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EDITORIAL

NOD2 in Crohn's disease – loss or gain of function mutations?

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KEYWORDS

Crohn's disease, cytokine, *NOD2*

The recognition of disease-associated alleles in the *NOD2/CARD15* gene has boosted research on the pathogenesis of Crohn's disease. Among the three risk alleles harbouring single nucleotide polymorphisms (SNPs), the C-insertion in the leucin-rich region of the gene has been most extensively studied. Although many questions about the role of *NOD2/CARD15* in the immune response have been elucidated, one seemingly simple question remains unanswered: do the risk alleles confer a gain or a loss of the protein function? The paper by Netea *et al.* in this issue of the *Netherlands Journal of Medicine*[1] adds to the discussion in favour of the last option, but the argument has not been settled yet.

NOD2 is a receptor for a pathogen-associated molecular pattern (PAMP) called muramyl dipeptide (MDP). MDP is a component of bacterial peptidoglycans and is bound by the leucin-rich region of the protein. As with the Toll-like receptors, a family of membrane bound molecules that bind all sorts of bacterial and viral compounds, NOD2 is thought to constitute an important link between innate immunity and the adaptive immune response. Upon binding of MDP, NF-κB is activated through a cascade of cytoplasmatic events and a proinflammatory immune response will occur.

The C-insertion at position 3020 of the gene leads to a truncated protein that lacks a lot of the MDP binding part of the protein.[2] Therefore, presumably, this defect in an NOD2 protein structure will lead to an impaired NF-κB activation and decreased production of proinflammatory cytokines. This is indeed supported by *in vitro* studies using cell lines transfected with the Crohn's disease-associated *NOD2* variants,[3] giving arguments for the hypothesis that these variants result in a loss of function of the protein.

However, Crohn's disease is characterised by an increased NF-κB activity. This apparent discrepancy led to the studies addressing this question *in vivo*, using different mice models.

Two studies based on experiments with *NOD2*-deficient mice provided two different hypotheses explaining the possible mechanism of a loss-of-function allele in the pathogenesis of Crohn's disease. Watanabe *et al.* observed reduced response of splenic macrophages to MDP in *NOD2*-deficient mice, yet found that PGN stimulation led to elevated levels of IL-12 in these mice.[4] Thus, an *NOD2*-mediated negative regulation of TLR2 signalling would be lost in an *NOD2*-deficient condition, leading to an enhanced cytokine response by macrophages to commensal bacteria and resulting in inflammation.

Another hypothesis was proposed by Kobayashi *et al.* who suggested that a loss-of-function *NOD2* allele might affect epithelial cells rather than macrophages.[5] In this study, *NOD2*-deficient mice developed more severe infection to *Listeria monocytogenes* when the pathogen was given orally compared with systemic administration. This, together with the finding of specific *NOD2* expression in intestinal crypt epithelial cells in wild-type, but not in *NOD2*-deficient mice, supported the hypothesis of epithelium-mediated impaired local control of pathogenic bacteria thus resulting in an inflammatory condition. Interestingly, in contrast to the findings of Watanabe *et al.*, in the study by Kobayashi *et al.* TLR2 stimulation of bone-marrow derived macrophages did not result in enhanced proinflammatory cytokine produc-

tion in *NOD2*-deficient mice, underlying the importance of the studied cell type for this kind of experiments.
On the other hand, Maeda *et al*. used a mice model with insertion of one of the mutations associated with Crohn's disease. In this model, enhanced production of IL-1β has been observed upon MDP stimulation of bone marrow-derived macrophages in mutated mice.[6] Based on these findings, a hypothesis of gain-of-function *NOD2*-associated mutations has been postulated, suggesting that in the presence of this mutation, the stimulation of antigen-presenting cells (i.e. macrophages or dendritic cells) with bacterial components binding *NOD2* would directly lead to the production of proinflammatory cytokines.
The mice studies mentioned have definitely provided interesting insights into the pathogenetic mechanisms of Crohn's disease-associated *NOD2* mutations. However, the question of the relevance of these models for human pathology remains. Moreover, these studies have lead to strikingly different hypotheses depending on the particular genetic modification approach used. Therefore, it is clear that for a better understanding of the *NOD2*-mediated pathogenesis of Crohn's disease, data from studies with human material are crucial.
Therefore, the report by Netea *et al*. published in this issue is of particular importance.[1] The authors used peripheral blood mononuclear cells to demonstrate decreased IL-1β production in homozygous *NOD2*-mutant Crohn's disease patients upon stimulation of these cells with MDP and TLR2 ligands. These results providing possible arguments for a loss-of-function hypothesis are in relative discrepancy with the study by Maeda et al. in mice with inserted Crohn's disease-related *NOD2* mutation. Besides the general differences between mice and human models, the contradictory findings can be explained by the different cell types used in these two studies. It has been shown that *NOD2* expression differs in monocytes and macrophages,[7] and may be enhanced by stimulating the cells with different TLR ligands and TNF-α.[8] Therefore, trying to make conclusions based on the results of these two studies using different cell types, might lead to mistaken interpretations.
On the other hand, it is not clear whether results obtained with stimulation of peripheral blood-derived monocytes may be translated into general statements on pathogenetic mechanisms of *NOD2* mutations in humans. Antigen-presenting cells, macrophages and dendritic cells seem to be particularly involved in the development of mucosal inflammation. Furthermore, the specific local inflammatory milieu in which these cells interact with luminal bacterial flora is not fully reflected in an *in vitro* experimental set-up using peripheral blood monocytes. Therefore, more human studies are needed, using different cell types, especially antigen-presenting cells, to resolve the paradigm of loss-of-function or gain-of-function mechanism of action of Crohn's disease-related *NOD2* allelic variants.

REFERENCES

1. Netea MG, Ferwerda G, de Jong DJ, Girardin SE, Kullberg BJ, van der Meer JWM. *NOD2* 3020insC mutation and the pathogenesis of Crohn's disease: impaired IL-1beta production points to a loss-of-function phenotype. Neth J Med 2005;63:305-8.
2. Tanabe T, Chamaillard M, Ogura Y, Zhu L, Qiu S, Masumoto J et al. Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. EMBO J 2004; 23(7):1587-97.
3. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003; 278(8):5509-12.
4. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004; 5:800-8.
5. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G et al. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005; 307(5710):731-4.
6. Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF et al. Nod2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1β processing. Science 2005; 307(5710):734-8.
7. Lala S, Ogura Y, Osborne C, Hor SY, Bromfield A, Davies S et al. Crohn's disease and the NOD2 gene: a role for paneth cells. Gastroenterology 2003; 125:47-57.
8. Gutierrez O, Pipaon C, Inohara N, Fontalba A, Ogura Y, Prosper F et al. Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kappa B activation. J Biol Chem 2002; 277(44):41701-5.

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EDITORIAL

National guidelines for the use of antibiotics in hospitalised adult patients: the SWAB guidelines revisited

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ABSTRACT

Since 1996, the Dutch Working Party on Antibiotic Policy (Stichting Werkgroep AntibioticaBeleid, SWAB) has been developing national guidelines for the use of antibiotics in hospitalised adult patients. As a result of both an inventory of the wishes of the users of these guidelines and the recently developed criteria for evidence-based guideline development, we have revised our format for the development of SWAB guidelines. By involving the members of the relevant professional societies and giving them the opportunity to comment on the guidelines at an early stage, we are aiming for a successful implementation of the guidelines in the hospitals.

KEYWORDS

Antibiotic policy, antibiotics, guidelines, infections

INTRODUCTION

In this issue of the *Netherlands Journal of Medicine* you will find the revised SWAB guideline for community-acquired pneumonia.[1] The Dutch Working Party on Antibiotic Policy (Stichting Werkgroep AntibioticaBeleid, SWAB) was founded in 1996 as an initiative of the Dutch Society for Medical Microbiology (NVMM), the Dutch Society for Infectious Diseases (VIZ) and the Dutch Association of Hospital Pharmacists (NVZA). Its major goal is to contribute to the containment of antimicrobial resistance and the expanding costs of the use of antibiotics. This is achieved by optimising the use of antibiotics by means of guideline development, education, and surveillance of antibiotic use and resistance.

In 2001, the SWAB was designated by the Ministry of Health to coordinate the surveillance of antibiotic resistance. In addition, the SWAB coordinates the surveillance of the use of antibiotics. Nethmap 2003, the first report with new information about consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands, was presented in April 2003, during the SWAB symposium 2003 in Utrecht.[2] Nethmap is updated annually (http://www.swab.nl → professional).

SWAB GUIDELINES

Since its conception, the SWAB has developed national guidelines for the use of antibiotics, which are aimed at the hospitalised adult patient. Guidelines are published in a Dutch medical journal (*Nederlands Tijdschrift voor Geneeskunde*).[3-7] Until now, the contents of these guidelines were prepared by a writing committee, consisting of five to ten experts. A proposal for the guideline was prepared by this committee and sent out for review to approximately 30 experts (medical microbiologists, infectious diseases

specialists, hospital pharmacists and medical specialists relevant to the specific topic). Based on their comments, a final guideline was prepared.

In 2001, a survey among hospital antibiotic policy committees revealed that the majority of respondents were aware of SWAB's guidelines, but it was suggested that the guideline concept should be made more broadly available, e.g. on the internet, and with a clearer method for grading the strength of the evidence on which the guideline was based.[8]

DEVELOPMENT OF GUIDELINES – RECENT INSIGHTS

As the number of clinical practice guidelines increases, there is a growing need to ensure that they satisfy certain minimum requirements. The AGREE (Appraisal of Guidelines, REsearch and Evaluation) collaboration has developed and validated an instrument that can be used to improve the quality of guideline development.[9] Six pivotal criteria of high-quality clinical practice guidelines have been identified:

- Scope and purpose: the overall objective, clinical questions, and target population should be specified.
- Stakeholder involvement: the composition, discipline, and expertise of the guideline development group should be specified. Target users should be defined.
- Rigour of development: the search strategy, inclusion/exclusion criteria for selecting the evidence, and the methods used to formulate the recommendations should be given. The recommendations should be externally reviewed before publication, and the information should be updated regularly.
- Clarity and presentation: the recommendations should be specific and unambiguous, different options should be presented, and key recommendations should be easily identifiable.
- Applicability: the organisational changes and cost implications of applying the recommendations should be discussed. Review criteria to monitor the use of the guidelines should be given.
- Editorial independence: the final recommendations should be independent of the views or interests of the funding body, and conflicts of interests should be stated.

A review of the development of clinical practice guidelines, especially in the field of infectious diseases, was published recently in this journal.[10] A distinct and specific feature of guidelines for infectious diseases is that local epidemiology and resistance data must be taken into account.

SWAB GUIDELINES: NEW FORMAT

As a result of both the survey of the users of the guidelines and the introduction of the evidence-based guideline development mentioned above, we recently revised the procedures for the development of SWAB guidelines (http://www.swab.nl → professionals → richtlijnen). Crucial elements in these procedures are:

- For each guideline, a writing committee is composed of members of all professional organisations involved: the Dutch Society for Medical Microbiology (NVMM), the Dutch Society for Infectious Diseases (VIZ) and the Dutch Association of Hospital Pharmacists (NVZA), the Dutch College of General Practitioners (NHG), and any other specialities that are relevant for the specific guideline under development.
- One of the first steps of the writing committee is to establish the main questions that should be addressed. Based on these questions, a systematic search of the literature is performed, according to the principles for literature searches described earlier.[9,11] This results in a systematic review, in which the literature is graded and the strength of the resulting conclusions is graded according to the level of evidence. Based on this systematic review, a draft guideline is proposed by the writing committee.
- This draft guideline is made available to all members of the professional societies involved in the development of the guideline. They can access the concept guideline through the SWAB website (www.swab.editline.nl) and the websites of the respective societies, and they can comment on the proposal through this website. Based on these comments, the writing committee finalises the guideline.
- As the hospitalised patients are the target population, local antibiotic policy committees remain the target users of the guidelines, because they are essential for the interpretation, the adaptation to local resistance patterns and policies, and the implementation of infectious diseases guidelines.[10]
- The final systematic review will be published in the *Netherlands Journal of Medicine*, or in other journals if applicable, for instance in the case of endocarditis, in Hartbulletin. A shortened version of the guideline will, as before, be submitted for publication in *Het Nederlands Tijdschrift voor Geneeskunde*.

In conclusion, this new procedure meets the criteria for evidence-based guideline development. By involving the membership of the relevant professional societies, and giving them the opportunity to comment on the guidelines at an early stage, we can also aim for a successful implementation of the guidelines in the hospitals. The new SWAB guidelines that have been fully developed

according to the above-mentioned procedures will be published in the coming year: guidelines on community-acquired pneumonia, acute infectious diarrhoea, and urinary tract infections.

NOTE

The authors are Board Members of the Dutch Working Party on Antibiotic Policy (Stichting Werkgroep AntibioticaBeleid, SWAB).

REFERENCES

1. Schouten J, Prins JM, Bonten M, et al. Revised SWAB guidelines for antimicrobial therapy in Community-acquired Pneumonia. Neth J Med 2005;63:323-35.
2. Verbrugh HA. Mapping antibiotic use and resistance in the Netherlands: SWAB and NethMap. Neth J Med. 2003;61:341-2.
3. Van Kasteren MEE, Wijnands WJA, Stobberingh EE, Janknegt R, van der Meer JWM. Optimaliseren van het antibioticabeleid in Nederland. II. SWAB-richtlijnen voor antimicrobiële therapie bij thuis opgelopen pneumonie en bij nosocomiale pneumonie. Ned Tijdschr Geneeskd 1998;142:952-6.
4. Van Kasteren MEE, Wijnands WJA, Stobberingh EE, Janknegt R, van der Meer JWM. Optimaliseren van het antibioticabeleid in Nederland. III. SWAB-richtlijnen voor antimicrobiële therapie bij volwassenen met bronchitis in het ziekenhuis. Ned Tijdschr Geneeskd 1998;142:2512-5.
5. Van Kasteren MEE, Stobberingh EE, Janknegt R, Wijnands WJA, van der Meer JWM. Optimaliseren van het antibioticabeleid in Nederland. IV. SWAB-richtlijnen voor antimicrobiële therapie in het ziekenhuis bij volwassenen met sepsis. Ned Tijdschr Geneeskd 1999;143:611-7.
6. Van Kasteren MEE, Gyssens IC, Kullberg BJ, Bruining HA, Stobberingh EE, Goris RJA. Optimaliseren van het antibioticabeleid in Nederland. V. SWAB-richtlijnen voor perioperatieve antibiotische profylaxe. Ned Tijdschr Geneeskd 2000;144:2049-55.
7. Bonten MJM, Kullberg BJ, Filius PMG. Optimaliseren van het antibioticabeleid in Nederland. VI. SWAB-advies:geen selectieve decontaminatie bij beademde patiënten op de intensive care. Ned Tijdschr Geneeskd 2001;145:353-7.
8. Van Kasteren MEE, van de Nieuwenhof L, Gyssens IC, van der Meer JWM. Vijf jaar Stichting Werkgroep Antibioticabeleid, een inventarisatie vanuit het veld. Ned Tijdschr Med Microbiol 2001;10:116-7.
9. The AGREE Collaboration. Writing Group: Cluzeau FA, Burgers JS, Brouwers M, Grol R, Mäkelä M, Littlejohns P, Grimshaw J, Hunt C. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines: the AGREE project. Qual Saf Health Care 2003;12:18-23.
10. Peetermans WE, Ramaekers D. Clinical practice guidelines in infectious diseases. Neth J Med 2002;60:343-8.
11. Kwaliteitsinstituut voor de gezondheidszorg CBO. Handleiding voor werkgroepleden, 2000. P 18-27.

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International guidelines for infectious diseases: a practical guide

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ABSTRACT

A growing number of organisations have become involved in the development of guidelines for infectious diseases (ID). The degree of acceptation of guidelines varies from one country to another. Some of these national differences are determining the practices of prescribing antibiotics, and infection control both in hospitals and in the community. This review provides updated information on ID guideline programmes, in particular on the topic of antimicrobial therapy. It is aimed at clinicians, both in their role as care providers and as designers of local antibiotic guidelines (antibiotic booklets). Definitions are given and the process of development is discussed. International and national ID guideline programmes in the English language are presented. Many URLs provide access to the different websites where most guidelines can be downloaded free of charge.

KEYWORDS

Antimicrobial therapy, clinical practice guidelines, guideline programme, review

INTRODUCTION

The phenomenon of guidelines in healthcare runs parallel with the evolution in medicine from experience-based medicine to evidence-based medicine (EBM).[1] The need for a guide for preventive, diagnostic, therapeutic and/or organisational procedures has been apparent since the last quarter of the past century. A growing number of organisations have become involved in the development of guidelines for infectious diseases (ID), in particular on the topic of antimicrobial therapy and infection control. A general review of evidence-based guideline development in ID was published by Peetermans and Ramaekers in this journal in 2002.[2] They concluded that ID guidelines must meet the international standards of guideline quality but, most importantly, they also require the integration of local epidemiology and resistance data.

The degree of acceptation of guidelines varies from one country to another. Italian physicians perceive practice guidelines as externally imposed and cost-containment tools rather than decision-supporting tools.[3] On the other hand, according to a survey carried out among Dutch physicians,[4] it appears that more than 75% of the responders do not or only sometimes dislike guidelines. The minor aversion appeared to depend on organisational and financial restrictions, legal aspects, and insufficient support from hospital management. National differences in acceptance of guidelines have an impact on the prescribing practices of antibiotics. Antibiotic use in the Netherlands is among the lowest in Europe.[5] In a survey on antibiotic control measures, local antibiotic guidelines were present in 95% of Dutch secondary care hospitals. However, in the process of development, antibiotic committees made use of international and national guidelines for local guidelines in only 36 and 19%, respectively.[6] There is a clear need for more information. The aim of the present paper is to provide an update on international and national guidelines on infectious diseases. In the section on guideline programmes, many links provide access to the websites where most guidelines can be downloaded free of charge.

AIM OF GUIDELINES ON INFECTIOUS DISEASES

The main motive for the development of clinical practice guidelines is to increase the quality of care. Guidelines aim to be a support for clinical decision-making, decrease the unwanted diversity of treatment procedures, and increase insight into clinical practice (for both the physician and the patient). Some bodies aim to achieve a broader goal than quality alone. Thus the Infectious Diseases Society of America (IDSA) maintains that guidelines must also promote cost-effectiveness. Guidelines can also serve as an educational tool and last but not least, for infectious diseases, the control of microbial resistance has become an important goal of guidelines.

DEFINITIONS

There is a large diversity in the terminology of guidelines, leading to confusion by users who are not familiar with the jargon. *Table 1* shows an actualised, referenced selection of authoritative definitions of terms related to guidelines on infectious diseases and antimicrobial therapy. Guidelines can be international, national or local. National guidelines can – and must – be translated into local/regional policies. National guidelines on antimicrobial therapy can constitute a framework for local hospital antibiotic policies.[7] According to current insights a guideline must include more than recommendations only; it must also include a description of the methodology used and supporting evidence. The older definition of clinical practice guidelines 'A systematically developed statement'[8] is now only applicable to that part of the guideline containing the recommendations. Protocols use national guidelines as a starting point, but formulate more specific recommendations that should be applied in certain local healthcare settings. In the development of a protocol, integrated care pathways are often used. Standards usually have a more specific goal than guidelines. The concept 'standards' as used in English literature refers to minimum norms which a professional is assumed to satisfy. As a rule, the preference for and the results of certain interventions are already known before a standard is created. Standards therefore leave little room for the physician who does not want to comply. They can thus also be called requirements. For example, the Dutch Inspectorate for Healthcare considers the guidelines of the Working Party on Infection Prevention (WIP) (see below) to be professional standards, on which hospital infection control protocols should be based. The IDSA initially published quality standards to be applied without controversy in most hospital settings to review the care of patients with certain infectious diseases problems.[9] Since 2001, however,

Table 1 *Definitions of guidelines on infectious diseases*

Term	Definition	Reference
Guideline programme	A structured and coordinated programme designed with the specific aim of producing several clinical practice guidelines	11
Guideline, clinical guideline, clinical practice guideline	A document that includes a set of statements about appropriate healthcare to support daily practice, based on evidence and critical appraisal, aimed at the explicit statement of good medical practice	11
Recommendation	A systematically developed statement to assist the practitioner in making decisions about appropriate healthcare for specific clinical circumstances	11
Standard (quality, standard of care)	Authoritative statements of (1) minimum levels of acceptable performance or results, (2) excellent levels of performance or results, or (3) the range of acceptable performance or results	8, 12
Protocol	A programmed and detailed description of a practice policy, with clear, well-defined decisions. Usually description of the process of care taking into account the specific local regional/clinical situation. Protocols often contain algorithms	11
Integrated care pathways	Structured multidisciplinary care plans with detailed essential steps in the care of patients with a specific clinical problem	33
Antibiotic guide, booklet	A local application of (inter)national guidelines on antimicrobial prophylaxis and therapy	34
Formulary, drug list	A list of drugs. Can be part of a policy by limitation of the number of drugs listed. Does not contain advice on indications	12
Performance measures	Methods or instruments to estimate or monitor the extent to which the actions of a healthcare practitioner or provider conform to practice guidelines or standards of quality (compliance)	12
Quality indicator	A measurable element of practice performance for which there is evidence or consensus that it can be used to assess, and hence change, the quality of care provided	35
Review criteria	Systematically developed statements that can be used to assess the appropriateness of specific healthcare decisions, services, and outcome	36

the IDSA no longer produces standards of care; it is stated that other organisations can adopt or adapt the guidelines of the IDSA for this purpose.[10] The Dutch College of General Practitioners (NHG) has up to now produced guidelines which they call 'standards', so that this concept has acquired a different meaning in the Netherlands than in general literature.[11]

In the past, there has been an indiscriminate use of various terms such as formularies, policies, antimicrobial booklets and guides to describe local guideline documents. To avoid confusion among users and evaluators, a clear distinction between formularies and antibiotic booklets should be made. A formulary is only a list of drugs and does not provide a judgment of their application (i.e. the indications).[12] So the word 'formulary' should not be used for an antibiotic guide or booklet. A formulary can be part of a chapter, for instance, or a guideline document. Worldwide, old-fashioned terms, such as *vademecum*, blueprint and compendium are still used to describe national or local consensus guidelines on antimicrobial therapy, although it is unclear what the different terms add or stand for.

DEVELOPMENT

Appraisal of Guidelines Research and Evaluation (AGREE) is a valuable checklist that has been developed to evaluate the quality of a guideline,[13] but it can also be used for design. In this issue of the journal, the application of the AGREE instrument in the design of the SWAB guidelines is described in an editorial by the Dutch Working Party on Antibiotic Policy of the SWAB guideline committee.[14] The AGREE instrument was developed through the cooperation of an international consortium of designers of guidelines. The instrument comes with a handbook and can be downloaded from the AGREE website www.agreecollaboration.org. Information and communication technology (ICT) is facilitating the process of guideline development by the consultation of stakeholders through a closed part of a website. Target users can log in from anywhere at any desired moment and insert their comments. A less sophisticated strategy is to post the draft guidelines on the internet for a period of time and request (electronic) commentaries. Guidelines can be revised more regularly, but the frequency is to a certain extent limited by how often the professionals can incorporate new material. However, even with these new approaches, the design phase of an evidence-based guideline can last one to three years depending upon the complexity of the subject. For multidisciplinary guidelines, i.e. antimicrobial therapy, many parties have to be consulted and this causes extra delay. One consequence of this long development process can be that the guideline is already out-of-date at the time it is finished, due to new available knowledge.

