal metabolism of vitamin D to polar metabolites other than (25-OH) vitamin D (25-OHD) and increase the metabolism of 25-OHD into biologically inactive products resulting in decreased active ($1,25(OH)_2$) vitamin D_3 concentrations.[7,11,20] In contrast to other steroid hormones, vitamin D is not produced by an endocrine organ with a hormonal feedback mechanism. PTH can stimulate hydroxylation of vitamin D3, but it cannot stimulate the production of vitamin D3 by the skin. A ($1,25(OH)_2$) vitamin D_3 deficiency results in an increased bone turnover activity in the presence of a secondary hyperparathyroidism. Longstanding vitamin D deficiency causes a progressive defective mineralisation in the newly formed bone matrix eventually ending in rickets in children and osteomalacia in adults.[10,12-14]

Similar negative effects of the second-generation AEDs (CBZ and VPA) on bone metabolism have been reported.[8,10,15,18] CBZ is also an enzyme-inducing AED. In contrast, VPA is a nonenzyme-inducing AED and thus its negative effects on bone metabolism cannot be explained by the mixed function oxidase induction. Several mechanisms for the negative effects of VPA on bone metabolism have been proposed. First, both the nonenzyme-inducing and the enzyme-inducing AEDs may have direct effects on the balance of osteoblast and osteoclast activity.[15] Second, VPA could induce hormonal changes, as suggested from the association of VPA treatment and the development of a polycystic ovary syndrome.[21] However, a definite explanation for the association between VPA use and the decreased BMD remains undefined.[15] To date there are several new nonenzyme-inducing AEDs on the market, but long-term follow-up of possible side effects on bone mass is still lacking.[10]

It is possible that in our patients a combination of the above-mentioned mechanisms eventually caused a lower BMD, leading to osteopenia and osteoporosis. They had both been on multiple AEDs for many years. The normal

vitamin D level in both patients, who had a normal feeding pattern, is less compatible with the enzyme-induction mechanism. However, one cannot exclude this mechanism as a cause of the bone metabolism abnormalities because both patients took the enzyme-inducing AEDs during their childhood. At that age, laboratory investigations for vitamin D were not carried out. It is known that the BMD continues to increase until about the age of 30 years. After this age, BMD slowly decreases. It is not unlikely that long-term AED use by our patients during their childhood and adolescence caused a lower peak BMD during their 20s to 30s. Consequently, this could impose a greater risk of sustaining fractures throughout their lives and not only during the period in which they were taking AEDs. Moreover, they were still on AEDs with known negative effects on bone metabolism during young adulthood.

In a recent study in the USA, 62% of the children/ adolescent patients and 77% of the adult patients had low vitamin D levels and one third of the children and adults with epilepsy treated with various forms of AEDs had serum concentration of <10 ng/ml. Furthermore, the BMD of the lumbar spine, femur head, femoral neck and trochanter was significantly decreased in these adults for both the T- and Z-score, and 59% had osteopenia at either the spine or the hip.[8] No correlation was observed between the type of AED (enzyme-inducing vs nonenzyme-inducing) and 25-OHD levels. However, no data are available about the (1,25-$(OH)_2$) vitamin D_3 levels, the most potent natural hormone analogue.

Several DEXA studies confirm the observed decrease in BMD during AED therapy.[2,10,15,22] DEXA facilities are widely available, generate a minimal radiation load, and are suitable for follow-up studies. Unfortunately, with DEXA one cannot differentiate between a decrease in the amount of bone (osteoporosis) and improper mineralisation of bone matrix (osteomalacia). Both conditions can only be differentiated by histomorphometric analysis of a bone biopsy.[12]

In the Netherlands no data are available about the prevalence of osteopenia, osteoporosis and osteomalacia nor of the vitamin D status in patients with epilepsy. Probably more relevant are data on the AED effects on the clinical end parameters BMD and fractures. Although the relation between long-term AED therapy and negative effects on bone metabolism has been known for many years, it has neither led to the development of national guidelines for prevention nor to the development of protocols for surveillance and treatment in the Netherlands. Despite the presence of around 120,000 patients with epilepsy in the Netherlands, neither its Council of General Practitioners (NHG), nor the Dutch Health Council[23] seem to be aware of this particular group at risk for the development of skeletal abnormalities/ bone diseases. Moreover, to date the Dutch Osteoporosis Foundation and the Dutch League against Epilepsy have not yet focused their attention on the prevention of metabolic bone abnormalities as a result of long-term AED therapy.

Extrapolating from an informal poll at a recent meeting of the Dutch League against Epilepsy it is estimated that only less than 5% of the neurologists perform active screening for bone disorders/osteoporosis in patients on long-term AED therapy. In the USA one third of the neurologists screen their patients for bone disorders.[24]

In the Netherlands, the estimated fracture incidence is about 5 pro mille, which means that in one year at least 500 to 600 patients with epilepsy have a bone fracture of any kind. It is not unlikely that, in agreement with the Farhat data,[8] the incidence is outnumbered in patients using AEDs chronically. It is not known how many patients on long-term AEDs will eventually develop either osteopenia or osteoporosis and how long they should take this medication. No data are available about the long-term effects of vitamin D treatment in these patients. However, recent studies show that even one month of treatment with 150,000 IE (25-OH) vitamin D_3 results in a significant increase in bone mass.[25,26]

The annual costs of treatment with 1 dd 800 IE cholecalciferol and 1 dd gram calcium[4] are estimated at around € 200 and just around € 30 for vitamin D alone. The direct costs of a spontaneous fracture of a vertebrae or forearm fracture are estimated at around € 1000 per year, for a hip fracture the direct costs are around € 12,000,[27] not counting the secondary costs due to loss of working capacity. Therefore, development of guidelines for screening and treatment for metabolic bone disease in epileptic patients at high risk seems reasonable and cost effective. We conclude that osteopenia and later on osteoporosis as a result of long-term AED use is an underestimated problem in patients taking AEDs, which theoretically can be quite easily prevented or treated.

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REFERENCES

1. Meinardi H, Deckers CLP, Hekster YA, et al. Praktische keuzecriteria voor anti-epileptica. Alphen aan de Rijn: Van Zuiden Communications, 1999.
2. Andress DL, Ozuna J, Tirschwell D, et al. Antiepileptic drug-induced bone loss in young male patients who have seizures. Arch Neurol 2002;59:781-6.
3. Hahn TJ. Bone complications of anticonvulsants. Drugs 1976;12:201-11.

4. Hahn TJ, Halstead LR. Anticonvulsant drug-induced osteomalacia: alterations in mineral metabolism and response to vitamin D3 administration. Calcif Tissue Int 1979;27:13-8.
5. Hahn TJ. Drug-induced disorders of vitamin D and mineral metabolism. Clin Endocrin Metab 1980;9:107-27.
6. Richens A, Rowe DJ. Disturbance of calcium metabolism by anticonvulsant drugs. BMJ 1970;4:73-6.
7. Christiansen C, Rodbro P, Tjellesen L. Pathophysiology behind anticonvulsant osteomalacia. Acta Neurol Scan Suppl 1983;94:21-8.
8. Farhat G, Yamout B, Mikati MA, Demirjian S, Sawaya R, El Hajj FG. Effect of anti-epileptic drugs on bone density in ambulatory patients. Neurology 2002;58:1348-53.
9. Sheth RD. Bone health in epilepsy. Epilepsia 2002;43:1453-54.
10. Stephen LJ, McLellan AR, Harrison JH, et al. Bone density and antiepileptic drugs: a case-controlled study. Seizure 1999;8:339-42.
11. Hamersvelt HW. Brains and bones. Nephrol Dial Transplant 1998;13:801-2.
12. Van Daele PLA, Pols HAP. Metabole botafwijkingen bij maag-, darm-, en leverziekten. Ned Tijdschr Geneeskd 2000;144:462-7.
13. Haussler MR, McCain TA. Basic and clinical concepts related to vitamin D metabolism and action (second of two parts). N Engl J Med 1977;297:1041-50.
14. Wauters IM, van Soesbergen RM. Disease caused by lack of sunlight: rickets and osteomalacia. Ned Tijdschr Geneeskd 1999;143:593-7.
15. Sato Y, Kondo I, Ishida S, et al. Decreased bone mass and increased bone turnover with valproate therapy in adults with epilepsy. Neurology 2001;57:445-9.
16. Dent CE, Richens A, Rowe DJ, Stamp TC. Osteomalacia with long-term anticonvulsant therapy in epilepsy. BMJ 1970;4:69-72.
17. Kroger H, Kotaniemi A, Vainio P, Alhava E. Bone densitometry of the spine and femur in children by dual-energy x-ray absorptiometry. Bone Miner 1992;17:75-85.
18. Sheth RD, Wesolowski CA, Jacob JC, et al. Effect of carbamazepine and valproate on bone mineral density. J Pediatr 1995;127:256-62.
19. Verrotti A, Greco R, Latini G, Morgese G, Chiarelli F. Increased bone turnover in prepubertal, pubertal, and postpubertal patients receiving carbamazepine. Epilepsia 2002;43:1488-92.
20. Perucca E. Pharmacokinetic interactions with antiepileptic drugs. Clin Pharmacokinet 1982;7:57-84.
21. Henderson RC, Lark RK, Gurka MJ, et al. Bone density and metabolism in children and adolescents with moderate to severe cerebral palsy. Pediatrics 2002;110(1 Pt 1):e5.
22. Rasgon N. The relationship between polycystic ovary syndrome and antiepileptic drugs; a review of the evidence. J Clin Psychopharmacol 2004;24(3):322-34.
23. Gezondheidsraad. Voedingsnormen: calcium, vitamine D, thiamine, riboflavine, nacine, pantotheenzuur en biotine. Nr. 2000/12 ed. Den Haag: 2000.
24. Valmadrid C, Voorhees C, Litt B, Schneyer CR. Practice patterns of neurologists regarding bone and mineral effects of antiepileptic drug therapy. Arch Neurol 2001;58:1369-74.
25. Van de Bergh JP, Smals AG, Schweitzer DH, Hermus AR. Ultrasound measurement of calcaneus: a promising method for prediction of osteoporotic fractures. Ned Tijdschr Geneeskd 2001;145:1105-9.
26. Pedrera JD, Canal ML, Carvajal J, et al. Influence of vitamin D administration on bone ultrasound measurements in patients on anticonvulsant therapy. Eur J Clin Invest 2000;30:895-9.
27. Kwaliteitsinstituut voor de Gezondheidszorg CBO. Tweede herziene richtlijn osteoporose. Alphen aan de rijn: Van Zuiden Communications, 2002.
28. Assessment of osteoporotic fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group. World Health Organ Tech Rep Ser 1994;843:1-129.

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CASE REPORT

Moraxella catarrhalis sepsis in a patient with juvenile spinal muscle atrophy

I.C.D. Westendorp[1*], M.A. Tiemessen[1,2], M. de Jong[1], A. Soomers[1], I.M.M.J. Wakelkamp[1], W.G. Boersma[2]

Departments of [1]Intensive Care and [2]Pulmonary Diseases, Alkmaar Medical Centre, Alkmaar, the Netherlands, [*]corresponding author: Department of Cardiology, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands, tel.: +31 (0)20-599 30 32, fax: +31 (0)20-599 39 97, e-mail: iwestendorp@cs.com

ABSTRACT

***Moraxella catarrhalis* rarely causes severe infections or bacteraemia in healthy subjects. In the literature only four cases of clinical sepsis with *M. catarrhalis* have been described, mostly in immunocompromised patients. We describe a case of a 34-year-old patient with Kugelberg-Welander disease and low body weight (28 kg) who developed clinical sepsis due to *M. catarrhalis* bacteraemia. A review of the literature is given.**

KEYWORDS

Juvenile spinal muscle atrophy, Kugelberg-Welander disease, malnutrition, *Moraxella catarrhalis*, pneumonia, sepsis

INTRODUCTION

After *Streptococcus pneumoniae* and *Haemophilus influenzae, Moraxella catarrhalis* is the third commonest pathogen of the respiratory tract among patients with chronic obstructive pulmonary disease (COPD).[1] Although *M. catarrhalis* is often present in the respiratory tract as a commensal micro-organism, it can clearly be pathogenic under certain circumstances.[2,3] Its importance as a pathogen, both in children and in adults, has been increasingly recognised in the past decades.[4]

Invasive infections with *M. catarrhalis* have seldom been described: in the literature only four cases of clinical sepsis (i.e. septic shock) with *M. catarrhalis* have been described in adults, although more cases (at least seven) have been reported in children. Bacteraemia (at least 61 reported cases) and severe infections with *M. catarrhalis* are also infrequent but have been described in immunocompromised patients, mainly those with haematological malignancies, in patients with chronic obstructive pulmonary diseases and in the elderly, as well as in healthy subjects. Our patient, with severe muscle atrophy, very low body weight and restrictive lung disease and therefore extremely limited (respiratory) reserves, demonstrates the favourable outcome with aggressive treatment of this rare sepsis.