Which topics to select?

In general, clinical practice guidelines are considered useful when physicians are uncertain about the appropriate treatment for which scientific evidence is available.[15] In infectious diseases there is usually a need for guidelines for empirical therapy and prophylaxis, and for diagnostics. In the field of empirical therapy, maximal efficacy of a blindly chosen broad-spectrum drug has to be balanced against the danger of selection of antimicrobial resistance. A topic can be chosen from the most common infectious diseases, such as respiratory tract or urinary tract infections, or the most threatening (high morbidity and mortality), such as bacteraemia. Topics can also be selected about which there are controversies, such as selective decontamination. A major reason to develop national guidelines for empirical therapy is the divergence of national antimicrobial resistance rates from those that support the choice of drugs in an authoritative international guideline. In connection with the support needed for implementation, the choice of a topic is best made in consultation with as large a group as possible.

Who should author guidelines on antimicrobial therapy?

There is a certain paradox in the fact that international guidelines usually have experts as their authors – therefore these guidelines have considerable authority. In contrast, because the users of local guidelines are themselves often involved at an early stage in the development process, these guidelines usually enjoy greater support because the providers have committed themselves at this early stage. An investigation by Grol *et al*. among Dutch medical specialists revealed that 80 to 90% appreciated and used the guidelines that were produced by their own scientific society in contrast to 50% for the same guidelines but produced by the Dutch Institute for Healthcare Improvement.[16]

DISTRIBUTION AND AVAILABILITY

Guidelines used to be distributed as printed matter, either as articles, a supplement to a medical journal or a book. Nowadays they are also placed on the internet, allowing more frequent actualisation. Almost all clinical practice guidelines can be downloaded free of charge from the websites of the organisations or the publisher. Many guidelines are distributed via multiple channels, to reach a maximum of healthcare workers.[9] IDSA guidelines are published in up to three journals: *Clinical Infectious Diseases* and two infection control journals. Since 2003, the SWAB guidelines are published both in Dutch and in English. In addition, the complete guideline containing the literature search and the recommendations can be downloaded from the SWAB website. Distribution of the guideline is the first step in its implementation, but this

is insufficient in practice. Discussions at (consensus) conferences or approval of the guideline at the general meeting of the relevant scientific society increases support. Adherence to the guideline can be increased by decision aids, summary cards and patient education and information leaflets. The Dutch College of General Practitioners provides CME packages for each practice guideline, complete with assessment and feedback procedures.

IMPLEMENTATION

Quality is only partially responsible for the successful application of a guideline. Whether and to what extent a guideline is followed therefore depends partially on the evidence presented in support of the recommendations, but also on the complexity of the requested action, the skills of, or the changes required in the organisation, and the clarity of the design.[16] A number of steps have been identified which lead to optimum use of the guidelines.[11] No single intervention works in all situations. Most of the methods of implementation are more or less effective, depending on the local situation and the presence of barriers. Education must be followed by interactive education, in which the participants can apply the proposed changes. Feedback alone is not effective, but it is often crucial at the beginning of the intervention. Face-to-face instruction (technique of the pharmaceutical industry) is especially effective when the 'detailer' is well-trained and a relationship based on trust has been established with the professionals. Interventions which are directed toward better organisation of care processes and changes in the culture of institutions, as well as interventions which attempt to improve care via the patient, have not been studied often.[16] Measures for limitation of the financial reimbursement for antibiotics have had a significant effect on the prescription conduct of general practitioners in Denmark[17] and surgeons in Belgian hospitals.[18] Recently, the importance of wording the guideline in precise behavioural terms has been stressed.[19]

CONFLICTS OF INTEREST AND DISCLAIMERS

Solid clinical practice guidelines should state possible conflicts of interest.[20] The highest level of evidence for a guideline is obtained by the most expensive form of research, the randomised controlled trial. The costs of such research on antimicrobial drugs can only be met by means of financial support from major drug companies. As a result, research scientists acquire bonds not only with large independent subsidising governmental institutions, but also with the industry. Recently it was reported that research that is paid for by the pharmaceutical industry more often results in a positive report for the drug under investigation than independent research.[21] In addition, a positive investigation is also published more often (publication bias). The conflicts of interest of designers of guidelines on therapy seem to be inevitable. The experts who are requested to formulate best practice are the same individuals who conduct sponsored research in the field in which they excel. There is certainly no reason to reject guidelines simply because of interests on the part of the designers. The users of the guideline, however, should be informed. A formal process built in to guideline development that forces authors to declare their financial interests and written declarations of competing interests was proposed in 2002.[22] At present, there are still many divergent practices. The Dutch Association of Respiratory Care Physicians (NVALT) gives complete transparency regarding its sponsors from the (pharmaceutical) industry on its website. The National Coordinator Infectious Disease Control (LCI) lists the details of an industry-sponsored project of the handheld version of the guidelines on their website. On the website of the Dutch College of General Practitioners, the NHG, it is difficult to find information about (governmental) grants for the clinical practice guidelines. The IDSA guidelines present lists of authors and their connections with diverse pharmaceutical companies in the paragraph 'Disclosure of Financial Interests'. The Cochrane collaboration www.cochrane.org/ has recently posted an extensive consensus document on its website which describes its structure, funding and the conditions for corporate sponsorship of all co-workers and sponsoring of reviews. Reassurance was sought that the conclusions of Cochrane reviews are not biased through the influence of funding by commercial entities that stand to benefit financially from the results of reviews. The SWAB policies regarding conflicts of interests and governmental sponsorship are clarified on the website.

Most websites of ID guideline providers present some form of disclaimer. The Scottish Intercollegiate Guidelines Network (SIGN) has a document entitled 'Notes for users' in which they explain that their guidelines should not be considered as standards. The WIP guidelines can only be downloaded after the user has declared that he agrees with the WIP declining the responsibility for the application of the guidelines to specific cases.

EVALUATION OF A GUIDELINE AND ITS USE

After the guideline has been developed and implemented, the degree to which the guideline is used in practice must be determined, i.e. what are the barriers to adherence.

Barriers effecting physician's knowledge, attitude and behaviour have been discussed by Peetermans and Ramaekers.[2] Evaluation of support by (potential) users can be achieved by means of questionnaires or interviews. In 2000 and 2002 the SWAB sent a written (anonymous) questionnaire to antibiotic committees to assess awareness and support of its guidelines.[6,23] In subsequent interviews, it appeared that systematic use of SWAB guidelines for the establishment of local antibiotic guidelines (booklets) is difficult due to the nonstandardised approach and the rather individualistic operating procedures of the antibiotic committees. The target group acknowledges the need for sound national guidelines on infectious diseases; however, the position of the SWAB guidelines is not always crystal clear (J. Bos, unpublished data). In reaction, the SWAB organised a workshop for members of hospital antibiotic committees in 2004 and is preparing an ICT programme to make the SWAB guidelines more accessible.

In order to measure whether a guideline has the desired result in practice, quality indicators must be developed (*table 1*). Quality indicators are measurable variables of care which give a signal about quality based on the guideline. One can differentiate between process indicators, which assess the outcome of medical-specialist care according to the guideline, and result indicators, which assess the results of medical-specialist care according to the guidelines. Process indicators such as the adherence of physicians to international guidelines have been investigated frequently. Less is known about the adherence of specialists to their own guidelines. Audits provide this type of information.[24] In the field of infectious diseases, the result indicators are given by patient results or microbiological results, such as resistance. The Surgical Prophylaxis and Surveillance project (CHIPS), which predominantly used audit and feedback, led to the successful implementation of the recommendations from the national SWAB guideline 'Perioperative Prophylaxis'. The fact that the patient result indicator, postoperative wound infections, remained stable suggests that a restrictive antibiotic policy, combined with correct timing, is just as effective as former practices.[25]

INFECTIOUS DISEASES GUIDELINES IN THE ENGLISH LANGUAGE

There are diverse guideline programmes for the field of infectious diseases in the English language, which make use of different methods (*table 2*).

Infection control

The American Centers for Disease Control (CDC) produces prevention guidelines. Sources for the CDC guidelines are variable. Two-thirds of the documents were originally published as CDC's Morbidity and Mortality Weekly Report (MMWR). A steering committee for the CDC selects documents to be included in the prevention guidelines database. The Society for Healthcare Epidemiology of America (SHEA) develops guidelines for hospital hygiene, termed position papers. The public part of the website provides little information on methodology. The Australian independent advisory body for healthcare, the National Health and Medical Research Council (NHMRC), has recently produced an elaborate infection control guideline for health professionals (*table 2*).

Management of infectious diseases

The IDSA started its programme for guidelines in 1994 with four quality standards. Since 1997, the society has developed guidelines only according to the method described in the Guide to the Development of Practice Guidelines.[10] The American Thoracic Society (ATS) publishes guidelines in the *American Journal of Respiratory* and *Critical Care Medicine*. These documents are also available free of charge in PDF format on their website. The Canadian Medical Association (CMA) has produced evidence-based guidelines on the topic of infectious diseases, of which many have been revised recently. Guidelines published by the Public Health Agency of Canada and the Canadian Task Force on Preventive Healthcare are downloadable via CMA infobase.

The SIGN designs guidelines which are methodologically very strong. Together with the National Institute for Clinical Excellence (NICE) www.nice.org.uk, SIGN has developed guidelines for the National Health Services (NHS) of Scotland and England/Wales, respectively. The British Thoracic Society (BTS) of respiratory medicine physicians has issued guidelines on pneumonia. The guidelines of The British Society for Antimicrobial Chemotherapy (BSAC) have been published in the *Journal of Antimicrobial Chemotherapy*. Draft guidelines are being issued for open consultation on the BSAC website. The New Zealand Guidelines Group (NZGG), a prominent organisation that develops guidelines according to evidence-based medicine, has only one topic in infectious diseases, a national tuberculosis control guideline (*table 2*).

The Cochrane Collaboration www.wileyeurope.com/go/cochrane provides important building blocks for the development of guidelines. This is an international nonprofit and independent organisation that involves more than 10,000 people worldwide. The formal structure of the Collaboration comprises Collaborative Review Groups (which produce systematic reviews) and Centres (with responsibilities that include support for the review groups within their area of geographical responsibility).

Table 2 *Organisations with evidence-based guideline programmes in the field of infectious diseases in the English language*

Name of organisation	Product, scope	Recent subjects (within the last two years)
Centers for Disease Control (CDC) www.cdc.gov	Guidelines on infection prevention and immunisations	Compendium of animal rabies prevention and control; medical examiners, coroners and biological terrorism; prevention and control of influenza; diagnosis and management of food-borne illnesses; prevention of healthcare associated pneumonia (2004)
Society for Healthcare Epidemiology of America (SHEA) www.shea-online.org	Guidelines, position papers on infection control	Infection control recommendations for patients with cystic fibrosis (2003) Preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus* (2003)
Public Health Agency of Canada www.phac-aspc.gc.ca/	Guidelines on infection prevention and immunisations	Statement on travel, influenza and prevention; update on meningococcal C conjugate vaccines (2005) Statement on bacille Calmette Guérin (BCG) vaccine (2004)
National Health and Medical Research Council of Australia (NHMRC), www.health.gov.au	Guidelines	Infection control guidelines for the prevention of transmission of infectious diseases in the healthcare setting (2004)
Infectious Diseases Society of America (IDSA) www.idsociety.org	Guidelines on clinical management of infectious diseases	Management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia; diagnosis and treatment of asymptomatic bacteriuria in adults (2005) Treating opportunistic infections among HIV-infected adults and adolescents; outpatient parenteral antimicrobial therapy; antimicrobial prophylaxis for surgery; treatment of candidiasis; diagnosis and treatment of diabetic foot infections; management of bacterial meningitis (2004) Treatment of tuberculosis (2003)
American Thoracic Society (ATS) www.thoracic.org	Guidelines on management of chest infections Handheld computer (PDA) programme	Management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia (2005) Tuberculosis treatment (2003) ATS-CDC-IDSA tuberculosis treatment (2004)
Canadian Medical Association (CMA) Infobase, all Canadian clinical guideline developers www.cma.ca	Guidelines on infectious diseases Physician summaries Patient guides	Acute otitis media (*revised* 2004) Clinical management of chronic hepatitis B and C (*revised* 2004)
Scottish Intercollegiate Guidelines Network (SIGN) www.sign.ac.uk/guidelines	Guidelines on prophylaxis and therapy containing: patient information leaflets, section on implementation and audit, quick reference guide	Diagnosis and management of childhood otitis media in primary care (2003)
British Thoracic Society (BTS) www.brit-thoracic.org.uk	Guidelines on management of chest infections	Management of pleural infections in children (2005) Community-acquired pneumonia (update 2004) Severe acute respiratory syndrome (2003)
British Society for Antimicrobial Chemotherapy (BSAC) www.bsac.org.uk	Guidelines	Guidelines for the prophylaxis and treatment of meticillin (methicillin)-resistant *Staphylococcus aureus* (MRSA) infections in the United Kingdom (2005) Antibiotic treatment of endocarditis in adults (2004)
New Zealand Guidelines Group (NZGG), www.nzgg.org.nz	Guideline	Guidelines for tuberculosis control in New Zealand (2003)

It systematically publishes and distributes literature surveys of interventions in healthcare. The Cochrane Library contains more than 50 Cochrane Database Systemic Reviews on antibiotic prophylaxis or therapy with recent updates. Many of these reviews highlight the lack of evidence for antibiotics already introduced in clinical practice and call for larger, well-designed randomised trials. Since 2003, the Cochrane Collaboration has commercially marketed the reviews. Residents in a number of countries get access for free through a 'national provision'. There are also several programmes that provide free access in Latin America and low-income countries. Abstracts can be accessed free of charge at www.cochrane.org/reviews. Examples of commercial applications of guidelines on antibiotic therapy are the Sanford Guide and the Johns Hopkins Guide. Both are written by excellent authors and contain many up-to-date literature references. However, neither booklet can be considered as an 'antibiotic guide

for a local hospital' as defined in *table 1*, since they do not take into account the local epidemiology of the causative agent, the pattern of sensitivity and the formulary and policy of the local hospital. The Sanford guide, www.sanfordguide.com, is a pocket-sized reference book. National editions attempt to compensate for the differences between the American and the European culture, and take into account some national differences in resistance rates.[26,27] Like the Sanford guide, the Johns Hopkins Guide www.hopkins-abxguide.org can be accessed after registration or be downloaded in a handheld version. Views on antibiotic policy can not usually be found in these booklets. The Sanford and Johns Hopkins guides list their sponsors on the website.

NATIONAL GUIDELINE PROGRAMMES: EXAMPLE OF THE NETHERLANDS

It is important to ensure that in a given country the guidelines for one subject for a specific professional group are attuned to one another. The simultaneous existence of several national guidelines for one subject in a particular country can cause the care provider overwork, confusion and discouragement. Fine tuning is very important. Ideally all designers of guideline programmes for a particular disease should cooperate to obtain one set of recommendations in that country.[28] In the Netherlands, there is a long tradition of guideline development, but it is only recently that there have been attempts towards systematic collaboration between guideline makers.

Infection control

The WIP issues guidelines for hospitals, nursing homes and other institutions. These guidelines are written by experts who are actively involved in the provision of healthcare. The draft guidelines are submitted to all members; they are also posted on the website for comment. If relevant, the comments are incorporated into the last draft which is presented to the National Health Council for review. The Dutch Inspectorate of Healthcare (IGZ) considers the guidelines of the WIP to be professional

Table 3 *Guidelines and programmes in the field of infectious diseases in the Netherlands*

Name of organisation	Product, scope	Recent subjects (and updates within the last two years)
Working Party on Infection Prevention (WIP), www.wip.nl	Guidelines on infection control for hospitals, policies	Policies on MRSA (2003)
National Coordinator Infectious Disease Control (LCI) www.infectieziekten.nl	Protocols, practice strategies, guidelines on technical care Handheld computer (PDA) programme	Hepatitis A, influenza, meningococcal disease, smallpox (2003) Adenovirus, aviary influenza, condyloma acuminata, EBV, genital warts, genital herpes, giardiasis, impetigo, leptospirosis, lice, malaria, *Mycoplasma pneumoniae*, plague, pneumococcal disease, rabies, SARS, scarlet fever, syphilis, tetanus, West Nile virus, yellow fever (2004) Botulism, Creutzfeldt Jakob, haemorrhagic fever, listeriosis, *S. aureus*, group A streptococci (2005)
Dutch College of General Practitioners (NHG) www.nhg.artsennet.nl	Practice guidelines, summary cards, education leaflets for patients	Acute cough (2004) Most ID guidelines are from the 1990s, no recent updates
Working Party on Antibiotic Policy (SWAB) www.swab.nl	Guidelines as a framework for antibiotic policy committees	Therapy of infective endocarditis (2003) Therapy of community-acquired pneumonia (2005) Treatment of acute diarrhoea (draft 2005)
The Quality Institute for Healthcare CBO/Consensus Multidisciplinary committee, 6-9 professional societies www.cbo.nl	Guidelines, summary cards	Varicella (2003) Lyme borreliosis (2004)
The Quality Institute for Healthcare CBO/Dutch Society of AIDS Physicians (NVAB) www.cbo.nl	Draft guideline	Guideline on antiretroviral therapy (update 2005)
The Quality Institute for Healthcare CBO/Organisations of Dutch Medical Specialists Dutch Society of Pulmonologists (NVALT) www.cbo.nl	Guideline	Guideline on diagnostics and treatment of community-acquired pneumonia (2003)
Dutch Society for Dermatology and Venereology (NVDV), www.soa.nl	Guideline, patient brochures	Guidelines on sexually transmitted diseases diagnosis and therapy (updated 2003 and 2004)

standards, to which healthcare workers are urged to comply. All guidelines of the WIP are available on the internet and can be downloaded free of charge. The guidelines on paper can be requested for a fee. Since 1995, the National Coordinator of LCI has been responsible for the 39 municipal health services and the national service in the Netherlands. LCI develops protocols that are endorsed by the Health Council, i.e. ID guidelines for the community and coordinates outbreak management. The programme includes protocols on infectious diseases and practice strategies.

Management of infectious diseases

The Dutch Institute for Healthcare Improvement (CBO) has developed consensus guidelines in the Netherlands. In the past few years the programme has become more closely related to the guideline programmes of the scientific societies. The Order of Medical Specialists involved the CBO closely in the elaboration of the long-term agreements project 'Development and implementation of guidelines for medical specialists'. In this document, it is established that the development of guidelines is based on available evidence that is obtained by systematic searching and is evaluated according to the principles of evidence-based medicine (EBRO). EBRO guidelines are developed according to this specific methodology. An EBRO Platform was established by the CBO and the Centre of Quality of Care Research www.wokresearch.nl; in 2003 many Dutch designers of guideline programmes joined. Together with a number of organisations from around the world, the EBRO recently founded a new international organisation for designers of guidelines, the Guidelines International Network G-I-N www.guidelines-international.net. The purpose is to promote cooperation between guideline organisations that use the AGREE method. The Working Party on Antibiotic Policy (SWAB) has been part of the EBRO network since 2003. The SWAB guidelines contain recommendations for therapy and prophylaxis with antimicrobial drugs. The principles of diagnostics are included insofar as they are essential for policy. The SWAB guidelines programme has been established for in-hospital use. The target group is the multidisciplinary antibiotic committee of a hospital which can use the SWAB guidelines to draw up their local antibiotic booklets and protocols. The SWAB guidelines differ in this respect from the CBO guidelines and those of the specialist societies which are directed toward the individual care provider, and include other aspects of the treatment of infection. The SWAB guidelines apply to the Dutch situation and the (low) resistance patterns in the country. They are based on published national resistance data and data from the national surveillance system NethMap www.swab.nl. Therefore, in many infectious diseases, choices of antibiotics in guidelines from other countries may not always be applicable to the Dutch situation. Besides differences in resistance rates, there are clear differences in recommendations compared with American guidelines,[29] for example not to treat mild cases of community-acquired pneumonia empirically due to the possibility of atypical pathogen infection.[30] The Practice Guidelines of the NHG are guidelines for general practitioners written by general practitioners. Three practice guidelines on infectious diseases have been translated into English.

The Blueprint for Paediatric Antimicrobial Therapy[31] is a collection of national guidelines of the Division of Paediatric Infectious Diseases of the Dutch Society for Infectious Diseases and is actually under revision.

There are two independent sources of information for guidelines for antimicrobial drugs: the Netherlands Drug Bulletin (NDB) (Geneesmiddelenbulletin) www.geneesmiddelenbulletin.nl and the Farmacotherapeutisch Kompas (national formulary) www.fk.cvz.nl. Both strive – for a fraction of the amount used by commercial sources – to provide impartial information about drugs in the Netherlands. The system of peer review of the NDB was described in a former issue of this journal. NDB feels very strongly about its independence.[32] Although NDB has a distribution of 50,000, to medical professionals and even to students, the impact of its recommendations has not been studied.

CONCLUSION

Guidelines enhance the quality of medical treatment in general. Care processes become more transparent and more easily managed. Infectious diseases guidelines are useful in clinical practice, for student training and post-graduate training. Guidelines are also important for development of quality indicators. Moreover, the process of the development of guidelines points out the direction for future scientific research because the gaps in knowledge and evidence become visible. Guidelines for antimicrobial therapy can be an important way to limit microbial resistance and to combat the spread of resistance.