CASE DESCRIPTION

A 34-year-old male, known to have juvenile spinal muscle atrophy type III (JSMA-III or Kugelberg-Welander disease) and moderately severe restrictive lung disease, was admitted with pneumococcal pneumonia, diagnosed by sputum cultures. He was treated with bronchodilators, prednisolone (50 mg/24 h) and amoxicillin, and later penicillin, intravenously. The patient had been treated with prednisolone twice before, but not recently. His condition improved at first, but later deteriorated and on day 5 of admission the patient became respiratory insufficient with recurrence of fever (39.2 °C). Laboratory investigations showed low creatinine (18 μmol/l), urea (2.0 mmol/l), albumin (31.1 mmol/l), and a severe hypophosphataemia (0.26 mmol/l), with an increased white blood cell count (24.2 x 10^9/mm^3) and C-reactive protein (126 μm/l). During his admission his body weight decreased from 28 to 22 kg, probably as a result of decreased dietary and

fluid intake. Differential diagnostic considerations consisted of nosocomial superinfection, aspiration – as the patient had weakened and had difficulty in swallowing – development of resistance to penicillin, ARDS or empyema.

The patient was transferred to the ICU for mechanical ventilation. Blood pressure was 85/45 mmHg and heart rate 135 beats/min. Measurements with a pulmonary artery catheter showed a septic profile with low systemic vascular resistance (450 dynes.sec/cm^5), a central venous pressure of 8 mmHg, a systolic pulmonary artery pressure (SPAP) of 18 mmHg and diastolic pulmonary artery pressure (DPAP) of 10 mmHg. Cardiac output and index were increased (CO 7.8 l/m, CI 5.9 l/min/m^2). Possible causes for the severe hypoposphataemia that were considered were, in the first place, respiratory alkalosis, which often occurs in sepsis. Secondly, the use of glucocorticoids can cause hypophosphataemia. Furthermore saline infusion, which can cause hypocalcaemia and parathyroid hormone release, or a decreased dietary intake were considered. Treatment consisted of intravenous substitution, restoration of acid/base balance, and rapid commencement of parenteral feeding. Additional treatment consisted of fluid replacement, dobutamine, and noradrenalin. On day 5 in the ICU (day 10 after admission), the sputum and two blood cultures that had been taken on admission to the ICU yielded *M. catarrhalis* and the penicillin was changed to amoxicillin/clavulanic acid, which improved the patient's condition markedly. After a total stay of 48 days the patient could be discharged from hospital, on ventilation via a tracheal cannula for 20 hours/day. Six months after discharge from our ICU, the patient is on night time ventilation only.

DISCUSSION

We describe a patient with benign JSMA-III and moderately severe restrictive lung disease who developed a nosocomial sepsis with *M. catarrhalis* after pneumococcal pneumonia. The diagnosis of JSMA-III, a disease of slowly progressive limb-girdle weakness, had been made in childhood in our patient. The prediction of progression and degree of disability is difficult and extremely variable, but most patients are bound to a wheelchair by their mid-thirties. The cause of death in these patients is often pneumonia. Our patient suffered severe wasting and muscle weakness and contractions, rendering him wheelchair bound.
The low body weight of our patient may well have played a role in the course of the disease. Defence mechanisms to infection have been shown to be strongly affected by nutritional status.[4] Both deficiency of protein energy and individual nutrients (trace minerals and vitamins, particularly zinc, iron, selenium, vitamins A, B_6, C and E) are associated with impairment of cell-mediated immunology, complement activation and secretory immunoglobulin antibody response. Complement plays an important role in host defenses against *M. catarrhalis*, as does the IgG3 response to *M. catarrhalis* outer membrane proteins.[2]
It has been estimated that approximately 1 to 5% of healthy adults is colonised with *M. catarrhalis*, and even higher percentages are found among children and among patients with COPD.[5] In the last decades *M. catarrhalis* has been recognised and a possible virulent pathogen, both in the respiratory tract as in other clinical situations.[2] While colonisation with *M. catarrhalis* is highly prevalent, over the last two decades only 61 cases of bacteraemia with *M. catarrhalis* have been described, whereas only four cases of clinical sepsis have been reported in adults, and at least seven cases in children. Although we must assume that the reported cases present only the 'tip of the iceberg' the *M. catarrhalis* sepsis remains relatively rare. Among adults 60 to 70% of the patients with bacteraemia were immunocompromised or had underlying pulmonary disease (COPD), but also other immunocompetent hosts have been described.[6,7] Bacteraemia was found to be associated with sinusitis, pharyngitis, pneumonia, meningitis, and endocarditis and without a focal source.[2,7] Clinical manifestations vary from mild febrile illness to severe and fatal disease.[2,7] Mortality from *M. catarrhalis* bacteraemia has been estimated in a recent review to be close to 20%,[7] with the highest mortality among patients with endocarditis (four out of five reported patients).[6,7]

Most cases described in the literature (at least seven) occurred in children.[8-10] Two cases were described as early as 1925 and 1928, but details of these cases are difficult to assess.[11,12] More recently only four cases of sepsis due to *M. catarrhalis* have been described in adults (*table 1*). In the three out of four adult cases comorbidity was present: lung cancer,[2] acute myelogenous leukaemia[13] and systemic lupus erythematosis.[14] The fourth patient was a previously healthy 68-year-old man who developed shock and disseminated intravascular coagulation after otitis media with *M. catarrhalis* in all blood and middle-ear cultures.[15] In our case the patient had severe muscle atrophy, restrictive lung disease, and severely reduced body weight, which probably played a role in the development of pneumonia. Only one of the four cases of sepsis in adults had a fatal outcome.
On admission to the ICU several treating physicians were hesitant to start intensive treatment and mechanical ventilation, as the prognosis of the patient appeared to be extremely poor. This case demonstrates that this sepsis can have a positive outcome with reasonable subsequent quality of life when treated, although the prognosis of sepsis with *M. catarrhalis* is strongly dependent on comorbidity.

Table 1 *Overview of adult cases of sepsis with Moraxella catarrhalis, as reported in the literature*

Authors	Year of publication	Sex	Age	Comorbidity	Focus of infection	Initiated therapy	Survival
Wallace *et al.*	1990	♂	71	Lung cancer	Pneumonia	1st erythromycine + piperacilline + amikacine	No
Zenklusen *et al.*	1985	♂	63	Aute myelogenous leukaemia	No focal source	Exact therapy unknown	Yes
Guthrie *et al.*	1988	♀	40	Lupus erythematosis (dormant)	Respiratory tract infection	1st erythromycin 2nd ceftazidime	Yes
Alaeus *et al.*	1991	♂	68	None	Otitis media	1st penicillin 2nd cefuroxim/gentamycin 3rd erythromycin	Yes
Westendorp *et al.*	2005	♂	34	Juvenile spinal muscle atrophy type III (Kugelberg-Welander syndrome)	Pneumonia	1st penicillin 2nd amoxicillin + clavulanic acid	Yes

1st = the first initiated therapy; 2nd = the second initiated therapy, after discontinuation of the first; 3rd = the third initiated therapy, after discontinuation of the first and second.