NOTE

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REFERENCES

1. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: what it is and what it isn't. BMJ 1996; 12:71-72.
2. Peetermans WE, Ramaekers D. Clinical practice guidelines in infectious diseases. Neth J Med 2002; 60:343-48.
3. Formoso G, Liberati A, Magrini N. Practice guidelines: useful and "participative" method? Arch Intern Med 2001; 161:2037-42.
4. Van Everdingen JJ. "Een hoop gedoe, maar wel de moeite waard". Medisch Contact 2003; 58:12-21.
5. Goossens H, Ferech M, Van der Stichele R, Elseviers M, the ESAC Project Group. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 2005;365:579-87.
6. Schouten JA, Hulscher MEJL, Natsch S, Grol RPTM, van der Meer JWM. Antibiotic control measures in Dutch secondary care hospitals. Neth J Med 2005; 63:24-30.
7. Van Kasteren MEE, Van der Meer JWM, Stobberingh EE, Verbrugh HA, Janknegt R. Stichting Werkgroep Antibioticabeleid (SWAB). Richtlijnen voor het gebruik van antimicrobiële middelen. Ned Tijdschr Geneeskd 1997; 142:949-52.
8. Field MJ, Lohr KN. Clinical practice guidelines: directions for a new program. Institute of Medicine Washington DC: National Academy Press, 1990.
9. Gross PA, Barrett TL, Dellinger EP, et al. Purpose of quality standards for infectious diseases. Clin Infect Dis 1994; 18:421.
10. Kish MA. Guide to development of practice guidelines. Clin Infect Dis 2001; 32:851-54.
11. Burgers JS. Quality of clinical practice guidelines. Thesis: Katholieke Universiteit Nijmegen, 2002.
12. Gross PA. The potential for clinical guidelines to impact appropriate antimicrobial agent use. Infect Dis Clin N Am 1997; 11:803-12.
13. The AGREE Collaboration. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines: the AGREE project. Qual Saf Health Care 2003; 12:18-23.
14. Prins JM, Kullberg BJ, Gyssens IC. National guidelines for the use of antibiotics in hospitalised adult patients: the SWAB guidelines revisited. Neth J Med 2005;63:288-90.
15. Woolf SH, Grol R, Hutchinson A, Eccles M, Grimshaw J. Clinical guidelines: potential benefits, limitations, and harms of clinical guidelines. BMJ 1999; 318:527-30.
16. Grol R, Grimshaw J. From the best evidence to best practice: effective implementation change in patients' care. Lancet 2003; 362.
17. Friis H, Bro F, Eriksen NR, Mabeck CE, Vejlsgaard R. The effect of reimbursement on the use of antibiotics. Scand J Prim Health Care 1993; 11:247-51.
18. Goossens H, Peetermans W, Sion JP, Bossens M. ['Evidence-based' perioperative antibiotic prophylaxis policy in Belgian hospitals after a change in the reimbursement system]. Ned.Tijdschr.Geneeskd. 2001; 145:1773-77.
19. Michie S, Johnston M. Changing clinical behaviour by making guidelines specific. BMJ 2004; 328:343-45.
20. Cluzeau F, Littlejohns P, Grimshaw J, Feder G, Moran S. Development and application of a generic methodology to assess the quality of clinical guidelines. Int J Qual Health Care 1999; 11:21-28.
21. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systemic review. BMJ 2003; 326:1167-70.
22. Choudhry NK, Stelfox HT, Detsky AS. Relationships between authors of clinical practice guidelines and the pharmaceutical industry. JAMA 2002; 287:612-17.
23. Van Kasteren MEE, Van de Nieuwenhof L, van der Meer JWM, Gyssens IC. Vijf jaar Stichting Werkgroep Antibioticabeleid; een inventarisatie naar bekendheid, draagvlak en visie vanuit het veld. Ned Tijdschr Med Microb 2002;10:116-7.
24. Van Kasteren MEE, Kullberg BJ, De Boer AS, Mintjes-de Groot J, Gyssens IC. Adherence to local hospital guidelines for surgical antimicrobial prophylaxis: a multicentre audit in Dutch hospitals. J Antimicrob Chemother 2003; 51:1389-96.
25. Van Kasteren MEE, Manniën J, De Boer AS, Wille J, Kullberg BJ, Gyssens IC. An intervention study to improve the prudent use of surgical prophylaxis in Dutch hospitals, Abstract K 1293, 43rd ICAAC, Chicago, 2003.
26. Peetermans W. Prophylactic, empiric and therapeutic use of antibiotics. Do we need a guide: a universal edition or a local one? Eur J Emerg Med 1997; 4:15-18.
27. Sanford JP, Gilbert DN, Sande MA. The Sanford guide to antimicrobial therapy 2002-2003. Belgian/Luxembourg Edition. Dallas, Texas, USA: Antimicrobial Therapy Inc, 2003.
28. Finch RG, Low DE. A critical assessment of published guidelines and other decision-support systems for the antibiotic treatment of community-acquired respiratory tract infections. Clin Microbiol Infect 2002; 8 Suppl 2:69-91.
29. Niederman MS, Mandell LA, Anzueto A, et al. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 2001; 163:1730-54.
30. Schouten JA, Prins JM, Bonten MJ, et al. Revised SWAB guidelines for antimicrobial therapy of community-acquired pneumonia. Neth J Med 2005;63:323-35.
31. Sectie Pediatrische Infectieziekten. Blauwdruk Pediatrische Antimicrobiële Therapie. In: Hartwig NG, Kornelisse RF, Verduin CM, eds. Zeist: Glaxo SmithKline BV, 2001.
32. Bijl D. Co-publication of articles from the Netherlands Drug Bulletin. Neth J Med 2003; 61:183-84.
33. Campbell H, Hotchkiss R, Bradshaw N, Porteous M. Integrated pathways. BMJ 1998; 316:133-37.
34. Gould IM, Hampson J, Taylor EW, Wood MJ, for the Working Party of the British Society for Antimicrobial Chemotherapy. Hospital antibiotic control measures in the UK. J Antimicrob Chemother 1994; 34:21-42.
35. Lawrence M, F. O. Indicators of quality in health care. Eur J Gen Pract 1997; 3:103-08.
36. Field MJ, Lohr KN. Clinical practice guidelines: directions for a new program. Institute of Medicine Washington DC: National Academy Press, 1992.

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REVIEW

Dehydroepiandrosterone administration in humans: evidence based?

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ABSTRACT

Dehydroepiandrosterone (DHEA) and its ester dehydroepiandrosterone sulphate (DHEAS) are produced by the adrenal glands. These hormones are inactive precursors that are transformed into active sex steroids in peripheral target tissues. After a peak in early adulthood, there is a marked decrease in plasma concentrations throughout adult life. These hormones are thought to affect mood and well-being, have neurosteroid effects and may influence the immune system. Animal experiments suggest that DHEA has many other effects, including anticancer, immune-enhancing, neurotropic and general antiageing effects, but information based on studies in humans is limited. In female patients with adrenal insufficiency, treatment with DHEA replacement doses of 20 to 50 mg results in improvements in mood, quality of life and libido. These studies usually lasted only a few months, so the effect of chronic DHEA treatment or its effectiveness in male patients is not known. Some studies suggest a favourable effect of pharmacological doses of DHEA in the treatment of depression. DHEA may have a very limited effect on cognitive function in elderly people, and some studies suggest a beneficial immunomodulatory effect of DHEA in patients with autoimmune diseases, but further studies are warranted before introducing DHEA for these indications in clinical practice.

KEYWORDS

Adrenal insufficiency, autoimmune diseases, cognitive function, DHEA, mood, well-being

INTRODUCTION

Dehydroepiandrosterone (DHEA) and its ester dehydroepiandrosterone sulphate (DHEAS) are the main secretory products of the adrenal zona reticularis, and are known as 'adrenal androgens'. DHEA is lipophilic, and can be converted to DHEAS by activity of the enzyme sulphotransferase in the liver and adrenal glands. DHEAS is the hydrophilic storage form that circulates in the blood, bound to albumin. DHEAS can be converted into DHEA by activity of the enzyme sulphatase in peripheral tissues. DHEA and DHEAS are inactive androgen precursors that are transformed into active sex steroids in peripheral target tissues, e.g. hair follicles, prostate, external genitalia, adipose tissue and the brain. These transformations depend on the tissue activity of metabolising enzymes and result in the production of active androgens (androstenedione, testosterone) and oestrogens (oestradiol and oestrone). In men, about 50% of androgens are derived from adrenal precursor steroids. In premenopausal women, about 75% of oestrogen synthesis occurs in peripheral target cells, and this is almost 100% in postmenopausal women.[1] The sex steroids that are derived from intracellular conversion of DHEA have their action mainly within the cells in which they are produced.

The production of DHEA(S) shows a strong association with age. Serum concentrations of these androgens are low during the prepubertal period, and reach a peak in early adulthood. This is followed by a decline throughout adult life, at the 7th decade the concentrations are only 10 to 20% of the earlier individual peak concentrations.[2,3] In contrast, cortisol levels in men and women show a progressive increase (20% overall) with ageing.[4] It has been suggested that the decrease in cortisol/DHEA ratio may cause a meta-

bolic shift towards a catabolic state during ageing and may negatively affect the cognitive function in the elderly.[4,5] DHEA crosses the blood-brain barrier and may act in the brain as a neurosteroid via effects on neurotransmitter function. A specific nuclear hormone receptor for DHEA has not been identified, but DHEA may act on the N-methyl-D-aspartate (NMDA) receptor as a modulator of the response to NMDA or as an allosteric antagonist at the level of the γ-aminobutyric acid (GABA) receptor.[6] DHEA increases neuronal excitability and initiates alterations in synaptic transmission in the hippocampus. Cognitive processes may therefore be influenced by DHEA.[7] Animal experiments suggest that DHEA has many effects, including anticancer, immune-enhancing, neurotropic and general antiageing effects. However, most experiments with DHEA(S) have been performed in rodents, which have little circulating DHEA(S). Therefore, as DHEA physiology in humans is clearly different from that in nonprimate mammals, these experiments are of limited significance for humans.[8] In the next paragraphs we focus on studies on the effects of DHEA administration in various conditions in humans.

DHEA REPLACEMENT IN ADRENAL INSUFFICIENCY

In patients with adrenal insufficiency, health-related quality of life is impaired despite adequate glucocorticoid and mineralocorticoid replacement.[9] As these patients are DHEA deplete, several studies have assessed the effects of DHEA administration in adrenal insufficiency. The most important are discussed below (see also *table 1*).
In a double-blind, placebo-controlled, crossover study, 24 women with primary or secondary adrenal insufficiency received 50 mg of DHEA or placebo orally, each for four months. Psychological and sexual functioning were evaluated using several validated questionnaires. Treatment with DHEA for four months resulted in significant improvements as compared with placebo. The greatest improvements occurred in the scores for depression and anxiety, compatible with a neurosteroidal action of DHEA. Sexuality improved as well. This was accompanied by an increase in peripheral androgen synthesis, as serum concentrations of DHEA(S), and testosterone returned to the normal range, while serum concentrations of sex hormone-binding globulin, and total and high-density cholesterol decreased. Side effects were transient and mild. The same authors did not find an effect of DHEA on carbohydrate metabolism, body composition or serum insulin.[10] DHEA administration did result in a significant increase in osteocalcin as compared with placebo, suggesting an osteoanabolic effect of DHEA. There was a nonsignificant improvement in physical capacity, and sense of well-being and self-perception improved significantly after four months of DHEA treatment. [11]

In another blinded, crossover study in 39 patients with Addison's disease, the effect of an oral daily dose of 50 mg DHEA for 12 weeks was evaluated. This was the only

Table 1 *DHEA therapy in adrenal insufficiency*

Study	**Arlt *et al.*[10] Callies *et al.*[11]**	**Hunt *et al.*[12]**	**Johannson *et al.*[13]**	**Løvås *et al.*[14]**
Subjects	24 women	24 women, 15 men	38 women	39 women
Age range (years)	23-59	25-69	25-65	18-70
Diagnosis	Primary and secondary AI	Primary AI	Secondary AI	Primary and secondary AI
Study design	1	1	2	2
Duration of DHEA therapy	9 months	7 months	6 months	9 months
Daily DHEA dose	50 mg	50 mg	30 mg (<45 years) 20 mg (>45 years)	25 mg
Results				
Body composition	No change	No change	No change	
Lipids	Decrease TC and HDL-C	No change	No change	No change
Bone markers and BMD	Increase osteocalcin level	BMD no change Osteocalcin level unchanged	Osteocalcin level unchanged	Osteocalcin and deoxypyridinoline unchanged
Mood/well-being	Improvement in scores for depression and anxiety	Self-esteem improved	No change	No change
Sexuality	Improved	No change	No change	No change

1 = double=blind, placebo-controlled, randomised, cross-over; 2 = double-blind, placebo-controlled, parallel; AI = adrenal insufficiency; BMD = bone mineral density; DHEA = dehydroepiandrosterone; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol.

study that also included men. DHEA administration resulted in significant improvement in some aspects of psychological functioning, such as self-esteem, and in improvements in mood and fatigue, especially in the evening. DHEA had no effect on libido and sexual function, memory or cognition. Further, no effect on bone metabolism was found, the authors attributed this to the short duration of treatment.[12]

In another study, 38 women with adrenal insufficiency due to hypopituitarism were treated with oral DHEA for six months in a randomised, placebo-controlled, double-blind study, followed by a six-month open treatment period. The DHEA dose was 30 mg/day when <45 years, and 20 mg/day when >45 years. The primary goal was to study the effect on quality of life, well-being and behaviour. Treatment with DHEA did not result in a change in general well-being as compared with baseline, but the partner questionnaire did reveal an overall improvement in the DHEA-treated group. Compared with placebo, DHEA treatment did not influence sexual interest and activity, whereas during the open phase most women reported an increase. No changes in glucose metabolism, lipoproteins, IGF-1, coagulation parameters, body composition or bone metabolism were observed.[13]

Løvås *et al*. treated 39 women with adrenal insufficiency with either placebo or DHEA, 25 mg/day for nine months.[14] In the DHEA group the levels of DHEAS, androstenedione and testosterone significantly increased as compared with baseline. Health status or sexuality did not improve in the DHEA group as compared with the placebo group. Several side effects, including sweating, hirsutism, acne and itching occurred more often in the DHEA group than in the placebo group.

To define a suitable replacement dose, nine patients with Addison's disease were randomised to oral DHEA 50 mg (n=5) or 200 mg (n=4) daily for three months. Oral doses of 50 mg/day increased circulating levels of DHEA from subnormal to the upper part of the reference range for young individuals, the 200 mg dose resulted in supra-physiological levels, suggesting that 50 mg/day is a replacement dose, and 200 mg/day a pharmacological dose. Five of the nine participants experienced a marked increase in psychological and general well-being. However, from the report it cannot be derived which dosage these five participants used.[15]

In conclusion, trials on DHEA replacement in patients with adrenal insufficiency have mainly been performed in female patients and usually lasted only a few months. Improvements in mood, health-related quality of life and libido were seen in some but not all studies and only if DHEA doses of 50 mg were administered. No convincing effects on bone density, body composition or serum lipid levels were found, and no long-term studies have been carried out to assess the possible effect of DHEA in either replacement or pharmacological dose on the incidence of cancer or cardiovascular disease.

EFFECT OF DHEA REPLACEMENT ON MOOD, WELL-BEING AND COGNITION

Ageing results in a decline in concentrations of DHEA, which may have physiological significance.[4] Morales *et al*. demonstrated an improvement in self-reported physical and psychological well-being in middle-aged and elderly men and women after administration of a daily dose of 50 mg DHEA for three months.[16] The same group also did a study on the effects of a DHEA 100 mg/day, but in this report the effect of this higher DHEA dose on well-being was not mentioned.[17] In a double-blind cross-over study, 22 healthy male volunteers received four months of DHEA (50 mg/day) and four months placebo treatment. In these healthy men with age-related physiological decline of DHEA secretion, no obvious benefit of DHEA replacement was found.[18] Wolkowitz *et al*. randomised 22 subjects with a major depression to receive either DHEA (30-90 mg/day) or placebo for six weeks.[19] They concluded that patients treated with DHEA showed a greater anti-depressant response (an overall enhancement of mood scores by 30.5%) than those who were treated with placebo. In a recently published study, six weeks of DHEA therapy in a pharmacological dose (90 mg/day for three weeks and 450 mg/day for three weeks), was compared with placebo for six weeks in men (n=23) and women (n=23) aged 45 to 65 years with midlife-onset major or minor depression. DHEA administration in a pharmacological dose was associated with a significant improvement compared with both baseline ($p<0.01$) and placebo treatment ($p<0.01$).[20]

The mechanism of the possible antidepressant effect of DHEA is unclear. DHEA crosses the blood-brain barrier and may interact directly with brain function by affecting serotonin and/or GABA receptors. Secondly, in brain cells DHEA is a precursor for testosterone and oestrogen formation, and increases in the levels of these hormones can enhance mood. Another theory suggests that a normal DHEA/cortisol ratio is required for a good balance between anabolic and catabolic steroid effects. Cortisol levels are increased in major depression, so DHEA administration may restore this ratio.[21]

DHEA ADMINISTRATION TO IMPROVE COGNITIVE FUNCTION IN ELDERLY PEOPLE

Serum levels of DHEA(S) decrease during ageing, resulting in a progressive decrease in the serum DHEA(S)/cortisol

ratio. It has been hypothesised that this may impair cognitive function. DHEA administration may restore the DHEA(S)/cortisol ratio, thus potentially improving cognitive function and preventing neurotoxic effects of cortisol. This hypothesis resulted in four placebo-controlled clinical trials that investigated the effect of DHEA administration on cognitive function in elderly people. These studies were reviewed by Huppert *et al.*[22] All four studies used a daily oral DHEA dose of 50 mg, for periods lasting less than three months. Few significant changes were found. In one study DHEA improved immediate and delayed recall in a visual memory test compared with placebo, while no improvement in a verbal memory test, nor in four other cognitive tests was seen.[23] Another study found a deterioration in a test following a psychological stressor in the placebo group, which was not seen in the DHEA group.[24] The other studies did not demonstrate any beneficial effects of DHEA administration.[25,26] The overall conclusion of Huppert *et al.* was that there is very limited evidence for a beneficial effect of a daily oral dose of 50 mg DHEA on cognitive function in elderly people.[22] Using a higher dose of 100 mg DHEA a day for three months, Flynn *et al.* did not find clinically meaningful changes in 39 healthy elderly men either.[27]

DHEA ADMINISTRATION IN POSTMENOPAUSAL WOMEN

In women the synthesis of DHEA occurs mainly in the adrenal cortex and DHEA serves as the main precursor of active oestrogens in postmenopausal women. The reduction in DHEA(S) formation by the adrenals during ageing results in a reduction in the formation of androgens and oestrogens in peripheral tissues. It has been suggested that this could influence well-being and the occurrence of osteoporosis, obesity and insulin resistance in postmenopausal women. In the last few years several trials have assessed whether administration of DHEA would improve interest in sex, sexual satisfaction, quality of life or osteoporosis in postmenopausal women. Barnhart *et al.* found no difference in improvements in mood, libido, cognition, memory or well-being after treatment with DHEA (50 mg/day for three months) *vs* placebo in 60 perimenopausal women.[25] Genazzani *et al.* studied the effect of oral administration of 25 mg DHEA daily in 20 postmenopausal women in a 12-month prospective study that was not blinded or randomised.[28] They found an increase in the circulating levels of all DHEA-derived steroids, osteocalcin, as well as growth hormone and IGF-1 during DHEA supplementation. An improvement in psychological functioning was also found. These authors concluded that DHEA administration may be considered in postmenopausal women.

DHEA ADMINISTRATION IN PATIENTS WITH AUTOIMMUNE DISEASES

Because of its immunomodulatory effects in animal studies, DHEA administration has been studied as a potential pharmacological tool in the treatment of human autoimmune diseases. In 21 patients with severe systemic lupus erythematosus (SLE), DHEA administration (200 mg/day *vs* placebo for six months) was added to conventional treatment. DHEA administration had a beneficial effect on lupus outcomes and protected against steroid-induced osteopenia.[29] This effect on bone density could not be confirmed by Hartkamp *et al.*[30] Chang *et al.* investigated 120 adult women with active lupus, who were treated with DHEA, 200 mg/day or placebo, for 24 weeks.[31] They found a reduction in disease flares and the patients perceived a decrease in disease activity. Side effects (acne, hirsutism) were mild.

CONCLUSION

DHEA is an adrenal androgen for which many effects in different clinical situations have been postulated. Many studies have been performed to assess the importance of DHEA administration. In most studies in patients with adrenal insufficiency, oral DHEA replacement is biochemically effective, well tolerated and associated with some improvement in well-being, mood and fatigue. This may at least partly be mediated by neurosteroid effects of DHEA. However, all studies have been small and short term, and studies in adrenal insufficiency have mainly been carried out in women. So far no studies have compared the effects of DHEA administration with those of other anabolic steroid hormones in patients with adrenal insufficiency. Side effects of treatment with DHEA were mild and androgen related.

Some studies suggest a favourable effect of DHEA in pharmacological doses in treatment of depression. So far, no consistent beneficial effect of DHEA administration on mood, general well-being and on cognitive function in elderly people has been found. DHEA administration in postmenopausal women needs to be assessed in larger and longer-lasting studies before therapy with DHEA is used for these purposes. Pharmacological therapy with DHEA in autoimmune diseases, such as SLE, may prove to be beneficial for these patients. More and longer-term studies are required to demonstrate the immunomodulatory effects of DHEA administration in patients with SLE. Further, long-term studies would also need to assess if long-term treatment with DHEA in either pharmacological or physiological doses will have any effect on the incidence of cardiovascular or malignant disease.