REFERENCES

1. Enright MC, McKenzie H. Moraxella (Branhamella) Catarrhalis - clinical and molecular aspects of a rediscovered pathogen. J Med Microb 1997;46:360-71.
2. Wallace MR, Oldfield III EC. Moraxella (Branhamella) Catarrhalis Bacteremia, a case report and literature review. Arch Int Med 1990;150:1332-4.
3. Domingo P, Puig M, Pericas R, Mirelis B, Prats G. Moraxella catarrhalis bacteremia in an immunocompetent adult. Scand J Inf Dis 1995;27:95.
4. Chandra RK. Nutrition and immunology; from the clinic to cellular biology and back. Proc Nutr Soc 1999;58(3):681-3.
5. Murphy T. Moraxella (Branhamella) Catarrhalis and Other Gram-Negative Cocci. In: Mandell GL (ed). Mandell, Douglas & Bennett's Principles and Practice of Infectious Diseases. 5th edition. Philadelphia: Churchill Livingstone, 2000: 2259-66.
6. Ioannidis JPA, Worthington M, Griffiths JK, Snydman DR. Spectrum and significance of bacteremia due to Moraxella catarrhalis. Clin Inf Dis 1995;21:390-7.
7. Thorsson B, Haraldsdottir V, Kristjansson M. Moraxella catarrhalis bacteremia. A report on 3 cases and a review of the literature. Scand J Inf 1998;30:105-9.
8. Burnett AK, Easton DJ, Gordon AM, Rowan RM. Neisseiria Catarrhalis septicaemia in acute lymphoblastic leukemia. Scott Med J 1985;20:37-8.
9. Wong VK, Ross LA. Branhamella catarrhalis septicaemia in an infant with AIDS. Scand J Inf Dis 1988 ;20:599-60.
10. Van Doern G, Miller MJ, Winn RE. Branhamella (Neisseiria) catarrhalis systemic infection in humans. Arch Int Med 1981;141:1690-2.
11. Endres VG. Der Mikrococcus catarrhalis als erreger einer sepsis mit Endocarditis und Nephritis. Munchener Medizinische Wochenschrift 1925;72:723-4.
12. Nagell H. Ueber eine sepsis, hervorgerufen durch micrococcus catarrhalis. Munchener Medizinische Wochenschrift 1928;75:124-5.
13. Zenklusen C, Erhard PH, Modde H, Siegenthaler P. Septicaemie a Branhamella (Neisseiria) Catarrhalis chez une patiente immunodeprimee. Schweis Med Wochenschr 1985;115:1106-7.
14. Guthrie R, Bakenhaster K, Nelson R, Woskobnick R. Branhamella Catarrhalis sepsis: a case report and literature review. J Inf Dis 1988;158:907-8.
15. Alaeus A, Stiernstedt G. Branhamella catarrhalis septicaemia in an immunocompetent adult. Scand J Infect Dis 1991;23:115-6.

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A patient with fever after a visit to South Africa

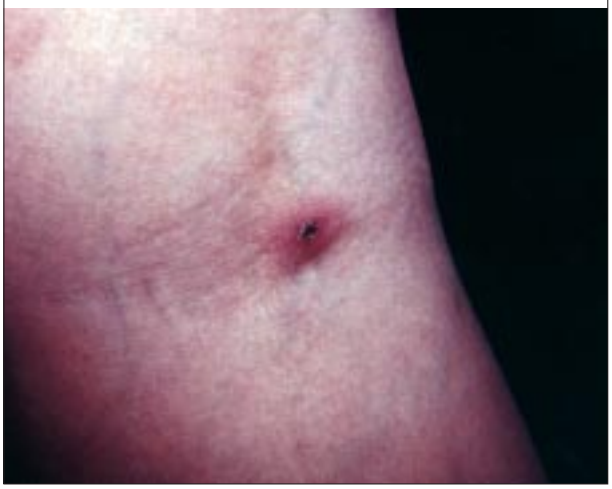
P.H.Th.J. Slee

Department of Internal Medicine, St. Antonius Hospital, Nieuwegein, the Netherlands,
e-mail: p.slee@antonius.net

CASE REPORT

A 37-year-old female patient visited Kruger National Park, South Africa, in March last year. One week later she developed low-grade fever, and a painful right knee and left hip. She noticed painful and enlarged lymph glands in her right inguinal region. She did not feel very ill and continued working. On physical examination an abnormality in the right knee hollow (*figure 1*) and painful and enlarged lymph glands in the inguinal region on the same side were noticed. Laboratory examination showed only an increased sedimentation rate of 23 mm.

Figure 1 *Knee hollow*



WHAT IS YOUR DIAGNOSIS?

See page 237 for the answer to this photo quiz.

ERRATUM

In the answer to the photo quiz 'A patient with dyspnoea, subfebrile temperature and electrocardiographic abnormalities' by H.J. Jansen, H. Haerkens-Arends and G. Vervoort, in the Netherlands Journal of Medicine 2005;63(3):118, the wrong figure was published as figure 2.
We therefore provide you with the correct version of the photo quiz in this issue. Our apologies for any inconvenience caused.

A patient with dyspnoea, subfebrile temperature and electrocardiographic abnormalities

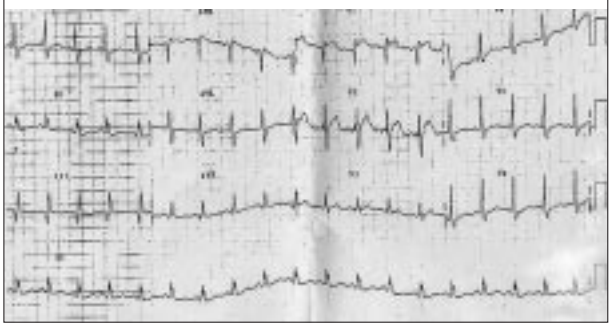
H.J. Jansen[1*], H. Haerkens-Arends[2], G. Vervoort[3]

Department of [1]Internal Medicine, Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands, Department of [2]Cardiology and [3]General Internal Medicine, Radboud University Nijmegen Medical Centre, the Netherlands, e-mail: Henry_jansen@hotmail.com, [*]corresponding author

CASE REPORT

A 66-year-old man was admitted to our hospital because of dyspnoea and slightly elevated body temperature (38.1°C). A month before admission the patient suffered an ischaemic cerebrovascular accident. His medical history also revealed a myocardial infarction seven years ago and a transient ischaemic attack.
On physical examination his blood pressure was 130/80 mmHg, pulse 102 beats/min and regular; the central venous pressure was clearly elevated. The respiratory rate was 36 breaths/min. Examination of the lungs and heart revealed no abnormalities. The right lower extremity was red, warm and swollen and painful on palpation.
Laboratory tests were performed. An arterial blood sample showed no abnormalities. Lactate dehydrogenase was 566 U/l and troponin I 0.51 μg/l. A chest X-ray showed no abnormalities. His electrocardiogram (ECG) is shown in *figure 1*.

Figure 1 *12-lead electrocardiogram of the patient*



WHAT IS YOUR DIAGNOSIS?

See page 238 for the answer to this photo quiz.

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If apoB is so good, why isn't everybody measuring it?

One reason why we need the Netherlands Journal of Medicine!

A.D. Sniderman[1]* M. Rosenbloom[2]

[1]Division of Cardiology, McGill University Health Centre, Montreal, Quebec, Canada, tel.: +514 934-19 34, ext. 34637, fax: +514 843-16 76, e-mail: allan.sniderman@muhc.mcgill.ca, [2]Laboratory for Cardiovascular Research, Royal Victoria Hospital, Montreal, Quebec, Canada, *corresponding author

INTRODUCTION

I recently had the privilege of giving a state-of-the-art lecture on the clinical value of apoB to the residents and staff of the Department of Internal Medicine of the Radboud University Nijmegen Medical Centre after which residents reviewed two recent key papers: the Northwick Park Heart Study[1] and the INTERHEART study.[2] One resident concluded his analysis with the question: if apoB is so good, why isn't everybody doing it?

All at once, I felt everyone's eyes on me: such a simple question, such a difficult answer. My answer was inadequate then and will almost certainly be inadequate now. But I know it is tied in some way to another question: Why do we need a journal such as the Netherlands Journal of Medicine?

I am going to try to answer both: the first by listing the relevant facts, the second by suggesting that we in modern academic medicine are less secure and less confident intellectually than we used to be. The remedy, I believe, is to rediscover our own strengths and by doing so to recover our independence. One way to do so is to encourage independent analyses of major issues in journals such as this.

APOLIPOPROTEIN B *VERSUS* CHOLESTEROL

What is plasma apoB?

Each atherogenic particle – that is to say, each VLDL, IDL, LDL and Lp(a) particle – contains one molecule of apoB100[3] Each chylomicron and chylomicron remnant particle contains one molecule of apoB48. All the standardised, automated assays that measure total plasma apoB recognise both apoB100 and apoB48. However, except in type III hyperlipoproteinaemia, there are so few apoB48 particles present in plasma, even during the peak postprandial period, that total apoB is not affected. This means that for clinical practice apoB does not have to be measured fasting, but can be determined at the patient's convenience. LDL, the most important of the atherogenic particles, account for more than 90% of total plasma apoB particles and so LDL particle number is the principal determinant of the atherogenic particle number.

What is the evidence that apoB is better than any of the other cholesterol indices for estimating the risk of vascular disease?

The evidence is overwhelming. To be sure, the initial generation of cross-sectional and nested case-control studies yielded mixed results, in part because the assays were not standardised, in part because the wrong question was asked (Did the indices being compared predict haemodynamically significant coronary disease – which is not the issue – *vs* just anatomic coronary disease – which is? Did apoB predict better than all the lipids combined including HDL – which is not the test? And finally, the initial types of studies – both cross-sectional and nested case-control studies – generate but do not establish hypotheses).

But time and knowledge have advanced. Multiple, large, prospective epidemiological studies are now in hand and the results are straightforward: apoB is superior to any of the cholesterol indices to predict the likelihood of vascular events. The Quebec Cardiovascular Study was the first of these,[4] followed by the THROMBO Study,[5] the AMORIS

Study,[6] the Northwick Park Heart Study,[1] the THROMBO Metabolic Syndrome Study[7] plus the placebo arms of a number of the statin clinical trials, including 4S,[8] AFCAPS/TexCAPS,[9] and LIPID.[10] Not only has apoB been shown to be better than any of the other cholesterol indices, the apoB/apoA-I ratio has also been shown to be superior to the other cholesterol indices – TC/HDL C, non-HDL C/HDL C, and LDL C/HDL .[5,9-11] The list of citations should be long enough and broad enough to justify the judgment at the beginning of this paragraph that the weight of evidence in favour of apoB as a predictor of vascular disease is, in fact, overwhelming.

What is the evidence that apoB is better than LDL C for judging the adequacy of statin therapy?

Depending on which analysis is examined, either LDL C or apoB was superior in the 4S study, the statin study in which cholesterol levels were highest.[12] However, there is no ambiguity in AFCAPS/TexCAPS[9] the Leiden Heart Study,[13] and LIPID.[10] On-treatment apoB was predictive of outcome, whereas on-treatment LDL cholesterol was not. Moreover, there is evidence for superiority of apoB over LDL cholesterol in CARE[14] and FATS.[15,16] Interestingly, a number of fibrate trials produced the same result. In the Bezafibrate trial, apoB was predictive of progression of coronary disease, whereas LDL cholesterol was not.[17] The same was observed in the DAIS trial.[18] Finally, when LDL particle number was estimated by nuclear magnetic resonance, a decrease in LDL particle number was shown to contribute to benefit in the VA-HIT trial, whereas there was no evidence that benefit correlated with a change in LDL cholesterol (Jim Otvos, personal communication). Non-HDL cholesterol has been proposed as a surrogate for apoB. However, while they are highly correlated, they are not highly concordant. That is, for any value of one, there is a considerable range of values for the other.[19] Moreover, the available evidence from epidemiological studies, noninvasive studies and clinical trials indicates that apoB is superior to non-HDL cholesterol as a marker of the risk of vascular disease and as an index of the adequacy of LDL-lowering therapy.[9-11,13,20-22] Finally, apoB is more closely associated with the other markers of the metabolic syndrome than either LDL or non-HDL cholesterol.[19, 23]

Why is apoB superior to any of the cholesterol indices for estimating risk and assess the adequacy of therapy?

Each atherogenic particle has one molecule of apoB; except for type III hyperlipoproteinaemia, LDL make up the vast majority of these, more than 90%.[24,25] Thus the first major advantage of apoB over LDL cholesterol is that it counts all the atherogenic particles, not just the majority of them. But the gain is much greater than this. The amount of cholesterol in LDL particles can vary substantially and so LDL cholesterol does not necessarily equal LDL particle number.[26-29] The discrepancy can be deadly in patients with predominantly small dense cholesterol-depleted LDL particles. In such patients, LDL cholesterol necessarily underestimates LDL particle number and the error is frequently substantial.[20]

Small dense LDL tend to be the rule with triglycerides >1.5 mmol/l, but the actual apoB cannot be guessed from the calculated LDL cholesterol.[27] Moreover, there is no triglyceride level that ensures small dense LDL are not present. Exceptions abound and no doctor should be confident the patient in front of him or her is not one more exception to a very porous rule. Indeed, the lipid profile may reveal normal plasma triglycerides and LDL cholesterol with low HDL cholesterol. But even in these patients, the apoB may be high or normal.[30] Skip apoB; miss the diagnosis. Skip apoB and therapy may be inadequate.

WHY DO WE NEED THE NETHERLANDS JOURNAL OF INTERNAL MEDICINE?