REFERENCES

1. Labrie F, Van Luu-The, Labrie C et al. Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocrine Reviews 2003;24;152-82.
2. Orentreich N, Brind JL, Vogelman JH, Andres R, Baldwin H. Long-term longitudinal measurements of plasma dehydroepiandrosterone sulfate in normal men. J Clin Endocrinol Metab 1992;75:1002-4.
3. Parker CR, Mixon RL, Brissie RM, Grizzle WE. Aging alters zonation in the adrenal cortex of men. J Clin Endocrinol Metab 1997;82:3898-901.
4. Laughlin GA and Barrett-Connor E; Sexual dimorphism in the influence of advanced aging on adrenal hormone level: the Rancho Bernardo Study. J Clin Endocrinol Metab 2000;85:3561-8.
5. Kalmijn S, Launer LJ, Stolk RP et al, A prospective study on cortisol, dehydroepiandrosterone sulfate, and cognitive function in elderly. J Clin Endocrinol Metab 1998;83:3487-92.
6. Demirgoren S, Majewska MD, Spivak CE, London ED. Receptor binding and electrophysiological effects of dehydroepiandrosteronesulfate, an antagonist of the GABAβ receptor. Neuroscience 1991;45:127-35.
7. Baulieu EE, Robel P. Dehydroepiandrosterone and dehydroepiandrosterone sulfate as neuroactive neurosteroids. J Endocrinol 1996;150:S221-39.
8. Cutler GB, Glenn M, Bush M, Hodgen GD, Graham CE, Loriaux DL. Adrenarche: a survey of rodents, domestic animals, and primates. Endocrinology 1978;103:2112-8.
9. Løvås K, Håvard Loge J, Husebye ES. Subjective health status in Norwegian patients with Addison's disease. Clin Endocrinol 2002;56:581-8.
10. Arlt W, Callies F, Vlijmen JC van et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency. N Engl J Med 1999;341:1013-20.
11. Callies F, Fassnacht M, Vlijmen JC van et al. Dehydroepiandrosterone replacement in women with adrenal insufficiency: effects on body composition, serum leptin, bone turnover, and exercise capacity. J Clin Endocrinol Metab 2001;86:1968-72.
12. Hunt PJ, Gurnell EM, Huppert FA et al. Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial. J Clin Endocrinol Metab 2000;85:4650-56.
13. Johannsson G, Burman P, Wiren L et al. Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women : a placebo-controlled trial. J Clin Endocrinol Metab 2002;87:2046-52.
14. Løvås K, Gebre-Medhin G, Trovik TS, et al. Replacement of dehydroepiandrosterone in adrenal failure: no benefit for subjective health status and sexuality in a 9-month, randomized, parallel group clinical trial. J Clin Endocrinol Metab 2003;88:1112-18.
15. Gebre-Medhin G, Husebye ES, Mallmin H et al. Oral dehydroepiandrosterone (DHEA) replacement therapy in women with Addison's disease. Clin Endocrinol 2000;52:775-80.
16. Morales AJ, Nolan JJ, Nelson JC, Yen SSC. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. J Clin Endocrinol Metab 1994;78:1360-67.
17. Morales AJ, Haubrich RH, Hwang JY, Asakura H, Yen SSC. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition, and muscle strength in age-advanced men and women. Clin Endocrinol 1998;49:421-32.
18. Arlt W, Callies F, Koehler I et al. Dehydroepiandrosterone supplementation in healthy men with an age-related decline of dehydroepiandrosterone secretion. J Clin Endocrinol Metab 2001,86:4686-92.
19. Wolkowitz OM, Reus VI, Keebler A, Nelson N, Friedland M, Brizendine L, Roberts E. Double-blind treatment of major depression with dehydroepiandrosterone. Am J Psychiatry 1999;156:646-49.
20. Schmidt PJ, Daly RC, Bloch M, et al. Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression, Arch Gen Psychiatry. 2005; 62 (2):154-62.
21. Wolkowitz OM, Epel ES, Reus VI. Stress hormone-related psychopathology: pathophysiological treatment implications. World J Biol Psychiatry 2001;115-43.
22. Huppert FA, Niekerk van JK. Dehydroepiandrosterone (DHEA) supplementation for cognitive function. Cochrane Review; The Cochrane Library, Issue 4, 2003. Chichester, UK: John Wiley and Sons, Ltd.
23. Wolf OT, Neumann E, Hellhammer DH et al. Effects of a two-week physiological dehydroepiandrosterone substitution on cognitive performance and well-being in healthy elderly women and men. J Clin Endocrinol Metab 1997;82:2363-67.
24. Wolf OT, Kudielka BM, Hellhammer DH, Hellhammer J, Kirschbaum C. Opposing effects of DHEA replacement in elderly subjects on declarative memory and attention after exposure to a laboratory stressor. Psychoneuroendocrinology 1998;23:617-29.
25. Barnhart KT, Freeman E, Grisso JA et al. The effect of dehydroepiandrosterone supplementation to symptomatic perimenopausal women on serum endocrine profiles, lipid parameters, and health-related quality of life. J Clin Endocrinol Metab 1999;84:3896-902.
26. Wolf OT, Naumann E, Hellhammer DH, Kirschbaum C. Effects of dehydroepiandrosterone (DHEA) replacement in elderly men on event related potentials (ERPs), memory and well-being. J of Gerontology 1998;53(5):M385-90.
27. Flynn MA, Weaver-Osterholtz D, Sharpe-Timms KL, Allen S, Krause G. Dehydroepiandrosterone replacement in aging humans. J Clin Endocrinol Metab 1999;84:1527-33.
28. Genazzani AD, Stomati M, Bernardi F, Pieri M, Rovati L, Genazzani AR. Long-term low-dose dehydroepiandrosterone oral supplementation in early and late postmenopausal women modulates endocrine parameters and synthesis of neuroactive steroids. Fertility and Sterility 2003;80:1495-1501.
29. Vn Vollenhoven RF, Park JL, Genovese MC, West JP, McGuire JL. A double-blind, placebo-controlled, clinical trial of dehydroepiandrosterone in severe systemic lupus erythematosus. Lupus 1999;8:181-87.
30. Hartkamp A, Geenen R, Godaert GL, Bijl M, Bijlsma JW, Derksen RH. The effect of dehydroepiandrosterone on lumbar spine bone mineral density in patients with quiescent lupus erythematosus. Arthritis Rheum 2004;50:3591-95.
31. Chang DM, Lan JL, Luo SF. DHEA in the treatment of women with mild-to-moderate systemic lupus erythematosus: a multi-center randomised, double-blind, placebo controlled trial. Arthritis Rheum 2002:46;2924-27.

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ORIGINAL ARTICLE

NOD2 3020insC mutation and the pathogenesis of Crohn's disease: impaired IL-1β production points to a loss-of-function phenotype

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ABSTRACT

Background: Mutations of the *NOD2* gene increase the susceptibility of humans to Crohn's disease. *NOD2* is a cytoplasmic receptor for the bacterial product peptidoglycan. There is considerable controversy in the literature whether the most common mutation in Crohn's disease, the 3020insC NOD2, leads to a loss of function, i.e. decreased cytokine production, or to the reverse, i.e. a gain of function. In previous papers we proposed the former, since we could show decreased cytokine production with a net proinflammatory status after exposure to muramyl dipeptide (MDP). Methods: Because of recent data in the literature showing increased interleukin-β (IL-1β) production in mice with the corresponding *NOD2* mutation, we investigated the production of this cytokine by cells of patients with Crohn's disease, either homozygous or heterozygous for the 3020insC mutation, and compared it with that of patients with Crohn's disease bearing the wild-type allele. Results: A strongly decreased production of IL-1β by peripheral mononuclear cells was found upon exposure to either peptidoglycan or peptidoglycan-derived MDP in homozygous patients bearing the 3020insC *NOD2* mutation. Conclusion: This sustains the hypothesis that the 3020insC mutation in the human *NOD2* gene leads to a loss-of-function phenotype.

KEYWORDS

Crohn's disease, cytokine, IL-1, *NOD2*

In recent years, the insight into genetic susceptibility to Crohn's disease has greatly increased. A susceptibility locus for Crohn's disease was detected on chromosome 16,[1] and subsequently the candidate *NOD2* gene has been identified as the susceptibility locus IBD1.[2-4] NOD2 is a member of the NOD-leucine-rich repeat (LRR) protein family (also called the CATERPILLER family), known to be involved in recognition of microbial structures, and is expressed intracellularly in antigen-presenting cells.[5] Initially, NOD2 was believed to be an intracellular pattern recognition receptor for lipopolysaccharide (LPS),[4] similar to NOD1,[6] but further investigations have demonstrated that NOD2 is the intracellular receptor for the muramyl dipeptide (MDP) component of bacterial peptidoglycan (PGN).[7,8]

The mutated *NOD2* associated with Crohn's disease has been reported to be unable to sense MDP and this would suggest that the mutation would result in a loss-of-function phenotype. This is consistent with the finding that peripheral blood cells of patients with the *NOD2* mutation exposed to NOD2 ligands produce low amounts of the proinflammatory cytokines tumour necrosis factor alpha (TNF), interleukin-6 (IL-6) and IL-8, as well as the anti-inflammatory cytokine IL-10.[9,10] Conceptually this poses an enigma, because Crohn's disease is an inflammatory disease. In essence, two basically opposite hypotheses have been put forward: one advocating that the *NOD2* mutation leads to defective anti-inflammatory control ('loss-of function'), the other advocating that the mutation

leads to activated inflammation ('gain of function'). So far, we have been more in favour of the loss-of-function hypothesis as we obtained experimental evidence for a defective response to peptidoglycan and MDP in cells with this mutation. Recently, however, Maeda *et al.* proposed a gain-of-function effect of *NOD2* mutations based on the finding of greater IL-1β release in MDP-stimulated cells of mice bearing an NOD2 mutation that corresponds to the human 3020insC mutation. Prompted by the intriguing findings of Maeda *et al.* on the increased IL-1β processing, we measured mature IL-1β released by the mononuclear cells of patients with the 3020insC mutation, after stimulation with either peptidoglycan, or a combination of MDP with the lipoprotein MALP-2, a Toll-like receptor-2 (TLR2) agonist. The latter stimulation was investigated, because we recently demonstrated synergy between the cell surface pattern recognition receptor TLR2 and the cytoplasmic NOD2.[11]

MATERIALS AND METHODS

Genotyping of NOD2 variants

Blood was collected from 74 patients with Crohn's disease and ten healthy volunteers. Polymerase chain reaction (PCR) amplification of *NOD2* gene fragments containing the polymorphic site 3020insC was performed in 50 μl reaction volumes containing 100 to 200 ng genomic DNA, as previously described.[10] The 3020insC polymorphism was analysed by Genescan analysis on an ABI Prism 3100 Genetic Analyser according to the manufacturer's protocol (Applied Biosystems, Nieuwerkerk a/d IJssel, the Netherlands).

Four patients with Crohn's disease were found homozygous for the 3020insC mutation, and they were further investigated in the cytokine studies. As control groups, five patients with Crohn's disease heterozygous for the 3020insC *NOD2* mutation, five patients with Crohn's disease bearing the wild-type allele, and five healthy volunteers homozygous for the wild-type *NOD2* allele were included.

Isolation of mononuclear cells and stimulation of cytokine production

After obtaining informed consent, venous blood was drawn from the cubital vein of patients and healthy volunteers into three 10 ml EDTA tubes (Monoject, s-Hertogenbosch, the Netherlands). The mononuclear cell (MNC) fraction was obtained by density centrifugation of blood diluted 1:1 in pyrogen-free saline over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). Cells were washed twice in saline and suspended in culture medium (RPMI 1640 DM) supplemented with gentamicin 10 μg/ml, L-glutamine 10 mM and pyruvate 10 mM. The cells were counted in a Coulter counter (Coulter Electronics, Mijdrecht, the Netherlands) and the number was adjusted to 5×10^6 cells/ml.

Next, 5×10^5 MNC in a 100 μl volume were added to round-bottom 96-well plates (Greiner, Alphen a/d Rijn, the Netherlands) and incubated with either 100 μl of culture medium (negative control), MDP (10 μg/ml, Sigma Chemical Co, St. Louis), purified staphylococcal peptidoglycan (1 μg/ml), or MALP2 lipopeptides (1 μg/ml, EMC Microcollections, Tübingen, Germany).

Cytokine measurements

Human IL-1β concentrations were determined by specific radioimmunoassays as previously described.[12]

Statistical analysis

The experiments were performed in triplicate with blood obtained from patients and volunteers. The differences between groups were analysed by the Mann-Whitney U test, and where appropriate by the Kruskal-Wallis ANOVA test. The level of significance between groups was set at $p<0.05$. The data are given as means ± SEM.

RESULTS

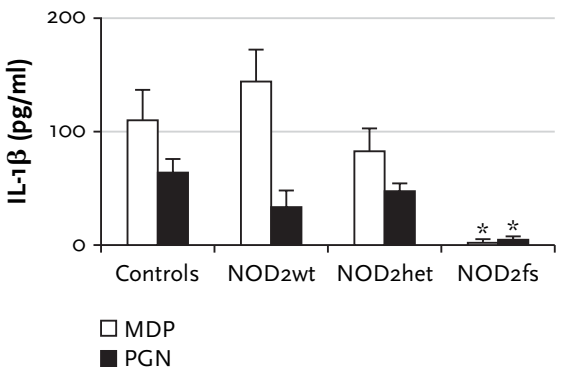
The release of IL-1β after stimulation with peptidoglycan or MDP did not differ between healthy volunteers and patients with Crohn's disease who were either heterozygous for the mutation or had no mutation (*figure 1*). In contrast, patients homozygous for the mutation exhibited a strongly decreased IL-1β synthesis in response to both peptidoglycan or MDP (*figure 1*).

To investigate whether the mutated *NOD2* leads to modified synergism between NOD2- and TLR2-mediated signalling, cells from the patients with the 3020insC mutation were stimulated with a combination of MDP and the lipoprotein MALP-2, a specific TLR2 agonist (*figure 2*). MDP and MALP-2 appeared to have synergistic effects on IL-1β release of normal cells; these effects were absent in patients with the 3020insC mutation, arguing that the 3020insC mutation induces a loss-of-function phenotype.

DISCUSSION

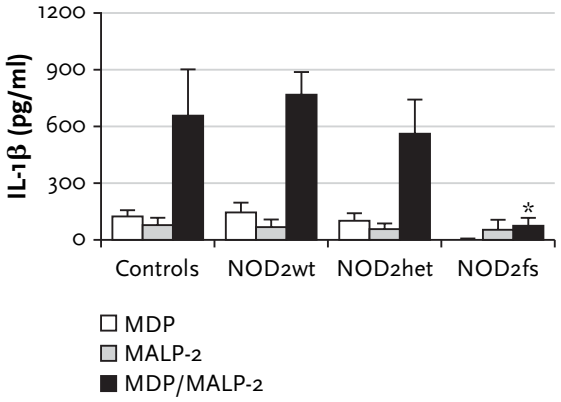
In this paper, we demonstrate that peripheral blood mononuclear cells of patients suffering from Crohn's disease with the 3020insC *NOD2* mutation are defective in terms of IL-1β production when stimulated with the NOD2 ligands MDP and peptidoglycan. These results argue for a lack-of-function character of the mutation and are fundamentally different from those obtained in mice with the equivalent mutation.[13]

Figure 1 *PGN and MDP stimulation of cytokines: requirement of NOD2*



MNC isolated from four patients with Crohn's disease homozygous for the 3020insC *NOD2* mutation (NOD2fs), five patients heterozygous for *NOD2* mutations (NOD2het), five patients with the wild-type *NOD2* allele (NOD2wt) and five healthy volunteers with wild-type *NOD2* (controls), were stimulated with either 5 μg/ml MDP (solid bars) or 10 μg/ml PGN (hatched bars), for 24h at 37°C. IL-1β concentrations were measured by ELISA. Data are presented as means ± SD, and compared by Mann-Whitney U-test (*p<0.05).

Figure 2 *TLR2 and NOD2 pathways have synergistic effects on IL-1β production*



MNC isolated from four patients with Crohn's disease homozygous for the 3020insC *NOD2* mutation (NOD2fs), five patients heterozygous for *NOD2* mutations (NOD2het), five patients with the wild-type *NOD2* allele (NOD2wt) and five healthy volunteers with wild-type *NOD2* (controls), were stimulated with either 5 μg/ml MDP (open bars), 5 μg/ml MALP-2 (hatched bars), or a combination of both (solid bars) for 24h at 37°C. IL-1β concentrations were measured by ELISA. Data are presented as means ± SD, and compared by Mann-Whitney U-test (*p<0.05).

The low IL-1β production in humans with the mutation is in agreement with previous studies from both our laboratory and others demonstrating decreased production of other proinflammatory cytokines in these patients.[9-11,14] Likewise, Li *et al.* have shown reduced IL-1β release in mononuclear cells from two patients with homozygous 3020insC mutation when stimulated with a combination of MDP and TNF.[15] These human studies are, however, at odds with the increased IL-1μ production in mice genetically engineered to have the same *NOD2* mutation as the human 3020insC mutation,[13] which suggested a gain-of-function phenotype of this mutation. Proponents of the gain-of-function hypothesis have argued that the cells from patients with the 3020inC mutation suffer from active inflammatory disease and therefore may have downregulated cytokine production,[16] as is commonly found in other inflammatory conditions.[17] This is, however, very unlikely for a variety of reasons. First of all, patients with Crohn's disease bearing the 3020insC mutation had lower cytokine production only after stimulation with the NOD2 ligands peptidoglycan and MDP, but not after the TLR2 agonist MALP-2 in this study (*figure 2*), or other TLR ligands as shown in previous studies.[11,14] If an inhibition of cytokine production due to inflammation had been present, a general downregulation of both NOD2- and TLR-induced cytokines should have been found. Secondly, an inflammation-driven downregulated proinflammatory cytokine production tends to be associated with an upregulated anti-inflammatory cytokine response.[17] This is not the case here: we have previously demonstrated that the anti-inflammatory response as exemplified by IL-10 production is strongly inhibited.[10] An explanation for the increased inflammation in mice bearing the variant *NOD2* could have been the lack of inhibitory signals on TLR2-induced cytokine release, leading to increased cytokine production, as recently proposed by Watanabe *et al.*[18] Unfortunately, Maeda *et al.* inappropriately tested this hypothesis by using PGN as a putative TLR2 ligand, and stimulating cells with a combination of MDP and PGN.[13] PGN is in fact the bacterial product containing the MDP motif, and thus an NOD2 ligand. The TLR2-dependent activity of PGN has been convincingly shown to be due to contamination with lipoteichoic acid.[19] To settle the argument whether the lack of functional NOD2 would lead to enhanced TLR2-mediated signals, we stimulated cells from the patients bearing the 3020insC mutation with a combination of MDP and the lipoprotein MALP-2, a specific TLR2 agonist (*figure 2*). We have shown here that MDP and MALP-2 have synergistic effects on IL-1β release, and these effects were absent in patients with the 3020insC mutation, arguing that an *NOD2*-mediated suppression of TLR2 signals does not play an important role in patients with Crohn's disease.

What could the explanation be for the apparent discrepancies between 3020insC-positive Crohn's patients and *NOD2* variant mice? Most likely, there are crucial differences between the murine and human *NOD2* systems. This may also explain why humans with *NOD2* mutations develop Crohn's disease, whereas mice deficient for *NOD2* do not show any signs of inflammation.[20] The mechanisms through which the mutations in the *NOD2* gene result in chronic intestinal inflammation in humans are likely mediated by two pathways: firstly through decreased defence against intestinal pathogens that trigger the initial inflammatory reaction,[21] and secondly through loss-of-control of the intestinal inflammation due to the defective release of anti-inflammatory cytokines such as IL-10 and TGFβ.[10]

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REFERENCES

1. Hugot J-P, Laurent-Puig P, Gower-Rousseau C, et al. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 1996;379:821-3.
2. Hugot J-P, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001;411:599-603.
3. Hampe J, Grebe J, Nikolaus S, et al. Association of NOD2 (CARD15) genotype with clinical course of Crohn's disease: a cohort study. Lancet 2002;359:1661-5.
4. Ogura Y, Bonen DK, Inohara N, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001;411:603-6.
5. Gutierrez O, Pipaon C, Inohara N, et al. Induction of Nod2 in myelomonocytic and intestinal epithelial cells via nuclear factor-kB activation. J Biol Chem 2002;277:41701-5.
6. Inohara N, Ogura Y, Chen FF, Muto A, Nunez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. J Biol Chem 2001;276:2552-4.
7. Girardin SE, Boneca IG, Viala J, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003;278:8869-72.
8. Inohara N, Ogura Y, Fontalba A, et al. Host recognition of bacterial muramyl dipeptide mediated through Nod2. J Biol Chem 2003;278:5509-12.
9. Bonen DK, Ogura Y, Nicolae DL, et al. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 2003;124:140-6.
10. Netea MG, Kullberg BJ, de Jong D, et al. NOD2 mediates induction of the antiinflammatory signals induced by TLR2-ligands: implications for Crohn's disease. Eur J Immunol 2004;34:2052-9.
11. Netea MG, Ferwerda G, De Jong DJ, et al. NOD2 modulates specific Toll-like receptor pathways for the induction of cytokine release. J Immunol 2005;174:6518-23.
12. Drenth JPH, van Deuren M, van der Ven-Jongekrijg J, Schalkwijk CG, van der Meer JWM. Cytokine activation during attacks of the hyper-immunoglobulinemia D and periodic fever syndrome. Blood 1995;85:3586-93.
13. Maeda S, Hsu L-C, Liu H, et al. NOD2 mutation in Crohn's disease potentiates NF-kB activity and IL-1beta processing. Science 2005;307:734-8.
14. Van Heel DA, Ghosh S, Hunt K, et al. Muramyl dipeptide and toll-like receptor sensitivity in NOD2-associated Crohn's disease. Lancet 2005;365:1794-6.
15. Li J, Moran T, Swansioson E, et al. Regulation of IL-8 and IL-1beta expression in Crohn's disease associated NOD2/CARD15 mutations. Hum Mol Genetics 2004;13:1715-25.
16. Eckmann L, Karin M. NOD2 and Crohn's disease: loss or gain of function? Immunity 2005;22:661-7.
17. Van Deuren M, Dofferhoff ASM, van der Meer JWM. Cytokines and the response to infections. J Pathol 1992;168:349-56.
18. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004;advanced online publication.
19. Travassos LH, Girardin SE, Philpott DJ, et al. Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition. EMBO Reports 2004;5:1000-6.
20. Pauleau AL, Murray PJ. Role of NOD2 in the response of macrophages to toll-like receptor agonists. Mol Cell Biol 2003;23:7531-9.
21. Kobayashi KS, Chamaillard M, Ogura Y, et al. NOD2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005;307:731-4.

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ORIGINAL ARTICLE

Regional differences in cardiovascular risk factor profile cannot fully explain differences in cardiovascular morbidity in the Netherlands: a comparison of two urban areas

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ABSTRACT

Background: Our objective was to investigate whether a region in the south of the Netherlands (Heerlen/Kerkrade) had a high burden of cardiovascular disease in comparison with a nearby region (Maastricht) and the average Dutch population, respectively. We also wanted to determine if there are interregional differences in cardiovascular risk factor profile.

Design: Cross-sectional study.

Methods: Data from a nationwide registry (CBS) were used to analyse cardiovascular mortality in the two regions and the average in the Netherlands. Data from a primary care morbidity registration network (RNH) were used to compare cardiovascular morbidity and cardiovascular risk factors in both regions. A standardisation procedure was carried out for age and sex. Data were analysed using logistic regression analyses.

Results: The overall cardiovascular mortality rate was higher in the Heerlen/Kerkrade region (7.8 ‰) compared with Maastricht (6.1‰, OR=1.3, 95% CI. 1.2-1.5) and the average in the Netherlands (5.7‰). Similarly, most cardiovascular morbidity rates for Heerlen/Kerkrade were more elevated compared with the RNH overall and with Maastricht. Prevalence rates of risk factors such as diabetes mellitus (7.2%, OR=1.5, 95% CI 1.3-1.7) and overweight (10.8%, OR= 2.0, 95% CI 1.8-2.2) were significantly higher in the Heerlen/Kerkrade region compared with Maastricht. There were no differences with regard to hypertension (15.2%, OR=1.0, 95% CI 0.9-1.1).

Conclusion: Heerlen/Kerkrade is indeed a region with a high burden of cardiovascular disease. Differences in morbidity between Heerlen/Kerkrade and Maastricht cannot be fully explained by differences in cardiovascular risk factor profile.

KEYWORDS

Cardiovascular diseases/mortality, Netherlands/epidemiology, morbidity/prevalence, risk factors

INTRODUCTION

Recently our group started the HIPPOCRATES project (Hypertension: Interaction and Prevalence of POlymorphisms related to Cardiovascular Risk and the Association to Treatment Efficacy Study).[1] The main objective of this study is implementation of genetic polymorphisms in the assessment of cardiovascular risk in primary care. This study utilises the population of a general practice centre located in the southeast of Limburg, i.e. an urbanised area around the cities of Heerlen and Kerkrade. From a population genetic point of view, this region is interesting in two aspects: (1) in unpublished Dutch reports a relatively high cardiovascular mortality

has been described; (2) in and out migration figures are relatively low. Therefore, we were interested in the cardiovascular mortality of this region compared with the average in the Netherlands as well as with a region geographically nearby. Comparisons as these are usually limited to mortality data due to scarcity of national morbidity data. However, morbidity data give a better estimation of the prevalence of cardiovascular disease. Fortunately, sources for regional morbidity data are available from general practice registration networks.[2] Since in the Netherlands almost everyone is on the list of a general practitioner, morbidity registrations in these practices reflect the health status of a general population in a specific area. Our department coordinates such a primary care morbidity registration network.[2] Consequently, we had the opportunity to compare the morbidity figures of various regions. Moreover, we could explore the prevalence rates of some important cardiovascular risk factors also registered by this network (hypertension, diabetes mellitus, overweight and lipid disorders). Variation in risk factor profile might explain possible differences in cardiovascular mortality and morbidity profile between regions.[3,4] Comparing data on cardiovascular mortality and morbidity as well as risk factors could give insight into the specific cardiovascular profile of the study region. In this study the main question was whether the Heerlen/Kerkrade region does indeed have a high burden of cardiovascular disease compared with a nearby region (Maastricht) and the average of the Netherlands. A second question was whether there were interregional differences in cardiovascular risk factor profile.