Listing the evidence that apoB is superior to any of the cholesterol indices is the easy part. Now I must turn to the difficult part: the resident's question, the audience's question, my question. If apoB is so much better, why isn't everybody doing it – or more accurately, why are so few doing it? The answer, if there is one, lies in understanding what governs modern medical decision-making. Many would say that the mark of modern medicine is that what we do is evidence based, by which they mean that what we do is the outcome, so far as possible, of the results of rigorously conducted clinical studies and clinical trials. It is the facts that direct us, nothing more, nothing less.

But is that the real sequence or is there another step that is decisive? Is it the bare and unadorned results from the studies that govern us or is it the interpretation and authorisation by 'expert' groups that really counts? How many of us have read the actual results of a study as opposed to the minimalist summary contained in a review by an 'expert'? For that matter, how many 'experts' really understand all the methods in the studies they review so often and so glibly? How many 'reviews' analyse methods and results, critique design and statistics, discuss opposing interpretations as opposed to merely presenting lists of positive *vs* negative?

Our present is different from our past.

As a young doctor, what I was taught and what we did in the hospital where I trained was based on my teachers' interpretation of the evidence which, in turn, was the

outcome of the interplay between the evidence of others and their own scholarly work. The academic faculty was made up of clinician-researchers. They were doers, analysers, innovators as well as appliers. They had the confidence to fulfil their responsibility as academics to measure the strengths and the weaknesses of new proposals. They could do so largely because they were integrally connected to the world from which change came.

Over the past 40 years, the clinician-researcher model – at least in North America – has been largely torn apart and discarded. Now clinicians may do some research, but they are not confident, and they do not love it. Their greatest commitment is to clinical trials, designed by others, organised by others, and interpreted by others. This is not to diminish the value of clinical trials, but in my opinion, except for the leadership, participation does not equal academic work.

There are clinician-scientists, although in ever-diminishing numbers. Their work is mainly fundamental. Such physician-researchers may do some clinical work, but often only when they must and frequently as little as possible. Research is now almost blind to the insights into biological regulation and dysregulation that can only come from clinical experience.

This loss of expertise has another critical consequence. We have surrendered the right my teachers had – the right to analyse and judge for ourselves. The rate at which medical knowledge has expanded has far outpaced the rate at which we have converted these facts to useful medical knowledge. This second step – conversion of facts to knowledge – is why I believe we need journals such as the Netherlands Journal of Medicine, which represent the broader rather than the narrower medical community.

Cholesterol is the most scientifically decorated word in modern medicine in that more Nobel prizes have been awarded for the study of this molecule than any other. Just as the one word 'penicillin' encompasses the transformation of infectious disease, so cholesterol, for both the profession and the public, has become the symbol of our mastery of vascular disease. But cholesterol is only a word and words have only assigned meanings and not intrinsic values. Cholesterol does not have to remain pre-eminent for order to exist in the universe. Which patient or physician would not choose change if change represents life rather than death?

What conclusion do I hope the reader will draw from this article? Only this: that perhaps we should not leave the evaluation of evidence entirely to the 'experts'. The 'experts' are not always right. We have innumerable examples in areas other than medicine (don't weapons of mass destruction and Iraq immediately come to mind?) when the 'experts' were absolutely confident, but utterly – and tragically – wrong. There is nothing so unique about our discipline that immunises it against similar error. As more and more issues come on the table, the competence and breadth of the expert, both intellectually and clinically, too often becomes more and more miniaturised and the result can be an awful gap between their conclusions and reality.

Is there an alternative? I think I saw one in Nijmegen: local analysis by a general internal medicine group that had the expertise and confidence to assess the merits of specific claims. They were a good and fair jury of the facts. Could the Netherlands Journal of Medicine be a formal multiplier of these values? Why not? The scientific process must be pluralistic to operate effectively.

Our best-known medical journals do much well. But intentionally or not, they have become high-earning vehicles competing for our attention. Profit is a major, sometimes perhaps a dominant, objective. Growing brand names are unquestionably a major objective. Our top line medical journals compete to publish the latest, largest clinical trials and their issues are replete with advertising. They assail others such as the pharmaceutical industry for their errors – as they should – but how developed are their processes to deal with errors that appear in the articles they publish? If we absolutely need post-marketing surveillance for medications, don't we also absolutely need post-publication surveillance for scientific articles? How confident are we that our best journals have made this commitment and that they are acting on it. Moreover, at least in cardiology, most of the major journals are controlled by the major professional societies. The guidelines from these societies appear automatically in their journals. How often do any critiques of these guidelines appear beside them? Given that guidelines often become public policy, how healthy is that relationship?

Globalisation of science should not – must not – mean homogenisation of opinion. The only antidote is to enlarge the presentation of reasoned opinion. The problems we face are difficult. The solutions – if there are such things – are not easy to find. That is why I am convinced that we need more, not fewer, avenues of medical expression. That is why I am proud to have the opportunity to publish my answer to the resident in Nijmegen in the Netherlands Journal of Medicine.

REFERENCES

1. Talmud PJ, Hawe E, Miller GJ, Humphries SE. Non-fasting apolipoprotein B and triglyceride levels as a useful predictor of coronary heart disease risk in middle-aged UK men. Arterioscler Thromb Vasc Biol 2002;22:1918-23.
2. Yusuf S, Hawken S, Ounpuu S, et al., on behalf of the INTERHEART Study Investigators. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.
3. Elovson J, Chatterton JE, Bell GT, et al. Plasma very low density lipoproteins contain a single molecule of apolipoprotein B. J Lipid Res 1988;29:1461-73.