Table 1 *Mortality and morbidity of categories of cardiovascular (CV) disease and risk factors studied and their ICD-10 and ICPC codes*

	Mortality (CBS database, ICD-10 codes)	Morbidity (RNH database, ICPC codes)
Disease category		
Ischaemic heart disease	I20 - I25	K74 - K76
Stroke	I60 - I69	K89 - K90
Other CV diseases:		
- Other heart disease	I00 - I09 I30 - I52	K70 - K73 K77 - K84
- Other vascular disease	I26 - I28 I80 - I89	I98 - I99 K93 - K99
- Peripheral arterial occlusive disease	I70 I73 - I74	K91 - K92
Risk factor category		
Hypertension	I10 - I13 I15	K85 - K87
Diabetes mellitus (I & II)	E10 - E14	T90
Overweight	E66	T82 - T83
Lipid disorders	E78	T93

CBS = Statistics Netherlands; RNH = Registration Network of General Practitioners.

METHODS

Cardiovascular mortality

From the official death certification data managed by Statistics Netherlands (CBS), the latest available mortality data (2000) were used on cardiovascular disease and risk factors for Heerlen/Kerkrade and Maastricht. Diagnoses were coded according to the International Classification of Diseases (ICD-10).[5] The validity of the death registry is generally considered sufficiently good for epidemiological use.[6,7] The disease categories studied are presented in *table 1*.

Cardiovascular morbidity

Cardiovascular morbidity rates were retrieved from the Registration Network of General Practitioners (RegistratieNet Huisartspraktijken, RNH). This is a continuous and computerised database in which 63 general practitioners (GPs) working in 22 different practices in the south of the Netherlands participate. All relevant health problems are registered. A health problem is defined as 'anything that has required, does or may require healthcare management and has affected or could significantly affect a person's physical or emotional well-being'.[8] Health problems are only coded by the GPs if they are permanent (no recovery expected), chronic (duration longer than six months), recurrent (more than three recurrences within six months), or when they have lasting consequences for the functional status or prognosis of the patient. Problems are coded according to the International Classification of Primary Care (ICPC) using the criteria of the International Classification of Health Problems in Primary Care for diagnoses.[9,10] The registered data are continuously updated and historically cumulated for each patient. Population membership only ends by migration or death. The quality of the data is ensured by instruction and training sessions, regional consensus groups, quality control experiments and by an automated thesaurus and automated checking for erroneous or missing entries.[11]

For this study, data from five general practices (n=10,587) in Heerlen/Kerkrade (index population) and from three general practices (n=8742) in Maastricht (control population) were used. For both regions and the RNH overall (n=56,976) prevalence rates were calculated. We compared the prevalence rates of Heerlen/Kerkrade with Maastricht. Age and sex distribution of the total RNH dataset was reported to be similar to that of the Netherlands.[8] Comparison of the

age and sex distribution of the total RNH dataset (2001) with the Netherlands (2001) still appeared to be the same. Age- and sex-specific data were drawn from the latest RNH dataset available (1 July 2001). The disease categories studied are presented in *table 1*.

Cardiovascular risk factors

Prevalence rates of cardiovascular risk factors were also retrieved from the eight general practices in Heerlen/ Kerkrade and Maastricht. Age- and sex-specific data were drawn from the latest RNH dataset available (1 July 2001). The risk factors studied are also presented in *table 1*.

Statistical analysis

To compare the prevalence of cardiovascular mortality and morbidity between both regions (Heerlen/Kerkrade vs Maastricht), a standardisation procedure was carried out for sex and age, in which the standard population was the population of all RNH practices. To determine whether the observed differences between both regions were statistically significant, logistic regression analyses were performed, using the statistical software programme SPSS 9.0 for Windows. In the analyses regarding mortality, the dependent variable was presence or absence of a specific cause or causes of death; in those regarding morbidity and risk factors, the dependent variable was presence or absence of the disease category or risk factor. The independent variable was 'region' (Heerlen/Kerkrade *vs* Maastricht) with potential confounders sex and age distribution. First, all variables were entered in the model, followed by all possible interaction terms (method: enter). The model was then further fitted, based on the statistical significance of the various interaction terms (region times age, region times sex, sex times age) according to the likelihood ratio test. In all analyses, age was entered as a categorical variable since this improved the Hosmer-Lemeshow goodness-of-fit tests considerably.[12]

RESULTS

General characteristics

The general characteristics of the study populations are summarised in *table 2*. With regard to mortality data (CBS), there were hardly any differences in age distribution between both regions. With regard to morbidity data (RNH), the population of Heerlen/ Kerkrade was slightly older than that of Maastricht. Both CBS and RNH populations contained slightly more females than males. Overall, both study populations were very comparable regarding to age and sex.

Cardiovascular mortality

Mortality figures of some cardiovascular diseases or risk factors were so small that no meaningful analyses could be performed. This was the case for 'other vascular disease' and 'peripheral arterial occlusive disease' and for all the risk factors. *Table 3* shows standardised mortality rates of cardiovascular diseases for both regions and the average in the Netherlands. The overall trend for all categories was that mortality rates were higher in Heerlen/Kerkrade compared with Maastricht. The most distinct difference was found for 'ischaemic heart disease'. *Figure 1* shows the age-specific mortality rates for the two regions. For every age group, the mortality rate was higher in Heerlen/Kerkrade compared with Maastricht. However, the pattern of the mortality rates was comparable in the two regions.

Table 2 *Sex and age distribution of the study populations for mortality and morbidity*

	Mortality analyses* %			Morbidity analyses** %		
Characteristic	The Netherlands (n=8,544,381)	Maastricht (n=67,975)	Heerlen/Kerkrade (n=87,444)	RNH (n=56,976)	Maastricht (n=8742)	Heerlen/Kerkrade (n=10,587)
Sex						
Male	48.2	47.3	47.7	48.1	46.9	48.3
Female	51.8	52.7	52.3	51.9	53.1	51.7
Age (years)						
35-44	29.6	27.2	27.8	27.5	28.8	26.5
45-54	26.7	25.4	25.1	26.4	23.7	25.9
55-64	18.5	18.9	18.9	19.6	18.7	19.5
65-74	14.0	15.8	16.0	15.4	16.4	15.9
75-84	8.6	9.8	9.8	9.0	9.6	10.0
≥85	2.6	2.8	2.4	2.1	2.8	2.1

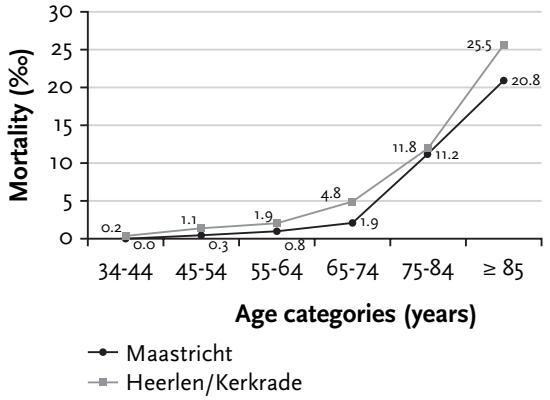
*Based on Statistics Netherlands (CBS) database 2000; **based on Registration Network of Family Practitioners (RNH) database 2001.

Table 3 *Standardised mortality rates of cardiovascular (CV) disease for two urban regions (n=155,419) and the Netherlands (n=8,544,381)*

	The Netherlands (‰)	Maastricht (‰)	Heerlen/Kerkrade (‰)	Significance of the variable 'region'
Mortality category				
Ischaemic heart disease	2.0	2.2	3.1	OR=1.4 (1.2-1.7)** †
Stroke	1.4	1.5	1.8	OR=1.2 (0.95-1.5)** †
Other CV disease	-*	2.4	2.9	OR=1.2 (1.03-1.5)** †
Other heart disease	-*	1.6	2.2	OR=1.3 (1.07-1.7)** †
Overall CV diseases	5.7	6.1	7.8	OR=1.3 (1.2-1.5)** †

*No comparable data available; **Heerlen/Kerkrade *vs* Maastricht; † 95% confidence interval.

Figure 1 *Age-specific mortality rates for ischaemic heart disease (CBS mortality data)*



Logistic regression analyses confirmed the statistical significance of the differences between the two regions. The variable 'region' had an independent association with mortality in the categories 'ischaemic heart disease' (OR=1.4 (1.2-1.7)), 'other heart disease and vascular disease' (OR=1.2 (1.03-1.5)), 'other heart disease' (OR=1.3 (1.07-1.7)). No independent association was observed for the mortality category 'stroke'.

Cardiovascular morbidity

In *table 4* standardised prevalence rates of cardiovascular morbidity are presented for both regions. Overall, the Heerlen/Kerkrade region consistently showed a higher prevalence of cardiovascular morbidity in comparison with Maastricht. The most distinct differences between the regions were found with respect to 'ischaemic heart

Table 4 *Standardised prevalence rates of cardiovascular (CV) disease and risk factors for two urban regions (n=19,329) and the RNH overall (n=56,976)*

	RNH (%)	Maastricht (%)	Heerlen/Kerkrade (%)	Significance of the variable 'region'
CV disease category				
Ischaemic heart disease	8.5	7.8	9.6	In interaction with age
Stroke	3.7	3.9	4.0	OR=1.0(0.9-1.2)** †
Other CV diseases:	19.6	20.1	22.3	OR=1.2 (1.1-1.3)** †
- Other heart disease	7.4	7.6	7.5	OR=1.0 (0.9-1.1)** †
- Other vascular disease	11.6	12.4	14.1	OR=1.2 (1.1-1.3)** †
- Peripheral arterial occlusive disease	3.2	2.8	3.9	OR=1.4(1.2-1.7)** †
Overall CV diseases	25.8	25.9	28.9	OR=1.2 (1.1-1.3)** †
CV risk factor category				
Hypertension	14.6	15.3	15.2	OR=1.0 (0.9-1.1)** †
Diabetes mellitus (I & II)	6.3	5.0	7.2	OR=1.5 (1.3-1.7)** †
Overweight*	7.5	5.8	10.8	OR=2.0 (1.8-2.2)** †
Lipid disorders	6.4	5.5	8.0	In interaction with age and sex

*BMI ≥25; **95% confidence interval; † Heerlen/Kerkrade *vs* Maastricht; RNH = Registration Network of Family Practitioners.

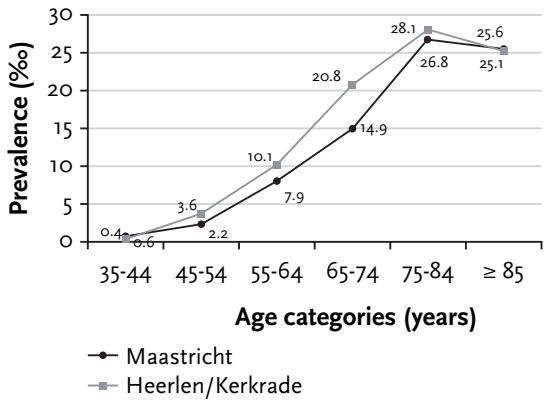
disease' and 'other heart and vascular diseases', in which the main contribution came from 'other vascular disease'. *Figure 2* shows the trend of the prevalence rates of ischaemic heart disease in both regions. The prevalence rate of Heerlen/Kerkrade for every age group was consistently above Maastricht, except for the youngest and the oldest age category. For all other cardiovascular morbidity categories, the prevalence rate of Heerlen/Kerkrade lay consistently above Maastricht. Comparatively, the relative risk was the highest for peripheral arterial occlusive disease. Logistic regression analyses confirmed the statistical significance of the differences between both regions in most cases. The variable 'region' had an independent association with morbidity in the categories 'other heart and vascular disease' (OR=1.2 (1.1-1.3)), 'other vascular disease' (OR=1.2 (1.1-1.3)) and 'peripheral arterial occlusive disease' (OR=1.4 (1.2-1.7)). It was significant in interaction with age for 'ischaemic heart disease', indicating that in all age categories the prevalence of ischaemic heart disease was higher in Heerlen/Kerkrade than in Maastricht, except for the age category 35 to 44 and ≥85. Differences in prevalence rates for stroke and 'other heart disease' were small, which was confirmed by the results of the logistic regression analysis (*table 4*).

Cardiovascular risk factors

In the second part of *table 4* standardised prevalence rates of available cardiovascular risk factors are presented for both regions. The prevalence of the cardiovascular risk factors studied was higher in Heerlen/Kerkrade compared with Maastricht. This holds especially for 'diabetes mellitus', 'overweight' and 'lipid disorders'. Regarding 'hypertension', the difference was small.

Logistic regression analyses confirmed the statistical significance of the differences between both regions in most cases. With respect to the cardiovascular risk factors studied, the variable 'region' had an independent association with 'diabetes mellitus' (OR=1.5 (1.3-1.7)) and with 'overweight' (OR=2.0 (1.8-2.2)). For 'lipid disorders', it was significant in interaction with age and sex, indicating that if age rises, more males develop lipid disorders in the region Heerlen/Kerkrade than in Maastricht. The difference in prevalence rates of hypertension was small, which was confirmed by the results of the logistic regression analysis.

Figure 2 *Age-specific prevalance rates for ischaemic heart disease (morbidity data from the RNH database)*



DISCUSSION

The present study showed that cardiovascular mortality rates were higher in the region Heerlen/Kerkrade compared with both the average in the Netherlands and in Maastricht in particular. Similarly, most cardiovascular morbidity rates for Heerlen/Kerkrade were more elevated compared with the RNH overall and more specifically with Maastricht. In accordance with these results prevalence rates of cardiovascular risk factors – in particular diabetes mellitus, overweight and lipid disorders, but not hypertension – were significantly higher in the Heerlen/Kerkrade region.

Before discussing possible explanations and the relevance and implications of these findings, it is important to discuss in short the validity of the registration systems used. In the case of mortality data (CBS), validity depends on the accuracy of the registration of the primary cause of death. Several studies have assessed the validity of the Dutch mortality registration and confirmed the completeness of this registration.[6,7,13] However, discrepancies have been found between the judgement of physicians and subsequent findings at autopsy and between physicians coding identical cases for research purposes.[6,14] Using broad categories, as was done in this study, is known to lead to fewer discrepancies than analysing single disorders.[14,15] We have no reason to assume there are regional differences with regard to registration of death certificates by doctors. Regarding data on morbidity and risk factors (RNH), validity depends on the accuracy of the registration of the diagnostic problems by the general practitioners involved. The quality is ascertained by instruction and training sessions, regional consensus groups, quality control experiments and by an automated thesaurus and automated checking for erroneous or missing entries.[8] There were no differences between the selected general practices in both regions with regard to participation in cardiovascular research projects over the last 13 years.

Our results demonstrate that Heerlen/Kerkrade is indeed a region with a high burden of cardiovascular disease, in comparison with Maastricht and the average in the

Netherlands. Given the fact that five general practices in Heerlen/Kerkrade are included in the RNH overall, the estimates of the prevalence rates for cardiovascular morbidity in the RNH overall are probably higher than the average in the Netherlands. Consequently, average cardiovascular morbidity figures for the Netherlands will probably be lower than presented here.

Data from the Framingham study show that important cardiovascular risk factors such as hypertension, diabetes mellitus, overweight and lipid disorders have a mutual amplifying effect.[16] Our results show a relatively high prevalence of diabetes mellitus, overweight and lipid disorders in Heerlen/Kerkrade and this will contribute to a higher prevalence rate of cardiovascular mortality and morbidity. However, the prevalence rates for hypertension did not differ in the two regions. A Dutch study using CBS data for the period 1950 to 1984 suggested that excess cardiovascular mortality appearing in the south of the Netherlands could be explained by Roman Catholic lifestyle and relatively lower income.[17] Unpublished reports for the Heerlen/Kerkrade region and Maastricht on these cardiovascular risk factors as well as for alcohol use, showed inconsistent differences. No data were available on risk factors such as elevated homocysteine levels and unfavourable nutrition patterns.[18-21]
Our results are consistent with the complex relationship between cardiovascular morbidity and multiple cardiovascular risk factors.[22] However, the excess risk observed in the Heerlen/Kerkrade region as compared with the Maastricht region can not be fully explained on the basis of a higher prevalence of risk factors. For instance, in subjects from Heerlen/Kerkrade with hypertension, the risk of coronary complications was substantially greater than that predicted from placebo-treated patient populations in major clinical trials.[23] In the pathogenesis of cardiovascular disease many factors, including genetic and environmental factors, play a role.[24,25] Genetic factors that modulate the individual susceptibility to cardiovascular disease are common, functionally different types of genes (polymorphisms).[26-29] These polymorphisms generally have a modest effect at an individual level, but because of their high frequency in the population can be associated with a high attributable risk.[30] Environmental factors can reveal or facilitate the phenotypic expression of such susceptibility genes. There is now accumulating evidence that most of the susceptibility genes for common diseases do not have a primary aetiological role in predisposition to disease, but rather act as response modifiers to exogenous factors such as stress, environment, disease, and drug intake.[30] A better characterisation of the interactions between environmental and genetic factors constitutes a key issue in the understanding of the pathogenesis of cardiovascular disease.[31-33] Therefore it is important to study all these factors on an individual level.

CONCLUSION

The Heerlen/Kerkrade region is indeed a region with a high burden of cardiovascular disease. However, the differences in morbidity between Heerlen/Kerkrade and Maastricht cannot be fully explained by differences in cardiovascular risk factor profile. Therefore a better characterisation of the interactions between environmental and genetic factors is important in cardiovascular research in the Heerlen/Kerkrade region.

REFERENCES

1. Henskens LH, Spiering W, Stoffers HE, Soomers FL, Vlietinck RF, de Leeuw PW, et al. Effects of ACE I/D and AT1R-A1166C polymorphisms on blood pressure in a healthy normotensive primary care population: first results of the Hippocrates study. J Hypertens 2003;21(1):81-6.
2. Metsemakers JF, Hoppener P, Knottnerus JA, Kocken RJ, Limonard CB. Computerized health information in the Netherlands: a registration network of family practices. Br J Gen Pract 1992;42(356):102-6.
3. Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. Lancet 2000;355(9205):675-87.
4. Nawawi HM, Nor IM, Noor IM, Karim NA, Arshad F, Khan R, et al. Current status of coronary risk factors among rural Malays in Malaysia. J Cardiovasc Risk 2002;9(1):17-23.
5. WHO. ICD-10: International statistical classification of diseases and related health problems. 10th ed. Geneva: World Health Organization; 1992.
6. Mackenbach JP, Van Duyne WM, Kelson MC. Certification and coding of two underlying causes of death in the Netherlands and other countries of the European Community. J Epidemiol Community Health 1987;41(2):156-60.
7. Schadé B. Reliability and validity of the classification of death in general practice. Scand J Prim Health Care 1987;5(2):109-12.
8. Metsemakers JFM. Unlocking patients' records in general practice for research, medical education and quality assurance. Maastricht: Rijksuniversiteit Limburg; 1994.
9. Lamberts H, Wood M, editors. ICPC: International Classification of Primary Care. Oxford: Oxford University Press; 1987.
10. Classification Committee of Wonca. Oxford: Oxford University Press; 1983.
11. van den Akker M, Franssen GHLM, Buntinx F, Metsemakers JFM, Knottnerus JA. The reliability of register-based patient characteristics. Archives of Public Health 1997;55:231-38.
12. Hosmer DW, Lemeshow S. Applied Logistic Regression. 2nd ed. New York: John Wiley and Sons; 2000. pp 147-156.
13. Mackenbach JP, Snels IA, Friden-Kill LM. Diabetes mellitus as cause of death. Ned Tijdschr Geneeskd 1991;135(33):1492-6.
14. Kircher T, Nelson J, Burdo H. The autopsy as a measure of accuracy of the death certificate. N Engl J Med 1985;313(20):1263-9.
15. Messite J, Stellman SD. Accuracy of death certificate completion: the need for formalized physician training. Jama 1996;275(10):794-6.
16. Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile: the Framingham Study. Am J Cardiol 1976;38(1):46-51.

17. Mackenbach JP, Kunst AE, Looman CW. Cultural and economic determinants of geographical mortality patterns in The Netherlands. J Epidemiol Community Health 1991;45(3):231-7.
18. Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE. Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 1997;337(4):230-6.
19. Bots ML, Launer LJ, Lindemans J, Hoes AW, Hofman A, Witteman JC, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. Arch Intern Med 1999;159(1):38-44.
20. van der Bom JG, Bots ML, Grobbee DE. Cardiovascular risk factors. Review of possible causes of heart and vascular diseases. Ned Tijdschr Geneeskd 2002;146(25):1169-74.
21. van der Griend R, Biesma DH, Banga JD. Hyperhomocysteinaemia as a cardiovascular risk factor: an update. Neth J Med 2000;56(3):119-30.
22. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, et al. European guidelines on cardiovascular disease prevention in clinical practice: third joint task force of European and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of eight societies and by invited experts). Eur J Cardiovasc Prev Rehabil 2003;10(4):S1-S10.
23. Neal B, MacMahon S, Chapman N. Effects of ACE inhibitors, calcium antagonists, and other blood-pressure-lowering drugs: results of prospectively designed overviews of randomised trials. Blood Pressure Lowering Treatment Trialists' Collaboration. Lancet 2000;356(9246):1955-64.
24. Sing CF, Stengard JH, Kardia SL. Genes, environment, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003;23(7):1190-6.
25. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJ, Stalenhoef AF. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59(4):184-95.
26. Wang JG, Staessen JA. Genetic polymorphisms in the renin-angiotensin system: relevance for susceptibility to cardiovascular disease. Eur J Pharmacol 2000;410(2-3):289-302.
27. Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, et al. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. J Hypertens 2002;20(10):2023-7.
28. Spiering W, Kroon AA, Vreugdenhil HA, Geraedts JP, Daemen MJ, de Leeuw PW. [The relationship between genetic polymorphisms and disease, illustrated by the renin-angiotensin-aldosterone system and cardiovascular disease]. Ned Tijdschr Geneeskd 1998;142(25):1445-50.
29. Spiering W, Kroon AA, Fuss-Lejeune MM, Daemen MJ, de Leeuw PW. Angiotensin II sensitivity is associated with the angiotensin II type 1 receptor A(1166)C polymorphism in essential hypertensives on a high sodium diet. Hypertension 2000;36(3):411-6.
30. Tiret L. Gene-environment interaction: a central concept in multifactorial diseases. Proc Nutr Soc 2002;61(4):457-63.
31. Stephens JW, Humphries SE. The molecular genetics of cardiovascular disease: clinical implications. J Intern Med 2003;253(2):120-7.
32. Herrmann SM, Paul M. Studying genotype-phenotype relationships: cardiovascular disease as an example. J Mol Med 2002;80(5):282-9.
33. D'Orleans-Juste P, Plante GE. ACE Inhibitors. Basel, Switzerland: Birkhäuser Verlag; 2001. pp. 11-27.