4. Lamarche B, Moorjani S, Lupien PJ, et al. Apoprotein A-1 and B levels and the risk of ischemic heart disease during a 5 year follow-up of men in the Quebec Cardiovascular Study. Circulation 1996;94:273-8.
5. Moss AJ, Goldstein RE, Marder VJ, et al. Thrombogenic factors and recurrent coronary events. Circulation 1999;99:2517-22.
6. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-1, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001;358:2026-33.
7. Corsetti JP, Zareba W, Moss AJ, Sparks CE. Apolipoprotein B determines risk for recurrent coronary events in postinfarction patients with metabolic syndrome. Atherosclerosis 2004;177:367-73.
8. Pedersen TR, Olsson AG, Faergeman O, et al., for the Scandinavian Simvastatin Survival Study Group. Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian simvastatin survival study (4S). Circulation 1998;97:1453-60.
9. Gotto AM, Whitney E, Stein EA, Shapiro DR, Clearfield M, Weis S. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). Circulation 2000;101:477-84.
10. Simes RJ, Marschner IC, Hunt D, Colquhoun D, Sullivan D, Stewart RAH. Relationship between lipid levels and clinical outcomes in the long-term intervention with pravastatin in the ischemic disease (LIPID) trial. To what extent is the reduction in coronary events with pravastatin explained by on-study lipid levels? Circulation 2002;105:1162-9.
11. Walldius G, Jungner I, Aastveit AH, Holme I, Furberg CD, Sniderman AD. The apoB/apoA-I ratio is better than the cholesterol ratios to estimate the balance between the plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. Clin Chem Lab Med 2004;42:1355-63.
12. Meijssen S, Castro Cabezas M, Twickler TB, Jansen H, Erkelens DW. In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia. J Lipid Res 2000;41:1096-102.
13. Van Lennep JE, Westerveld HT, van Lennep HW, et al. Apolipoprotein concentrations during treatment and recurrent coronary artery disease events. Arterioscler Thromb Vasc Biol 2000;20:2408-13.
14. Packard CJ. Apolipoproteins: the new prognostic indicator? Eur Heart J 2003;suppl 5:D9-D16.
15. Brown G, Albers JJ, Fisher LD, et al. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990;323:1289-98.
16. Sniderman AD, Brown BG, Stewart FF, Cianflone K. From familial combined hyperlipidemia to hyperapoB: Unravelling overproduction of heptic apoB lipoproteins. Curr Opin Lipidol 1992;3:137-42.
17. Ruotolo G, Ericsson CG, Tettamanti C, et al. Treatment effects on serum lipoprotein lipids, apolipoproteins, a low density lipoprotein particle size and relationships of lipoprotein variables to progression of coronary artery disease in the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT). J Am Coll Cardiol 1998;32:1648-56.
18. Vakkilainen J, Steiner G, Ansquer JC, et al., for the DAIS Group. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease in the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation 2003;107:1733-7.
19. Sniderman AD, St-Pierre A, Cantin B, Dagenais GR, Depres J-P, Lamarche B. Concordance/discordance between plasma apolipoprotein B levels and the cholesterol indexes of atherosclerotic risk. Am J Cardiol 2003;91:1173-7.
20. Sniderman AD, Furberg CD, Keech A, et al. Apolipoproteins versus lipids as indices of coronary risk and as targets for statin therapy treatment. Lancet 2003;361:777-80.
21. Keulen ET, Kruijshoop M, Schaper NC, Hoeks APG, de Bruin TWA. Increased intima-media thickness in familial combined hyperlipidemia associated with apolipoprotein B. Arterioscler Thromb Vasc Biol 2002;22:283-8.
22. Simon A, Chironi G, Gariepy J, Del Pino M, Levenson J. Differences between markers of atherogenic lipoproteins in predicting high cardiovascular risk and subclinical atherosclerosis in asymptomatic men. Atherosclerosis 2005;179(2):239-44.
23. Connelly PW, Poapst M, Davignon J, et al. Reference values of plasma apolipoproteins A-I and B, and association with nonlipid risk factors in the populations of two Canadian provinces: Quebec and Saskatchewan. Canadian Heart Health Surveys Research Group. Can J Cardiol 1999;15:409-18.
24. Sniderman AD, Vu H, Cianflone K. The effect of moderate hypertriglyceridemia on the relation of plasma total and LDL apoB levels. Atherosclerosis 1991;89:109-16.
25. Durrington PN, Bolton CN, Hartog H. Serum and lipoprotein apolipoprotein B levels in normal subjects and patients with hyperlipoproteinaemia. Clin Chim Acta 1978;82:151-60.
26. Packard CJ, Shepherd J. Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler Thromb Vasc Biol 1997;17:3542-56.
27. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeniety. J Lipid Res 2002;43:1363-79.
28. Chapman MJ, Paplaud PM, Luc G, et al. Further resolution of the low density lipoprotein spectrum in normal human plasma: physicochemical characteristics of discrete subspecies separated by density gradient ultracentrifugation. J Lipid Res 1988;29:442-58.
29. Teng B, Thompson GR, Sniderman AD, Forte TM, Krauss RM, Kwiterovich PO. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinaemia, normolipidaemia and familial hypercholesterolaemia. Proc Natl Acad Sci USA 1983;80:6662-6.
30. Sniderman AD, Dagenais GR, Cantin B, Despres JP, Lamarche B. High apolipoprotein B with low high-density lipoprotein cholesterol and normal plasma triglycerides and cholesterol. Am J Cardiol 2001;87:792-3, A8.

Sniderman. The need for the Netherlands Journal of Medicine.

SPECIAL REPORT

Awards for best articles published in the Netherlands Journal of Medicine in 2004

In 2004 we started a fine tradition of awarding prizes for three categories of papers published in the Journal: the best original article, case report and review.[1] As established by the mission statement, the primary goal of the Journal is to inform the practicing internist about current developments in healthcare. Another important goal is to provide the young researcher with a forum to publish his/her first study results. We hope that this tradition stimulates young researchers to publish their work in the Journal. In 2005, the jury consisted of colleagues P.W de Leeuw, A.E. Meinders, and C. Gaillard and they had the difficult task of selecting the best paper from each category.

Review article

Articles from this category were judged according to clinical relevance, comprehensiveness, educational value, and value for the general internist. The jury awarded the first prize in this category to the article on prevention of infections in hyposplenic and asplenic patients as written by Drs Melles and De Marie.[2] The jury emphasised that this paper dealt with this important topic in a practical and highly enticing way.

Case report

In this category, the jury selected the article on a boy with autosomal recessive hypercholesterolaemia by Dr Rodenburg *et al*.[3] This paper contained an astute clinical description of the case with autosomal recessive hypercholesterolaemia and is an excellent example of clinical reasoning that led to the correct diagnosis, finally confirmed by novel molecular diagnostic techniques.

Original article

The article written by Dr Spoelstra-De Man *et al*. was chosen as the best paper from the category for original articles.[4] This article shows that treatment with folic acid in patients with type 2 diabetes mellitus and mild hyperhomocystenaemia does not result in amelioration of the markers of endothelial dysfunction or inflammation. Although the results of this study are negative, it has immediate clinical implications.

At the 2005 Convention for Internal Medicine (Internistendagen) in Maastricht, the Netherlands on 21 and 22 April 2005, the prizes were awarded by the Editor in chief Anton F. Stalenhoef to the first authors from these papers. The award is unique in its kind and consisted of a choice of one of the original prints of the graphic art as published on the cover of each issue of the Journal.

The editors,
The Netherlands Journal of Medicine

REFERENCES

1. Anonymous. The Netherlands Journal of Medicine awards for best original article, case report and review. Neth J Med 2004;62:176.
2. Melles DC, de Marie S. Prevention of infections in hyposplenic and asplenic patients: an update. Neth J Med 2004;62:45-52.
3. Rodenburg J, Wiegman A, Vissers MN, Kastelein JJ, Stalenhoef AF. A boy with autosomal recessive hypercholesterolaemia. Neth J Med 2004;62:89-93.
4. Spoelstra-de MA, Brouwer CB, Terheggen F, Bollen JM, Stehouwer CD, Smulders YM. No effect of folic acid on markers of endothelial dysfunction or inflammation in patients with type 2 diabetes mellitus and mild hyperhomocysteinaemia. Neth J Med 2004;62:246-53.