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Serotonin syndrome and rhabdomyolysis in venlafaxine poisoning: a case report

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ABSTRACT

Newer, more selective, antidepressant agents are increasingly being used as first-line treatment. However, clinical experience in patients after a deliberate overdose is limited. We present a case of venlafaxine intoxication complicated by a late rise in creatine kinase, seizures and serotonin syndrome. Rhabdomyolysis prolonged the hospital stay in our patient but had no other serious consequences. Physicians should be aware of this late phenomenon in patients with venlafaxine poisoning.

KEYWORDS

Rhabdomyolysis, self-poisoning, serotonin syndrome, venlafaxine

INTRODUCTION

The development of antidepressant medications has proceeded through several historical phases. The older antidepressants such as monoamine oxidase inhibitors (MOAs) and early tricyclic antidepressants (TCAs) affected a wide range of neurotransmitter systems and caused many undesirable side effects. In the late 1980s new classes of more selective antidepressant agents, starting with the selective serotonin reuptake inhibitors (SSRIs) and more recently serotonin noradrenergic reuptake inhibitors (SNaRIs), noradrenergic specific serotonergic antidepressants (NaSSA) and noradrenaline reuptake inhibitors (NaRI) were introduced and are currently widely used. Because they do not interact with histaminic, muscarinic, adrenergic or cholinergic receptors, they tend to have a more benign side-effect profile and have improved compliance. These newer drugs have proven efficacy and are often considered to be safer.[1,2] We present a case of an intoxication to the SNaRI venlafaxine (Efexor) complicated by a late rise in plasma creatine kinase (CK) and the serotonin syndrome.

CASE REPORT

A 21-year old white female with a history of depression was admitted to the emergency department five hours after intoxication with the antidepressant venlafaxine. She reported to have ingested a total of 7.8 g of the extended-release formulation. She had not taken any other medications or alcohol. On presentation she was somnolent and disorientated with a Glascow Coma Scale of 4-6-3. Her pupils were dilated and responsive to light. She was haemodynamically stable with a blood pressure of 147/55 mmHg and a sinus tachycardia of 160 to 170 beats/min. Her rectal temperature was 38.9°C. There were no signs of respiratory distress. During the initial presentation she suffered a tonic-clonic seizure, which responded well to 1 mg of clonazepam. Routine blood analysis was performed and besides a leucocytosis (19.3 x 10^9/l), which returned to normal within three days, no abnormalities were found. She was admitted to the intensive care unit and treatment was started with activated charcoal and a laxative.
Two hours after admission she suffered a second tonic-clonic seizure, which was again terminated with a single

dose of clonazepam 1 mg. Throughout the rest of the admission there were no signs of muscle tenderness. Nausea, vomiting and sweating were prominent. The following day her heart rate was 140 beats/min and her body temperature had dropped to 37.8°C. It took another day before she became fully orientated. On the second day her temperature and heart rate returned to normal (<100 beats/min). We did not detect any intraventricular conduction abnormalities.

Initial plasma CK, taken five hours after ingestion, was 43 U/l (normal range 0-50 U/l). Nineteen hours after ingestion, the plasma CK had increased to 13,653 U/l and it rose progressively over the next few days. A peak of 42,340 U/l was measured 41 hours after ingestion (*figure 1*). Isoenzymes of CK were CK-MM (muscle) 100%, CK-MB 0% and CK-BB (brain) 0%. Maximum troponin level measured on day 2 was 1.6 μg/l (<2 μg/l). Together with the raised CK of 13,653 U/l blood analysis showed a slight increase in creatine level (118 μmol/l). Treatment was started with hydration and bicarbonate infusion. Renal function was at no time affected. After 51 hours, the CK levels started to decrease (34,841 U/l) and after three days of admission in the intensive care unit she was referred to a psychiatric ward. CK level and creatine level at the time of referral were 22,740 U/l and 68 μmol/l, respectively. After seven days on the psychiatric ward the CK level had decreased further to 1276 U/l.

DISCUSSION

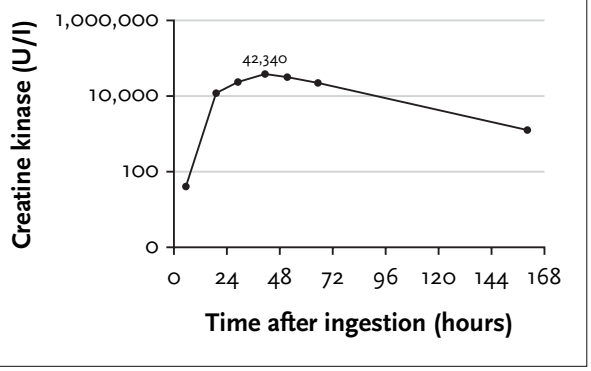
Venlafaxine is an antidepressant that causes selective inhibition of neuronal reuptake of serotonin and norepinephrine with little effect on other neurotransmitter systems. It is less prone to cause anticholinergic symptoms and sedation than tricyclic antidepressants (TCAs). Venlafaxine is well absorbed and extensively metabolised in the liver by cytochrome P450 enzyme system. Because of genetic polymorphisms, the metabolism of venlafaxine varies between patients. O-desmethylvenlafaxine (ODV) is the only major active metabolite. The half-lives of the extended-release formulations of venlafaxine and ODV are approximately five hours and 11 hours, respectively. The primary route of excretion of venlafaxine and its metabolites is renal elimination. The most common adverse effects are nausea, asthenia, dizziness, insomnia, somnolence, headache, dry mouth, sweating, hypotension, hypertension, nervousness and abnormal ejaculation.[1,3] Several cases of seizures indicating neurological toxicity, tachycardia and QRS prolongation indicating cardiac toxicity as well as serotonin syndrome have been reported following a venlafaxine overdose.[4-6] The serotonin syndrome is a potentially life-threatening disorder of excessive serotonergic activity. It presents as a triad of altered mental status, neuromuscular abnormalities, and autonomic dysfunction.[7] It occurs most frequently when two serotonergic agents are given in combination, but may also occur with a single agent.[8] The patient reported in this case report had clinical signs and symptoms of an altered mental status, autonomic disorders (tachycardia, pupillary dilatation, nausea, hyperthermia and sweating) and a neurological disorder (seizures). Guided by the Sternbach criteria,[9] the diagnosis of the serotonin syndrome was made in our patient. Therapy is supportive and symptoms usually resolve after discontinuing the offending agent.

The raised plasma CK reported in our patient is most likely to have originated from skeletal muscle. It is conceivable that the seizures might account for some of the CK rise, but the magnitude and late peak levels suggest another mechanism as well. Troponin levels were not elevated. A late rise in CK level has previously been reported with a peak CK level of 10,475 U/l four days after ingestion and also without renal impairment.[10] The mechanism of this rare complication of a venlafaxine overdose remains to be elucidated.

A venlafaxine overdose may be more serious than an overdose with SSRIs. An analysis in the United Kingdom of deaths per million prescriptions due to acute single drug poisoning, the so-called fatal toxicity index (FTI), suggested that the FTI of venlafaxine was significantly higher than that of SSRIs and similar to some tricyclic antidepressants.[11] Concerns have also been raised that venlafaxine may increase the risk of suicidal ideation and behaviour.[12] In 2004, the US Food and Drug Administration (FDA) asked manufactures to make labelling changes to include a warning about a possible increased risk of suicidality.[13]

Due to the large distribution volume of venlafaxine, forced diuresis, dialysis, haemoperfusion and exchange transfusion are of no benefit. Activated charcoal should be administered to prevent absorbtion and might be

Figure 1 *Plasma concentrations during hospital admission*



useful when administered timely. No specific antidotes are known.

The increase in the plasma CK concentrations prolonged the hospital stay for our patient, but had no other serious consequences. Our case serves to illustrate some of the consequences of venlafaxine overdose, such as seizures and serotonin symptoms. In addition, venlafaxine can cause a delayed rise in plasma CKs. Physicians should be aware of this late phenomenon.

REFERENCES

1. Rudolph RL, Derivan AT. The safety and tolerability of venlafaxine hydrochloride: analysis of the clinical trials database. J Clin Psychopharmacol 1996;16:54S-61S.
2. Kent JM. SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. Lancet 2000;355:911-18.
3. Gutierrez MA, Stimmel GL, Aiso JY. Venlafaxine: a 2003 update. Clin Ther. 2003; 25(8):2138-54.
4. Kelly CA, Dhaun N, Laing WJ, Strachan FE, Good AM, Bateman DN. Comparative toxicity of citalopram and the newer antidepressants after overdose. J Toxicol Clin Toxicol. 2004;42(1):67-71.
5. Blythe D, Hackett LP. Cardiovascular and neurological toxicity of venlafaxine. Hum Exp Toxicol. 1999;18(5):309-313.
6. White CM, Gailey RA, Levin GM, Smith T. Seizure resulting from a venlafaxine overdose. Ann Pharmacother. 1997;31(2):178-180.
7. Boyer EW, Shannon MPH. The Serotonin Syndrome. N Engl J Med. 2005;352:1112-20.
8. Pann JJ, Shen WW. Serotonin syndrome induced by venlafaxine. Ann Pharmacother. 2003;37(2):209-211.
9. Sternbach H. The serotonin syndrome. Am J Psychiatry. 1991;148:705-713.
10. Oliver JJ, Kelly C, Jarvie D, Denieul S, Bateman DN. Venlafaxine poisoning complicated by a late rise in creatine kinase: two case reports. Human & Experimental Toxicology 2002;21:463-466.
11. Buckley NA, McManus PR. Fatal toxicity of serotoninergic and other antidepressant drugs: analysis of United Kingdom mortality data. BMJ 2002;325:1332.
12. Healy D. Lines of evidence on the risk of suicide with selective serotonin reuptake inhibitors. Psychother Psychosom 2003;72:71.
13. www. fda.gov/antidepressants.

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CASE REPORT

Rat-bite fever

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ABSTRACT

A 23-year-old woman presented with fever, arthralgias and a skin rash. She possessed nine pet rats, and denied that she had been bitten. Blood culture was positive for *Streptobacillus moniliformis*, which can cause rat-bite fever. The patient fully recovered after treatment with clarithromycin.

KEYWORDS

Rat, rat-bite fever, *Streptobacillus moniliformis*

INTRODUCTION

Rat-bite fever is a disease characterised by fever, arthralgias and a skin rash. Both *Streptobacillus moniliformis* and *Spirillum minus* have been identified as causative agents. Rat-bite fever is a zoonosis, and the rat is the most important reservoir. The bacterium is transmitted by a rat bite, or by ingestion of contaminated food or water. Little is known about the prevalence of rat-bite fever. We report the case history of a patient with the disease.

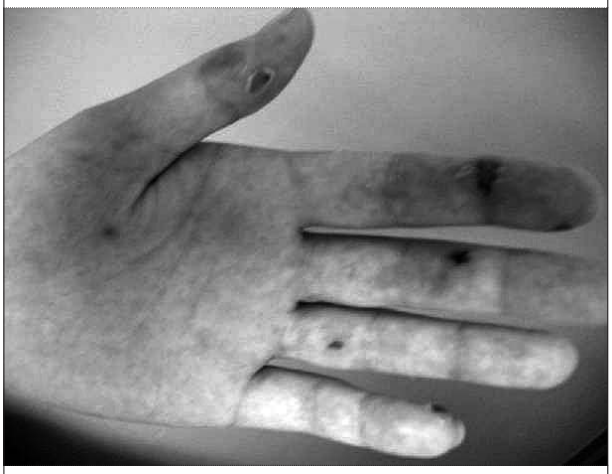
CASE REPORT

A 23-year-old woman without a previous medical history presented to the outpatient clinic with fever (up to 39.0°C), accompanied by rigors. She had been feeling unwell for several weeks and had noticed painful and swollen joints with her knees and wrists most prominently affected. The swelling had subsided spontaneously, but she was unable to use her joints properly. Prior to the onset of symptoms she had noticed a large red lesion approximately 8 cm in size on the skin of her right upper arm, which had resolved in a couple of days. Several days before her visit to the outpatient clinic she had developed painful, small, red spots on her hands and feet. Especially the palmar side of the hands was affected, with red clear marked lesions located on the hand and fingers. She had not been abroad, and no-one else in her family had been ill. She had a steady relationship with her boyfriend and did not have a history of sexually transmitted diseases. She had not noticed an insect bite. At home she kept nine pet rats, a couple of rabbits and a cat. In her spare time she worked as an assistant at a veterinary clinic, but she had no recollection of being bitten by her rats or other animals.

On examination the patient was not acutely ill. Her temperature was 38°C. Lymphadenopathy was absent. On the lateral edge of the tongue there was a small aphthous lesion. She had slight swelling of the left wrist, with diminished flexion, but without discoloration of the skin. On both hands and feet she had a maculopapular rash with numerous small, dark-red eruptions, some of which with a blister-like appearance (*figure 1*).

Extensive laboratory testing only revealed a mild acute-phase response with a C-reactive protein of 22 mg/l. Chest X-ray and abdominal ultrasound were normal. The patient was admitted to evaluate the fever, skin abnormalities and joint complaints. As differential diagnosis we thought of systemic lupus erythematosus,

Figure 1 *Maculopapular rash with small dark-red eruptions on hands of patient with rat-bite fever*



A colour version of this figure can be found on our website www.njmonline.nl.

Henoch-Schonlein purpura, a cytomegaly or Epstein-Barr virus infection, or a toxic drug reaction. Secondary syphilis seemed very unlikely. After admission she did not develop any new episodes of fever and the pain in her joints subsided. A gram-negative rod was grown from blood cultures taken on admission, which was identified as *Streptobacillus moniliformis* three days later. A polymerase chain reaction (PCR) for *Streptobacillus moniliformis* using the saliva of our patient's rats yielded a positive result. Treatment with oral clarithromycin was started and the patient recovered fully and was discharged two days later. When she visited the outpatient clinic two months later, she did not have any joint symptoms, and the skin lesions had resolved completely. During that visit she told us that she had bought four new rats from a local pet shop two months before she became ill. At that moment all the rats appeared to be in good health, along with the rats she already owned. We reasoned that the red lesion on her right upper arm, seen on admission, was most likely the result of a bite or scratch by one of the rats, although the patient did not recall this having happened.

DISCUSSION

Rat-bite fever, a zoonosis, is caused by one of two bacteria, *Streptobacillus moniliformis* or *Spirillum minus*.[1,2] The gram-negative rod *Streptobacillus moniliformis* can be found in the nasopharynx of small rodents, especially rats. Both wild and laboratory rats can be carriers. The percentage of laboratory rats that carry *Streptobacillus moniliformis* varies from 10 to 100%.[3] Rats that are carriers have no symptoms. Rat-bite fever due to *Streptobacillus moniliformis* is predominantly reported after a bite or scratch from a carrying animal. There are no large series and the literature mainly consists of case reports.[4-6]
Infections due to contaminated food or water have been reported. Two epidemics of rat-bite fever have been described. In both cases *Streptobacillus moniliformis* was identified as the cause. In the first epidemic, people were infected through water that was contaminated with the excreta of rats (Haverhill, USA, 1926). In the second epidemic contaminated milk was the cause of the disease spreading (Essex, UK, 1983). This form of rat-bite fever is called Haverhill fever.[7]
Rat-bite fever in itself is a systemic disease. Symptoms start after an incubation period of two to ten days, with fever, followed by asymmetrical arthalgias mainly involving the larger joints (50%) and a maculopapular rash. The rash occurs predominantly on the palmar side of hands and feet.[1,8] After a bite or scratch the skin region becomes inflamed and on laboratory testing there is evidence of an acute-phase response, with leucocytosis. The Venereal Disease Research Laboratory (VRDL) test for syphilis yields false-positive results in 25% of the patients. The bite usually heals spontaneously. Numerous complications have been reported such as pericarditis, endocarditis, myocarditis, meningitis, septic arthritis and focal abscesses. These complications predominantly occur in immune-compromised hosts.[9,10]
Cultures taken from skin lesions, blood and aspirated joint fluid are the most commonly used methods to identify the bacteria. *Streptobacillus moniliformis* is a slow-growing bacterium, which is difficult to grow *in vitro*. PCR is a reliable method to identify *Streptobacillus moniliformis* in rats.[11] In laboratory rats this is a common technique to screen rats for the presence of the bacteria. In our patient *Streptobacillus moniliformis* was identified from blood cultures. Cultures taken from the maculopapular lesions as seen on admission remained negative. At that time we were no longer able to identify the location where the patient had probably been bitten or scratched. Since it was unclear whether a bite by one of her rats caused the disease, we decided to test the patient's rats for the presence of *Steptobacillus moniliformis*. A PCR was performed on the rats' saliva. (Dr R. Boot, National Institute for Public Health and the Environment, Bilthoven), and all rats were found to be carriers of the bacteria. The patient was not willing to part with her rats. She stated that she would try to avoid bites in the future.
Penicillin is the number one antibiotic.[1] If the patient is allergic to penicillin, a tetracycline is second choice.
When our patient's blood culture became positive our microbiologist advised a macrolide as therapy of choice. A two-week course was advised.

CONCLUSIONS

Rat-bite fever caused by *Streptobacillus moniliformis* is a systemic disease. Given the intimate contact rat owners and laboratory workers have with their pets, it is unclear why rat-bite fever is relatively rare.[12] Moreover, considering the fact that the bacteria lives as a commensal in the nasopharyngeal flora of many rats, and that these bacteria can be transmitted not only through a bite but also through saliva, it is very unlikely that the infection occurs as infrequently as described. One explanation could be that the bacteria are difficult to grow *in vitro*, and maybe *in vivo* as well, and that, even in infected patients, it rarely leads to symptoms. As the disease can occur without obvious fever, arthralgias and skin changes, many physicians may not take blood cultures, or perform other laboratory tests which results in missed diagnosis. Most cases that have been described are associated with a clear rat bite. In every patient with fever, arthralgias with or without maculopapulous skin rash and who is in close contact with rats, rat-bite fever should be considered, even though there is no evidence of a bite or scratch.

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We would like to thank Dr R. Boot for performing the PCR on the saliva of the rats.

REFERENCES

1. Ronald G Washburn in Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases, fifth edition, volume 2, 2422-24.
2. Ojukwu IC, Christy C, Rat-bite fever in children: case report and review. Scand J Infect Dis. 2002;34(6):747-7 Review.
3. Anderson LC, Leary SL, Manning PJ. Rat-bite fever in animal research laboratory personnel. Lab Anim Sci 1983;33:292-4.
4. Torres L, Lopez AI, Escobar S, Marne C, Marco ML, Perez M, Verhaegen J. Bacteremia by Streptobacillus moniliformis; first case described in Spain. Eur J Clin Microbiol Infect Dis. 2003 May;22(5):258-60.
5. Frans J, Verhaegen J, Van Noyen R. Streptobacillus moniliformis; case report and review of the literature. Acta Clin Belg.2001 May-Jun;56(3):187-90.
6. Schuurman BM, van Griethuysen AJ, Marcelis JH, Nijs AM. Rat bite fever after a bite from a tame pet rat. Ned Tijdschr Geneeskd. 1998 Sep ;142(36):2006-9.
7. Shanson DC, Gazzard BG, Midgley J, Dixey J, Gibson GL, Stevenson J et al, Streptobacillus moniliformis isolated from blood in four cases of Haverhill fever Lancet. 1983 Jul 9;2(8341):92-4.
8. Stehle P, Dubuis O, So A, Dudler J. Rat bite fever without the fever Ann Rheum Dis 2003 Sep;62(9):894-6.
9. Hockman DE, Pence CD, Whittler RR, SMith LE. Septic arthritis of the hip secondary to rat bite fever: a case report. Clin Orthop. 2000 Nov;(380):173-6.
10. Rordorf T, Zuger C, Zbinden R, von Graevenitz A, Pirovino M. Streptobacillus moniliformis in an HIV positive patient. Infection 2000 Nov-Dec:28(6):393-4.
11. Boot R, Oosterhuis A, Thuis HC. PCR for detection of Streptobacillus moniliformis. Lab Anim. 2002 Apr;36(2):200-8.
12. Graves MH, Janda JM. Rat bite fever (Streptobacillus moniliformis) a potential emerging disease. Int J Infect Dis. 2001 ;5(3) :151-5.

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PHOTO QUIZ

Pneumothorax?

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KEYWORDS

Acute myeloblastic leukaemia, mass effect, noninvasive mechanical ventilation, pneumothorax

CASE REPORT

A 56-year-old man was diagnosed with acute myeloblastic leukaemia (WHO, M5) and had started on ARA-C for induction treatment. He had been well until he developed this illness, apart from oesophageal cancer some ten years earlier for which he had been cured.

After granulopenia had set in, he developed fever accompanied by slight dyspnoea on exertion. His condition remained stable but his fever did not subside, despite empirical broad-spectrum antimicrobial and antifungal treatment (piperacillin-tazobactam combination and itraconazole). His chest radiograph showed diffuse abnormalities (*figure 1*).

He underwent bronchoscopy with bronchoalveolar lavage in an attempt to reach a diagnosis, which he initially tolerated well. Six hours later, however, he developed acute respiratory failure and was admitted to the intensive care unit.

His respiratory rate on arrival on the ICU was >40 breaths/min, and despite supplemental oxygen using 60% O_2 rebreathing mask, pulse oxymetric saturation remained poor (SpO_2, 70 to 80%). As treatment limitation had been agreed based on his prognosis at this early stage of granulopenia, he was treated palliatively by applying a full-face mask (noninvasive) bi-level positive pressure ventilation (BiPAP), combined with intravenous morphine (2 mg/h).

He tolerated the mask ventilation well; his respiratory rate gradually came down to 20 breaths/min and his SpO_2 rose to 90%. The chest radiograph now showed a gas configuration in the left hemithorax (*figure 2*).

Figure 1 *Postero-anterior chest radiograph of patient showing the presence of a left subclavian vein access port, with interstitial densities in right middle and upper lung fields; right costo-phrenic filling, probably representing pleural effusion; and left lower lobe consolidation*

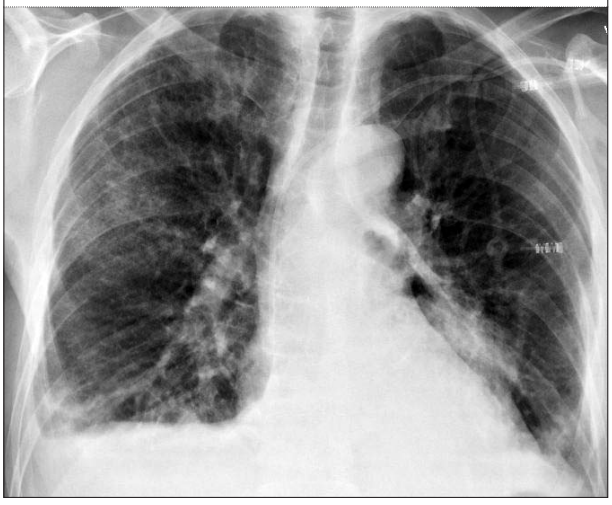
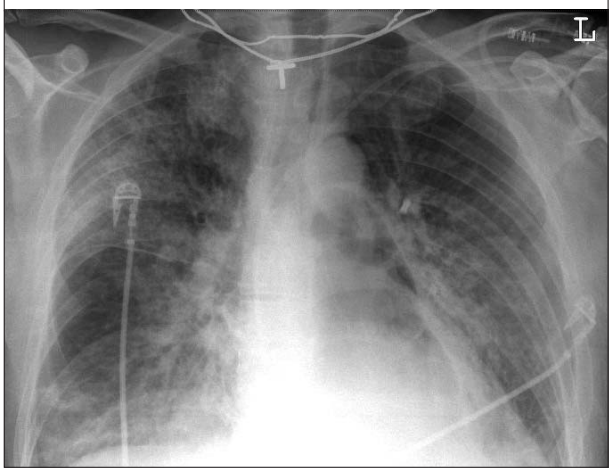


Figure 2 *Chest radiograph showing a gas configuration in the left hemithorax*



WHAT IS YOUR DIAGNOSIS?

See page 336 for the answer to this photo quiz.

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Revised SWAB guidelines for antimicrobial therapy of community-acquired pneumonia

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ABSTRACT

The Dutch Working Party on Antibiotic Policy (SWAB) develops evidence-based guidelines, aimed at optimalisation of antibiotic use and limitation of the spread of antimicrobial resistance.

A revision of the SWAB guideline for the treatment of community-acquired pneumonia (CAP), published in 1998, was considered necessary because of changes in resistance patterns and new insights into the epidemiology, diagnostics and treatment of CAP.

In contrast to the former version, this guideline is transmural and has been drawn up according to the recommendations for evidence-based guideline development by a multidisciplinary committee consisting of experts from all relevant professional societies. The 'severity of disease' exhibited by the patient with pneumonia on admission is considered important for the choice of the optimum empirical treatment strategy. Severely ill patients are treated empirically with a drug directed against multiple potential pathogens, including *Legionella* spp.

Classification according to 'severity of disease' can be accomplished with a validated scoring system (Pneumonia Severity Index or CURB-65 score) or pragmatically, based on the site of treatment: an outpatient setting, a clinical ward or an intensive care unit. The *Legionella* urine antigen test plays an important role in decisions on the choice of initial antibiotic treatment.

KEYWORDS

Antimicrobial therapy, community-acquired pneumonia, guidelines

INTRODUCTION

The Dutch Working Party on Antibiotic Policy (SWAB; Stichting Werkgroep AntibioticaBeleid), established by the Dutch Society for Infectious Diseases (VIZ), the Dutch Society for Medical Microbiology (NVMM) and the Dutch Association of Hospital Pharmacists (NVZA), coordinates activities in the Netherlands aimed at optimalisation of antibiotic use, management of the development of antimicrobial resistance, and limitation of the costs of antibiotic use. By developing evidence-based guidelines, SWAB offers local antibiotic and formulary committees a guideline for the development of their own, local antibiotic policy.

Community-acquired pneumonia (CAP) is defined as an acute symptomatic infection of the lower respiratory tract which develops outside a hospital or nursing home, whereby a new infiltrate is demonstrated on a chest X-ray. In primary care, the diagnosis is usually established on the grounds of clinical criteria, such as those described in the practice guideline 'Acute coughing' of the Dutch College of General Practitioners (NHG).[1]

The current guideline for community-acquired pneumonia is a revision of the SWAB guideline, published in 1998.[2]

Revision was considered necessary because of important new developments, including the increased resistance of pneumococci to penicillins and macrolides, the development of new quinolones, and new insights into epidemiology and diagnostics, partly as a result of the *Legionella* epidemic at the West Friesian Flora in 1999.
In contrast to the first version, this guideline focuses on the treatment of outpatients (by a general practitioner or at an outpatient hospital clinic) as well as hospitalised patients up to 72 hours after admission, and is in full accordance with the NHG practice guideline. The guideline is applicable for adult patients with a community-acquired pneumonia in the Netherlands with the exception of immunocompromised patients, such as those who have undergone organ transplantation, HIV-positive patients and patients receiving immunosuppressive therapy. The guideline focuses specifically on recommendations for antibiotic treatment. Other aspects of care for the patient with CAP are described extensively in the 2003 guideline by the professional society for respiratory care physicians (NVALT).[3]

METHODS

This guideline was drawn up according to the recommendations for evidence-based development of guidelines[4] (EBRO) and the AGREE instrument (www.agreecollaboration.org). The guideline is derived from a systematic review of literature based on six essential research questions about the treatment of CAP. Recommendations for the guideline were assigned a degree of evidential value according to the handbook of the Dutch Institute for Healthcare Improvement (CBO).[5] For each question a survey of existing guidelines was performed by the main author (JS) for purposes of orientation.[2,6-10] In addition, a literature search was performed for each research question in the PubMed database (January 1966 to January 2005), the Cochrane Register of Controlled Trials (CENTRAL), Clinical Evidence® and Sumsearch® engine. When scientific verification could not be found, the guideline text was formulated on the basis of the opinions and experiences of the members of the guideline committee. For the research question about the choice of optimum therapy (question 5), the interactive Informatrix® procedure was carried out by the members of the guideline committee as a supplementary consensus procedure.[11] Preparation of the guideline text was carried out by a multidisciplinary committee consisting of experts, delegated from the professional societies for infectious diseases, medical microbiology, hospital pharmacy, pulmonary diseases and general practice. After consultation with the members of the involved professional societies via a web-based module, the definitive guideline was drawn up by the delegates and SWAB. The full text of the guideline and literature review is available at www.swab.nl.[12]

SYSTEMATIC LITERATURE REVIEW

In order to develop recommendations for optimum treatment of CAP, answers were sought to six key questions:

- What are the causative micro-organisms of CAP in the Netherlands and what is their susceptibility to commonly used antibiotics?
- Is it possible to predict the causative agent of CAP on the basis of simple clinical data at first presentation?
- Which prognostic factors (e.g. comorbidity, age, medical history) are important for the choice of initial treatment?
- Is the severity of disease upon presentation of importance for the choice of initial treatment?
- What is the optimum initial treatment for patients with CAP?
- What is the role of rapid diagnostic tests in the initial treatment decision for patients with CAP?

WHAT IS THE AETIOLOGY OF CAP IN THE NETHERLANDS?

In ambulatory patients the most commonly demonstrated causative agent is *S. pneumoniae*, followed by *H. influenzae* and *M. pneumoniae*, while an unknown diagnosis is present in 40 to 50% of all patients.[12] Comparison of the relative frequency of causative agents is dependent upon the sensitivity and specificity of the tests used in the studies and whether there was an epidemic at the time (e.g. *M. pneumoniae*). The aetiological spectrum of agents that cause CAP among patients who were admitted to a general hospital ward is comparable throughout the world and agrees closely with the data from Dutch studies (*table 1*).[12] *S. pneumoniae* is the most commonly identified pathogen (demonstrated in 18.5 to 41.8%), *H. influenzae* (3.4 to 8%) and *M. pneumoniae* (5.4 to 12.6%) take the second place. Among patients with CAP who are admitted to the intensive care unit, the most frequently identified pathogens are *S. pneumoniae* (16 to 28%), *Legionella* spp. (4 to 24%), *S. aureus* (5 to 14%) and *Enterobacteriaceae* (0 to 10%) (*table 2*).[12] Several studies have put the importance of these specific causative agents for severe CAP into perspective.[13-15]

WHAT IS THE SUSCEPTIBILITY OF MICRO-ORGANISMS THAT MOST COMMONLY CAUSE CAP IN THE NETHERLANDS?

S. pneumoniae

Throughout the world, increasing resistance of pneumococci to penicillin has been noted. In the Netherlands this effect is as yet very limited (0.5 to 1.0%), but increases to 3.6% for patients admitted to a Pulmonology Department.[16,17]

Table 1 *Aetiology of CAP in Dutch hospitals (patients on a general ward)*

	Boersma[78] (n=90) Mean (%)	Bohte[79] (n=334) Mean (%)	van Eerden[80] (n=260) Mean (%)	Oosterheert[74] (n=302) Mean (%)	Braun[81] (n=157) Mean (%)
S. pneumoniae	38	27	37	25	34
H. influenzae	2	8	10	2	12
M. catarrhalis	1	1	2	2	1
S. aureus	1	1	5	4	3
Legionella spp.	0	2	5	3	8
Enterobacteriaceae	2	0	2 (*E. coli*)	-	2
M. pneumoniae	4	6	8	3	24
Chlamydia spp.	6	3	<1	5	4
Coxiella burnetii	0	0	0	-	1
Influenza A & B, parainfluenza	7	4	2	-	22
Other viruses	4	3	2	-	10
M. tuberculosis	1	0	0	-	1
Bordetella pertussis	-	-	-	-	18
Other	0	0	3	14	10
None	38	45	24	51	13

Table 2 *Aetiology of severe CAP (patients on ICU)*

	UK[10] (4 studies, n=185)		The Netherlands[82] (1 study, n=62)		Europe[10] (10 studies, n=1148)	
	Mean (%)	95% CI	Mean (%)	95% CI	Mean (%)	95% CI
S. pneumoniae	21.6	15.9-28.3	35	-	21.8	19.4-24.2
H. influenzae	3.8	1.5-7.6	11	-	5.3	4.1-6.8
Legionella spp.	17.8	12.6-24.1	5	-	5.5	4.2-7.2
S. aureus	8.7	5.0-13.7	7	-	7.0	5.6-8.6
M. catarrhalis	?	?	-	-	3.8	2.4-5.9
Enterobacteriacae	1.6	0.3-4.7	11	-	8.6	7.1-10.4
M. pneumoniae	2.7	0.9-6.2	0	-	2.0	1.3-3.0
C. pneumoniae	?	?	-	-	6.6	2.5-13.8
C. psittaci	2.2	0.6-5.4	-	-	0.9	0.4-1.9
C. burnetii	0	0-2.0	-	-	0.7	0.3-1.4
Viruses	9.7	5.9-14.9	-	-	4.0	2.7-5.6
Influenza A & B	5.4	2.6-9.7	-	-	2.3	1.1-4.2
Mixed infections	6.0	3.0-10.4	-	-	5.0	2.4-9.1
Others	4.9	2.3-9.0	14	-	8.4	6.8-10.1
None	32.4	25.7-39.7	34	-	43.3	40.4-46.2

Macrolide resistance in the Netherlands is widespread: surveillance studies of hospital isolates report resistance percentages of 6.5 to 10% for macrolides in 2002 *vs* 2 to 3% in 1996.[17,18] In the Netherlands, the resistance of pneumococci to tetracycline was 4.2% in 2001, which is about the same as in 1996. In 2001 there was (as yet) very little resistance to the new generation of quinolones such as levofloxacin and moxifloxacin.[18]

H. influenzae

The prevalence of *H. influenzae* resistance to amoxicillin is about 9 to 14% among patients admitted to a Pulmonology Department.[17] *H. influenzae* resistance to claritromycin has been 18 to 23% in recent years.

WHICH COMORBID CONDITIONS AND/OR RISK FACTORS ARE IMPORTANT FOR THE CHOICE OF INITIAL TREATMENT?

The pathogens that cause CAP can differ in populations with specific risk factors. There are no Dutch studies on this subject.

- The frequency of most causative agents among the elderly is not significantly different from that found

for younger patients with mild as well as severe CAP. Probably, however, *Legionella* spp. and *M. pneumoniae* will be found less frequently in the elderly.[12]

- There is an ongoing discussion about the true incidence of Gram-negative causative agents among COPD patients with CAP. There are no studies that confirm that CAP in COPD patients is caused more frequently by *H. influenzae* or *Moraxella catarrhalis* than in patients without COPD.[19] *Pseudomonas aeruginosa* remains a rare cause of CAP and can only be expected among patients with serious structural lung disease such as cystic fibrosis or bronchiectasis.[20]
- Patients with diabetes mellitus have the same spectrum of causative pathogens of CAP as the normal population, although a pneumococcal pneumonia is more often accompanied by bacteraemia.[21]
- The results of studies on causative agents in alcoholics are neither in agreement nor consistent with the advantage of one or more specific pathogens.
- Most CAP studies do not include patients with aspiration pneumonia. In this group, *Enterobacteriaceae* and anaerobes are more common.[22,23]
- When *S. aureus* is isolated as the causative agent, 39% of the hospitalised patients to 50% of those admitted to the intensive care unit have a concomitant influenza virus infection.[12]

CAN THE CAUSATIVE AGENT BE PREDICTED ON THE BASIS OF CLINICAL DATA AT PRESENTATION?

Some specific causative agents have been described to be associated with characteristic clinical symptoms, but the core question is whether it is possible to predict the causative agent at presentation on the basis of the symptoms. Bohte *et al.*[24] describe an algorithm to differentiate between *S. pneumoniae* and 'other' causative agents. One of the findings essential for a correct prediction is a Gram stain of sputum; however, on admission this is often not obtained or unreliable due to previous use of antibiotics. Previous studies by Farr[25] were also unable to confirm the prediction of the causative agent on the basis of clinical parameters. Sopena investigated whether *Legionella* spp. can be predicted reliably as causative agent on the basis of clinical signs.[26] In a multivariate analysis there was a significant difference for only one symptom (diarrhoea) in the occurrence of *Legionella* compared with the other causative agents. Finally, studies show that the causative agent for elderly patients and patients with comorbidities is even more difficult to predict than in the normal population.[27-29]

IS THE SEVERITY OF DISEASE AT PRESENTATION OF IMPORTANCE FOR THE CHOICE OF INITIAL TREATMENT?

There are theoretical arguments for the choice of empirical antibiotic therapy for patients with CAP according to the severity of illness at initial presentation. On the basis of the medical history and physical examination alone, it is impossible to reliably distinguish the causative agent. In addition, choosing an initial antibiotic regimen that is directed toward one specific agent with the intention to adjust therapy later on ('wait and see' policy), is not clinically justifiable for severely ill patients. The core question is: at which degree of 'severity of illness' is antibiotic therapy that provides coverage against both atypical and classical causative agents required, assuming that in the event of severe CAP the prescription of initial narrow-spectrum therapy and later adjustment ('wait and see' policy) is not clinically justifiable.

There are various scores that can predict the chance of death (30-day mortality) and/or ICU admission of patients with CAP (*figure 1* and *table 3*). The easiest score is the modified British Thoracic Society rule, known as the CURB-65 score (Confusion, Urea, Respiratory rate, Blood pressure, age (65 years of age),[30] recommended in the British Thoracic Society guidelines for the management of CAP 2004 update (www.brit-thoracic.org.uk/guidelines).

An alternate scoring system, the Pneumonia Severity Index (PSI) was validated in 2287 patients.[31] Via two steps, the patient is assigned to one of five risk categories. Both scores have been validated in national and supranational databases, but never in a primary care setting.[30,32,33]

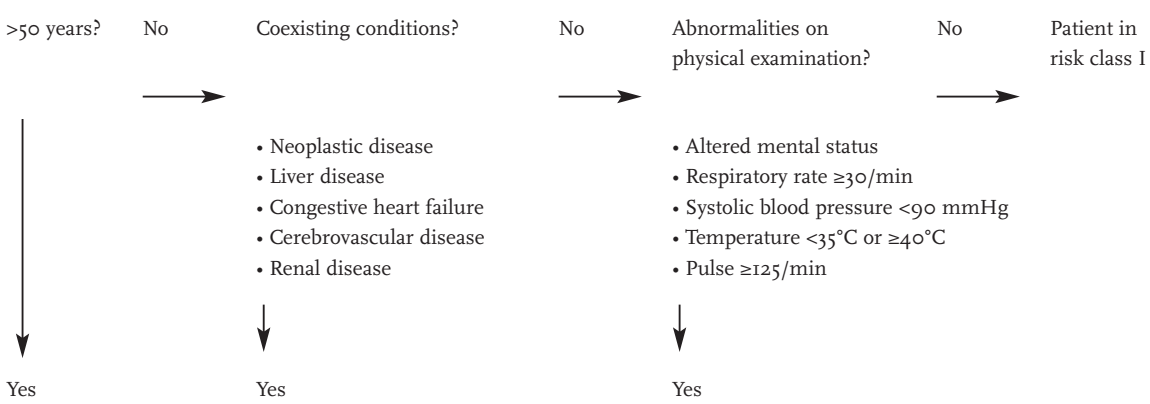
Table 3 *CURB-65 score*[30]

Core criteria*	Score CURB-65	30-day mortality
No core criteria	0	0.7%
One core criterion	1	3.2%
Two core criteria	2	13%
Three core criteria	3	17%
Four core criteria	4	41.5%
Five core criteria	5	57%

*Confusion: defined as a new disorientation in person, place or time, urea >7 mmol/l, respiratory rate ≥30/min, blood pressure: systolic blood pressure <90 mmHg or diastolic blood pressure ≤60 mmHg, age ≥65.

Figure 1 *Pneumonia severity index (PSI)*[31]

Step 1: Patient with community-acquired pneumonia



Risk class II - V, dependent of score in step 2

Step 2: Point scoring system

Characteristic	**Points assigned**
• Age	Age in years (male) Age in years -10 (female)
Underlying diseases	
• Neoplastic disease	+30
• Liver disease	+20
• Congestive heart failure	+10
• Cerebrovascular disease	+10
• Renal disease	+10
Physical examination	
• Altered mental status	+20
• Respiratory rate ≥30/min	+20
• Systolic blood pressure <90 mmHg	+20
• Temperature <35°C or ≥40°C	+15
• Pulse ≥125/min	+10
Laboratory and radiological findings	
• Arterial pH <7.35	+30
• Urea ≥11.0 mmol/l	+20
• Sodium <130 mmol/l	+20
• Glucose ≥14.0 mmol/l	+10
• Haematocrit <30%	+10
• Partial oxygen pressure <60 mmHg	+10
• Pleural effusion	+10

Mortality (30 days) per PSI risk class

Risk class	**Total score**	**Mortality**
I	Not applicable	0.1%
II	≤70	0.6%
III	71-90	0.9%
IV	91-130	9.3%
V	>130	27.0%

WHAT IS THE OPTIMUM TREATMENT OF PATIENTS WITH CAP?

Recent developments

In recent literature, there are indications that treatment with a combination of a macrolide plus a β-lactam antibiotic or monotherapy with a fourth-generation quinolone yields a survival benefit and a decreased hospital stay for patients with mild to moderately severe CAP compared with reference monotherapy such as third-generation cephalosporin.[34] The differences in favour of combination therapy or monotherapy with a fourth-generation quinolone in uncontrolled, mainly retrospective studies[34-37] can partially be explained by selection bias: prescription on the basis of the severity of the illness at first clinical presentation. In addition, the resistance pattern for pneumococci in the United States (where most of the large retrospective studies were carried out) could be the reason that combination therapy in these studies scored better than monotherapy. In the Netherlands, however, there is limited penicillin resistance. A number of retrospective studies have suggested that even in the event of proven penicillin-sensitive pneumococcal pneumonia, better results are obtained with combination therapy.[38-40] A recent prospective study has confirmed this, although it is subject to important methodological flaws: it is a nonrandomised study, including 10% nosocomial pneumonia patients and HIV patients, and only 20% of patients were >65 years.[41] Various, as yet unproven, hypotheses have been proposed to explain this effect: synergism between antibiotics, an anti-inflammatory effect of macrolides and the presence of combinations of infections.[42]

Many prospective trials have been carried out to compare the efficacy of fourth-generation quinolones or macrolides with that of β-lactam antibiotics. The results of these trials are not in agreement. File *et al.* compared levofloxacin with a second- or third-generation cephalosporin, with or without erytromycin in an unblinded trial.[43] The cure rates were 96% for the levofloxacin group and 90% for the β-lactam group. Finch *et al.* carried out a similar unblinded multicentre trial in which moxifloxacin was compared with amoxicilllin-clavulanate with or without claritromycin; the cure rates were 93.4 and 85.4%, respectively (p=0.004).[44] These results appeared to be independent of the severity of CAP and the combination with a macrolide. Comparable studies, however, did not demonstrate a treatment advantage for levofloxacin *vs* ceftriaxone (Norrby[45]), moxifloxacin *vs* amoxicillin (Petitpretz[46]), sparfloxacin *vs* amoxicillin (Aubier[47]) or the combination of ceftriaxone and azitromycin *vs* levofloxacin.[48] A recent meta-analysis in patients with mild to moderately severe pneumonia did not reveal any difference in outcome between treatment with a β-lactam and treatment with an antibiotic that is active against atypical pathogens (relative risk for therapeutic failure 0.97, CI 0.87-1.07).[49] A systematic review of trials in hospitalised patients with CAP showed no benefit of survival or clinical efficacy of empirical regimes with 'atypical' coverage, but the included trials were mostly comparisons of quinolone monotherapy and β-lactam monotherapy. No trials at all were found comparing a β-lactam with a β-lactam combined with a macrolide or quinolone.[50] Almost all of the trials were carried out in areas where pneumococci resistance to penicillin is common, and are therefore not applicable in the Netherlands. The only Dutch trial (Bohte[51]) has insufficient power to demonstrate significant differences between the treatment groups, although there was a trend toward higher effectivity of azitromycin compared with penicillin. Two randomised trials demonstrated that doxycycline as initial monotherapy for mild CAP is equivalent to a β-lactam or a quinolone.[52,53]

Severe pneumonia

No randomised double-blind placebo-controlled trials to investigate initial treatment of patients with severe CAP have been carried out. Some retrospective studies suggest a reduction in mortality for treatment of severe CAP with combination therapy consisting of a β-lactam antibiotic and a macrolide or quinolone.[34,54] In a recent prospective study, the subset of patients with severe CAP (Fine risk category IV and V) exhibited a clinical cure rate of 87.0% (20/23) for gemifloxacin *vs* 83.3% (20/24) for ceftriaxon/cefuroxim (not significant).[55] In Finch's study, about half of the patients had severe CAP (265/538). In this subgroup, the cure rate for moxifloxacin was 92.2 *vs* 84.7% for the control group (amoxicillin-clavulanate, with or without claritromycin).[44] Other studies have reported identical results for ceftriaxon and erytromycin *vs* levofloxacin (92.3 vs 94.1%) for moderately severe and severe CAP[48] and penicillin plus ofloxacin *vs* amoxicillin-clavulanate with erytromycin[56] for severe CAP.