ANSWER TO PHOTO QUIZ (ON PAGE 230)

A PATIENT WITH FEVER AFTER A VISIT TO SOUTH AFRICA

DIAGNOSIS

In the knee hollow a nodus with a central, slightly black crust, known as an eschar (tâche noire), was seen. The clinical picture characterised by an elevated temperature, an eschar in the knee hollow, painful regional lymph glands and myalgia – in the absence of a rash – after a visit to sub-Saharan Africa suggest African tick bite fever, a rickettsiosis, by *Rickettsia africae*. The patient received 2 x 100 mg doxycycline for one week and the clinical picture improved after three days. The first serological tests were negative, but 14 days later the clinical diagnosis was conformed by an IgG (1:125) and IgM (>1:125) positive for *R. conorii*, which is seen with infections with *R. africae* and *R. conorii*.

The time lag between tick bite and onset of symptoms is usually five to seven days but may be as long as ten days. Patients present with flu-like symptoms such as fever, nausea, fatigue, headache and myalgia, prominent neck muscle myalgia. A black crust surrounded by a red halo at the site of the tick bite – inoculation eschar – is present in most patients and in more than 50% of patients multiple eschars are noted. Regional lymphadenopathy is common. A generalised cutaneous rash, sometimes vesicular and usually best seen close to the eschar, is present in 15 to 46% of the patients. Serological tests (immunofluorescence) show late seroconversion, frequently more than three weeks after the onset of symptoms. The responsible tick, *Amblyomma hebraeum* (*figure 2*), is only present in southern Africa. In South Africa the species is distributed along the coast of the Indian Ocean, including the KwaZula-Natal province, as well as in the north-eastern regions where many popular wildlife attractions are located.

R. conorii is the aetiological agent of Mediterranean spotted fever (fièvre boutonneuse), which is prevalent in southern Europe and is also transmitted by ticks. No specific serological test for *R. africae* is commercially available, but due to extensive cross-reactions, a commercial kit based on *R. conorii* and *R. africae* is used.

The conventional treatment is doxycyclin 2 x 100 mg for seven days.

Figure 2 *Amblyomma hebraeum, the characteristic tick for R. africae (picture from Dr Peter G. Jupp, Special Pathogens Unit, National Institute for Communicable Diseases and Department of Virology, University of the Witwatersrand Johannesburg, South Africa)*

REFERENCE

Jensenius M, Fournier P-E, Kelly P, Myrvang B, Raoult D. African tick bite fever. Lancet Infect Dis 2003;3:557-64.

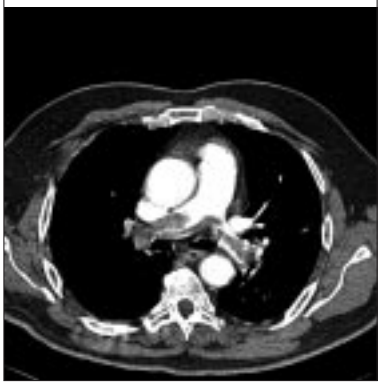
ANSWER TO PHOTO QUIZ (ON PAGE 231)

A PATIENT WITH DYSPNOEA, SUBFEBRILE TEMPERATURE AND ELECTROCARDIOGRAPHIC ABNORMALITIES

DIAGNOSIS

The ECG showed a sinus tachycardia of 111 beats/min, an incomplete right bundle branch block and a so-called McGinn-White S1Q3T3 pattern.[1] There were no signs of acute ischaemia.
Because a pulmonary embolism was considered, a high-resolution spiral computed tomography angiography was performed.[2] This showed a massive pulmonary embolism presenting as a classic saddle embolus at the bifurcation of the main pulmonary artery extending into the left and right pulmonary arteries (*figure 2*).

Figure 2 *High-resolution spiral CT-scan angiography presenting the saddle embolus*



A transthoracic echocardiography was performed which showed dilatation of the right ventricle with tricuspid regurgitation and systolic pulmonary artery hypertension of 58 mmHg, compatible with massive pulmonary embolism.[3]
The patient was treated with intravenous heparin/low-molecular-weight heparin and acenocoumarol. Because of a recent cerebrovascular accident we considered thrombolytic therapy to be contraindicated.[4,5] After eight days the patient was discharged. During follow-up the patient remained asymptomatic.

Classic features of massive pulmonary embolism at electrocardiography[1] and echocardiography[3]:
- Sinus tachycardia
- (Incomplete) right bundle branch block
- McGinn-White S1Q3T3 pattern, which means S wave in lead I, Q wave in lead III and a flattened or negative T wave in lead III
- Right ventricular dilatation and systolic pulmonary hypertension

REFERENCES

1. Daniel KR, Courtney M, Kline JA. Assessment of cardiac stress from massive pulmonary embolism with 12-lead ECG. Chest 2001;120:474-81.
2. Van Strijen MJL, de Monye W, Schiereck J, et al. Single-detector helical computed tomography as the primary diagnostic test in suspected pulmonary embolism: a multicenter clinical management study of 510 patients. Ann Intern Med 2003;138:307-14.
3. Haenen N, Odekerken D, Jaarsma W. Images in cardiology: Imaging of massive pulmonary embolism. Heart 2002;88(1):111.
4. Dalen JE, Alpert JS. Thrombolytic therapy for pulmonary embolism: is it effective? Is it safe? When it is indicated? Arch Intern Med 1997;157:2250-6.
5. Konstantinides S, Geibel A, Olschweski M, et al. Association between thrombolytic treatment and the prognosis of hemodynamically stable patients with major pulmonary embolism: results of a multicenter registry. Circulation 1997;96:882-8.

ABOUT THE COVER

'Vernis mou' print

Ruud Mathes

This month's cover shows a 'vernis mou' print by Ruud Mathes. Ruud was born in Amsterdam in 1948. While studying social pedagogy at the University of Amsterdam from 1972 to 1979, he also pursued studies in printmaking at the Free Academy in The Hague, the Netherlands (1975), at the international summer Academy in Salzburg, Austria (1976) an at S.W. Hayter's Atelier 17 in Paris, France (1978). In 1989 he moved to Thessalonica in Greece. He usually presents his work in group exhibitions in Thessalonica and Athens. For the last 20 years he has been inspired by the Greek landscape, which you will find in his prints. Recently, his work was exhibited in Wuppertal, Germany.

An original print of this month's cover is available at a price of € 210. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK BEEK-Ubbergen, the Netherlands, by e-mail: galerie-unita@planet.nl, or see the website: 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 proprietary 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

Bijsluiters

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.

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