Quinolone therapy

There are sufficient indications that *S. pneumoniae* can become resistant to quinolones during monotherapy with these drugs.[57] There is concern about the development of resistance and cross-resistance due to the large-scale use of the newer fluoroquinolones.[58] There are theoretical arguments for a preference for moxifloxacin on the basis of the high intrinsic activity against pneumococci[59] and its favourable pharmacodynamic characteristics,[60] associated with decreased selection of antimicrobial resistance[61] and good penetration into tissues.[62-64] Prolongation of the QT interval has been described for moxifloxacin.[65]

WHAT IS THE ROLE OF RAPID DIAGNOSTIC TESTS IN THE INITIAL TREATMENT DECISION FOR PATIENTS WITH CAP?

Gram stain of sputum

A rapid Gram stain of sputum can contribute to faster determination of the causative agent and possibly therefore also to early streamlining of the initial therapy.[66] There are no prospective comparative studies that have investigated the results of a rapid Gram stain as only criterion for immediate streamlining (or not) to narrow-spectrum therapy.

Legionella urinary antigen test

Detection of *L. pneumophila* antigens in urine is now generally available. With the current test, only *L. pneumophila* type 1 can be detected.[67] In the early phase of the disease, the test can be false-negative. The sensitivity is about 70 to 80% and the specificity 95 to 100%.[67,68] A negative antigen test does not exclude legionellosis. Antigen tests are not influenced by previous antimicrobial therapy.[69]

Pneumococcal urinary antigen test

The pneumococcal antigen test in urine can be performed easily and quickly. Compared with conventional methods for diagnosis of pneumococcal pneumonia, sensitivity varies from 50 to 80%.[70-73] The pneumococcal antigen test can contribute to a more rapid determination of the causative agent and possibly therefore to early streamlining of the initial therapy, but it is not yet sufficiently validated to be able to use it as a definite decision tool.

APPLICATION OF THE EVIDENCE INTO A PRACTICAL GUIDELINE

In *table 4*, the most important conclusions per research question and their grades of evidence are presented. Based on these findings the committee has designated the following as basic assumptions:

1. The 'severity of disease' in patients with pneumonia is important for the choice of an optimum initial treatment strategy. For severely ill patients, initial monotherapy – directed toward one specific causative agent with the intention to change the therapy later ('wait and see') – is clinically not justifiable. The choice was made to classify patients into three categories: mild, moderately severe and severe pneumonia.
2. Classification according to 'severity of disease' on the basis of a validated scoring system is to be preferred. For this purpose, the Pneumonia Severity Index[31] or the CURB-65 score[30] are suggested. Equally, a more pragmatic classification in three categories may be used: treatment at home, admission to a general medical ward, and admission to an intensive care unit. The user of the guideline may choose the scoring system which he/she prefers.
3. The *Legionella* urine antigen test plays an important role: this test can contribute to important policy decisions on initial treatment.

On the basis of these considerations, the committee drew up the following guideline. A flow chart for the guideline is shown in *figure 2,* and *table 5* presents an overview of the different antibiotic regimens. The full text of the guideline is available at www.swab.nl.[12]

Mild pneumonia (category I)

Mild CAP is defined as pneumonia with a PSI score of 1 or 2 or the presence of 0 or 1 CURB-65 criteria. These patients can usually be treated at home. Patients with mild CAP who are admitted to the hospital for reasons other than a strictly medical indication also belong to category 1. For this group, initial therapy with a narrow-spectrum β-lactam antibiotic or doxycycline is recommended. For patients in category I who receive amoxicillin as initial therapy but do not improve within 48 hours, therapy is switched to monotherapy with a macrolide or with doxycycline. If at the start of therapy doxycycline was administered, then failure of therapy means that macrolides cannot be given. In that case, referral to hospital must be considered.

Moderately severe pneumonia (category II)

Moderately severe CAP is defined as pneumonia with a PSI score of 3 or 4 or the presence of two CURB-65 criteria or CAP, necessitating admission to a general ward on clinical grounds. The initial therapy for this category consists of monotherapy with a β-lactam antibiotic: the first choice is intravenous penicillin or amoxicillin. For patients in category II with a PSI score of 4 or 2 CURB-65 criteria, a urinary *Legionella* antigen test must be performed within 12 hours of admission. If the test is positive, therapy must be switched to monotherapy directed against *Legionella* spp. If a patient satisfies one or more of the risk factors listed below, then therapy that also covers *Legionella* spp. must be initiated immediately: 1. recent visit to a foreign country, 2. comes from an epidemic setting of *Legionella* spp. infections, 3. treated >48 hours with a β-lactam antibiotic in adequate dosages with normal resorption and compliance without clinical improvement.

Severe pneumonia (category III)

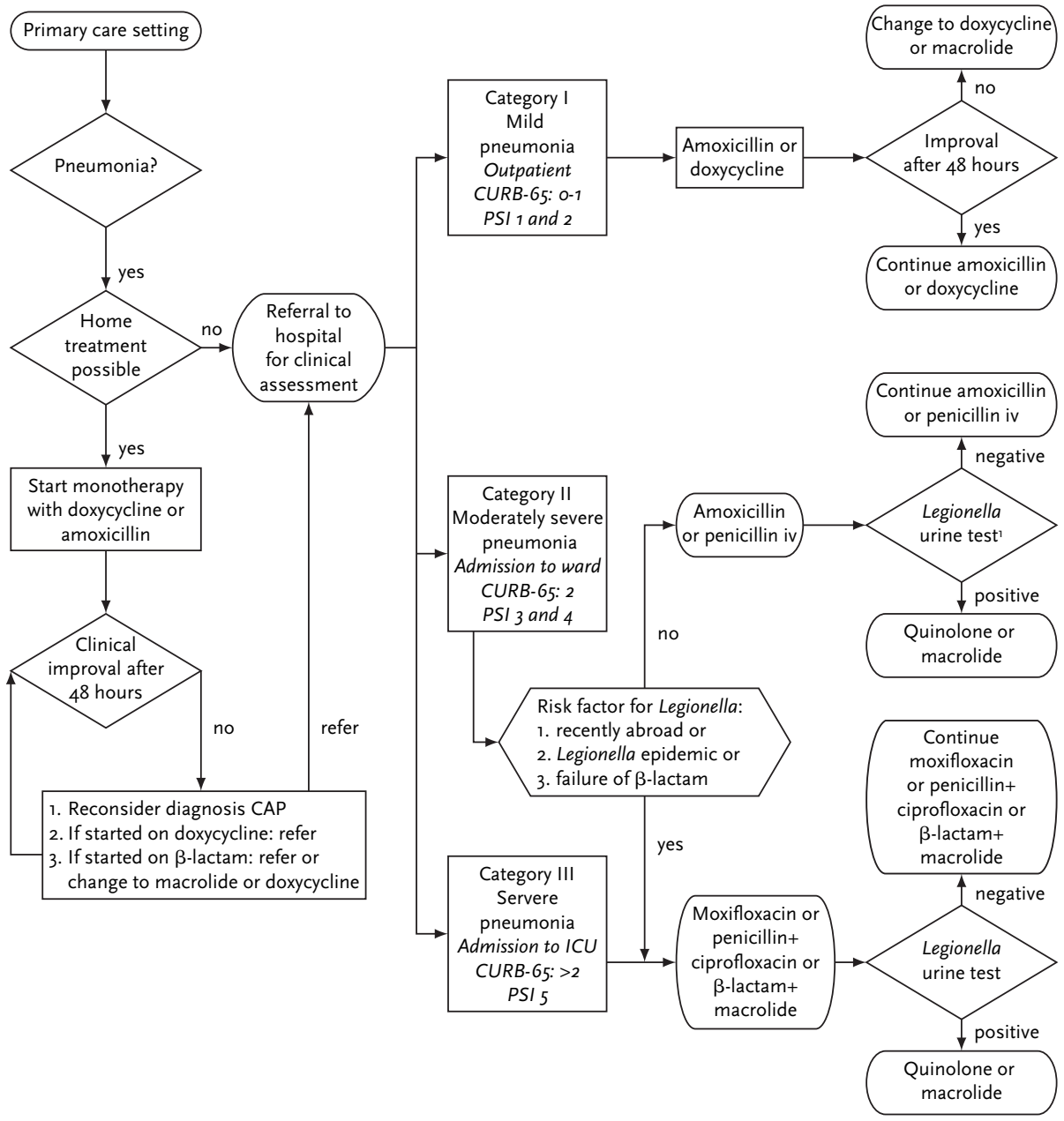
Severe CAP can be defined as CAP with a PSI score of 5, CAP with three or more CURB-65 criteria, or CAP requiring admission to an intensive care unit on clinical grounds. In this group, therapy is always directed against *S. pneumoniae* and *Legionella* spp. For this purpose there are four equally acceptable choices. The choice is dependent, on the one hand, on the risk of development of antimicrobial resistance at the population level; on the other hand, the costs, ease of administration and profile of side effects play an

Table 4 *Most important conclusions of a literature review on initial antibiotic therapy for community-acquired pneumonia*

Conclusions	Level of evidence[1]
1. What are the most frequently occurring causative agents of CAP and what is their sensitivity for the most commonly used antibiotics?	
• In view of the use of different diagnostic methods and study populations, the low percentage of demonstrated causative agents, asymptomatic carrier state, influence of epidemics and pretreatment of the patient population, the incidence of causative agents of CAP is not easily determined. In almost all of these studies *S. pneumoniae* is the most common causative agent in the Netherlands (27-38%)	2
• There are indications that in patients with severe CAP or patients who must be admitted to the intensive care unit, in addition to *S. pneumoniae, Legionella* spp. (4 to 24%) and *S. aureus* (5 to 14 %) are encountered more frequently	2
• *Mycoplasma pneumoniae* (1.3 to 34%) and *Chlamydia* spp. (1.3 to 21.5%) occur in significant percentages in the nonhospitalised population with CAP. The validity of the diagnostic methods for these causative agents is subject to discussion as well as the importance of co-infections with atypical and classical bacterial causative agents	2
• In 2005 in the Netherlands, it is not necessary to take into account a decreased sensitivity of *S. pneumoniae* for penicillin, except for patients who have recently returned from a foreign country. There is an increase in the resistance of pneumoccoci against macrolides	2
2. Which factors (such as comorbidity, age, medical history) are important for the choice of an initial therapy?	
• In the case of aspiration, anaerobes and enterobacteriacae are more often identified	2
• CAP caused by *S. aureus* is often preceded by an influenza virus infection; however the incidence of an *S. aureus* pneumonia is very low among patients treated at home	2
• *P. aeruginosa* as cause of CAP is only expected among patients with severe structural lung disease. There is no convincing evidence that *H. influenzae* and *M. catarrhalis* are more common causes of CAP among patients with COPD	2
• For patients with CAP who have recently visited a country with a high prevalence of penicillin-resistant pneumococci (PRSP), this must be taken into account when initial therapy is chosen	4
3. Is it possible to predict the causative agents of CAP on the basis of the clinical data at first presentation?	
• Information obtained from the medical history about geographical and environmental factors can be worthwhile when considering a particular causative agent of CAP, but it is not sensitive and specific enough to guide initial therapy	2
• Clinical presentation on admission is not sufficient for prediction of the causative agent of CAP. Concepts such as 'typical' and 'atypical' should no longer be used	2
4. Is the severity of the disease at presentation of importance for the choice of initial treatment?	
• For severely ill patients, initial monotherapy directed against one specific causative agent with the intention to change therapy later ('wait and see') is clinically not justifiable	2
• It is recommended to classify initial antibiotic therapy on the grounds of the severity of the disease at presentation	4
• A validated scoring system that can predict mortality is useful for the determination of the severity of CAP. The Pneumonia Severity Index (Fine score) is the best validated and most widely used system of all scoring systems.	1
• The CURB-65 is also useful for measuring severity of CAP	2
5. What is the optimum empirical treatment of patients with CAP?	
• There are indications that doxycycline as empirical therapy is equivalent to monotherapy with a β-lactam for mild pneumonia	2
• Macrolides and β-lactam antibiotics are equally effective as treatment for CAP but because of the increasing risk of resistance of pneumococci for macrolides, macrolides should not be recommended	2
• For patients with a mild to moderately severe pneumonia, treatment with a β-lactam antibiotic is equivalent to an antibiotic with activity against atypical causative agents	1
• There is no benefit in survival or clinical efficacy of empirical regimes with 'atypical' coverage compared with those without 'atypical' coverage in hospitalised patients with CAP	1
• There are no prospective trials studying monotherapy with a β-lactam antibiotic compared with therapy with a β-lactam in combination with a macrolide or in combination with a quinolone	1
• Retrospective studies suggest that empirical treatment with a combination of a macrolide plus a β-lactam antibiotic or monotherapy with a 4th generation quinolone for patients with mild to moderately severe CAP will lead to improved survival and shortened hospitalisation in comparison with monotherapy with β-lactams	2
• Early causal therapy for infections with *Legionella* spp. decreases mortality. It is therefore recommended that patients with severe CAP should be treated with empirical combination therapy which is directed against both *S. pneumoniae* and *Legionella* spp.	2
• There are theoretical arguments to have a preference for moxifloxacin when a 4th generation quinolone is chosen	3
6. What is the role of rapid diagnostics for the empirical treatment of CAP?	
• It is worthwhile to carry out a urinary antigen test for *Legionalla* spp. for all patients with severe CAP, if a *Legionella* infection is suspected in an epidemic setting or if there is no response to empirical treatment with a β-lactam antibiotic	2
• In the early phase of the disease, the urinary antigen test for *Legionella* spp. can be false-negative. Sensitivity is not optimal (70-80%), especially in mild pneumonia	2
• The rapid Gram stain on sputum can give an early indication of the cause of the CAP. The test is however not sufficiently validated to be used as a decisive diagnostic tool	3
• The pneumococcal antigen test for urine has reasonable sensitivity and good specificity for the presence of pneumococcal pneumonia. The test is however insufficiently validated to be used as a decisive diagnostic tool	2

[1]Recommendations in the guideline are given a level of evidence according to the CBO manual.[5] Level 1: conclusion or recommendation is supported by at least two independent randomised studies of good quality or by a meta-analysis. Level 2: supported by at least two randomised trials of moderate quality or insufficient size or another comparative study (not randomised, cohort studies, patient control studies). Level 3: not supported by research of the above-mentioned levels. Level 4: based on the opinion of members of the guideline committee.

Figure 2 *Flowchart of guideline recommendations on antibiotic treatment of CAP*



[1]**Always perform a *Legionella* urine antigen test in patients with a PSI score 4 or presence of 2 CURB-65 criteria.**

important role. On the basis of proven efficacy against all expected causative agents, its easy use and limited side effects, monotherapy with a fourth-generation quinolone (levofloxacin or moxifloxacin) is feasible. A second possibility is combination therapy with penicillin G and ciprofloxacin. The combinations of penicillin and a macrolide or (second- or third-generation) cephalosporin plus macrolide are equal third and fourth choices. For all patients in category III, a *Legionella* urinary antigen test is carried out as a routine procedure within 12 hours of admission. If the test is positive, monotherapy directed against *Legionella* spp. is prescribed. If the test is negative, the patient is still treated further with combination therapy because the sensitivity of the urinary antigen test is not 100%.

Comorbidity and risk factors

A review of the literature reveals no associations between specific pathogens and comorbidity and/or risk factors, with the exception of the situations described below: in the event of aspiration of gastric contents, an infection

Table 5 *Guideline for the choice of initial therapy for community-acquired pneumonia*

	Antibiotic	IV/oral	Dose	Frequency	SWAB comments
Category I					Macrolides should not be used as initial therapy. They can be used in the event of penicillin allergy and when doxycycline cannot be used due to pregnancy or lactation. If doxycycline is given, start with a loading dose of 200 mg
1st choice	Amoxicillin	oral	500-750 mg	Q6-8h	
	Doxycycline	oral	100 mg	QD	
2nd choice	Feneticillin	oral	500 mg	Q6h	
Category II					
1st choice	Penicillin	IV	1 million IU	Q6h	In the event of penicillin allergy, give a 2nd or 3rd generation cephalosporin or moxifloxacin
2nd choice	Amoxicillin	IV	1000 mg	Q6h	
Category III					
Monotherapy	Moxifloxacin	IV/oral	400 mg	QD	In the event of aspiration, the possibility of anaerobes or enterobacteriacae should be taken into account: penicillin is replaced by amoxicillin-clavulanate
Combination therapy	Penicillin + Ciprofloxacin	IV IV/oral	1 million IU 400 mg (IV)/ 500 mg (oral)	Q4h Q12h	
Combination therapy	Penicillin + erytromycin	IV IV	1 million IU 500 mg	Q4h Q6h	In the case of fulminant pneumonia after an episode of influenza, penicillin is replaced by a β-lactam antibiotic with activity against *S. aureus*
Combination therapy	Ceftriaxone or cefotaxime + erytromycin	IV IV IV	2000 mg 1000 mg 500-1000 mg	QD Q6h Q6h	Patients with demonstrated colonisation of the respiratory tract with *Pseudomonas* spp. receive penicillin + ceftazidime or penecillin + ciprofloxacin for category II and penicillin + ciprofloxacin for category III For patients with CAP who have recently visited a country with a high prevalence of penicillin-resistant *S. pneumoniae* (PRPS) the dose of penicillin is increased to 2 million IU Q4h (or continuous infusion) or 2000 mg ceftriaxone QD is given

Table 6 *Pathogen-directed therapy in CAP*

Pathogen	Oral	Intravenous
S. pneumoniae	1. Amoxicillin 2. Feneticillin 3. Macrolide or doxycycline*	1. Penicillin G 2. Amoxicillin 3. 2nd or 3rd generation cephalosporin or 4th generation quinolone*
H. influenzae		
β-lactamase negative	1. Amoxicillin 2. Macrolide or doxycycline*	1. Amoxicillin 2. 2nd or 3rd generation cephalosporin*
β-lactamase positive	1. Amoxicillin-clavulanate 2. Doxycycline or macrolide*	1. Amoxicillin-clavulanate 2. 2nd or 3rd generation cephalosporin
Legionella spp.	1. Quinolone 2. Azitromycin or claritromycin 3. Doxycycline	1. Quinolone 2. Erytromycin
M. pneumoniae, C. psittaci, or *C. pneumoniae*	1. Doxycycline 2. Macrolide	1. Doxycycline 2. Macrolide
S. aureus (non-MRSA)	1. Flucloxacillin 2. Amoxicillin-clavulanate 3. 1st generation cephalosporin	1. Flucloxacillin 2. Amoxicillin-clavulanate 3. 1st generation cephalosporin 4. Vancomycin* + aminoglycoside or rifampicin
P. aeruginosa	1. Ciprofloxacin	1. Ceftazidim 2. Ciprofloxacin
K. pneumoniae	1. Amoxicillin-clavulanate 2. Trimethoprim/sulfamethoxazole	1. Amoxicillin-clavulanate 2. 2nd or 3rd generation cephalosporin 3. Trimethoprim/sulfamethoxazole
Anaerobe bacteria**	1. Amoxicillin-clavulanate 2. Clindamycin 3. Metronidazole	1. Amoxicillin-clavulanate 2. Clindamycin 3. Metronidazole

***In the event of penicillin allergy; ****usually polymicrobial.**
Table based on literature and NVALT, BTS and IDSA guidelines.[3,10,83,84]

with anaerobes and enterobacteriacae can develop. Such patients are treated with amoxicillin-clavulanate. In the event of a fulminant pneumonia after an episode of influenza, the possibility of *S. aureus* as causative agent must be considered. Such patients are treated with a β-lactam antibiotic, active against *S. aureus*. Patients with demonstrated colonisation of the respiratory tract with *Pseudomonas* spp. are treated with an antibiotic with antipseudomonas activity (*table 5*). For patients with CAP who have recently visited countries with a high prevalence of penicillin-resistant *S. pneumoniae* (PRSP), this should be taken into account when choosing the initial therapy: the dose of initial therapy is increased to 2 million IU penicillin six times daily or either cefotaxime or ceftriaxone.

Treatment when causative agent is known

In the event of a culture-proven causative agent, a pathogen-directed antibiotic treatment is to be preferred at all times (*table 6*).

Oral therapy

An early switch from intravenous to oral antibiotic therapy for CAP as soon as clinical improvement occurs is safe and cost-effective.[74-76] Pneumonia caused by *S. aureus* or *Pseudomonas aeruginosa*, a lung empyema or lung abscess that has not been drained, and disturbed gastrointestinal resorption are contraindications for oral therapy.[3,10]

Optimum duration of therapy

There are no controlled studies on the optimum duration of treatment for pneumonia. The trend is to shorten the duration of treatment on the basis of the clinical response.[77] Based on experience, a pneumococcal pneumonia is treated up to 72 hours after normalisation of the temperature. It is recommended that pneumonia caused by *S. aureus* be treated for at least 14 days, and pneumonia caused by *L. pneumophila, M. pneumoniae* or *Chlamydia* spp. for 14 to 21 days.[8]

REFERENCES

1. Verheij TJ, Salome PL, Bindels PJ, Chavannes AW, Ponsioen BP, Sachs AP. NHG-standaard Acuut hoesten. Huisarts en Wetenschap 2003;46:496-506.
2. van Kasteren ME, Wijnands WJ, Stobberingh EE, Janknegt R, van der Meer JW. [Optimization of the antibiotics policy in the Netherlands. II. SWAB guidelines for the antimicrobial therapy of pneumonia in patients at home. The Netherlands Antibiotic Policy Foundation]. Ned Tijdschr Geneeskd 1998;142:952-6.
3. NVALT (National Society for Respiratory Physicians). Guideline for Diagnosis and Treatment of Community-acquired Pneumonia (CAP). Alphen aan den Rijn: Van Zuiden Communications, 2003.
4. Everdingen JJE, Burgers JS, Assendelft WJJ, Swinkels JA, Barneveld TA van ea. Evidence-based richtlijnontwikkeling. Een leidraad voor de praktijk. Houten: Bohn Stafleu van Loghum, 2004.
5. CBO. Richtlijnontwikkeling binnen het kwaliteitsinstituut voor de gezondheidszorg CBO. handleiding voor werkgroepleden. Utrecht: CBO, 2000.
6. Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD et al. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 2001;163:1730-54.
7. Mandell LA, Bartlett JG, Dowell SF, File TM, Jr., Musher DM, Whitney C. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin Infect Dis 2003;37:1405-33.
8. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis 2000;31:347-82.
9. ERS Task Force Report. Guidelines for management of adult community-acquired lower respiratory tract infections. European Respiratory Society. Eur Respir J 1998;11:986-91.
10. BTS Guidelines for the Management of Community Acquired Pneumonia in Adults. Thorax 2001;56 Suppl 4:IV1-64.
11. Janknegt R. Using health outcomes data to inform decision-making: formulary committee perspective. Pharmacoeconomics 2001;19 Suppl 2:49-52.
12. Schouten J.A., Prins J.M., Bonten M., Janknegt R., Kullberg BJ, Verheij T et al. Revised SWAB guidelines for antimicrobial therapy of Community-acquired pneumonia (www.swab.nl). Amsterdam: SWAB, 2005.
13. El Solh AA, Sikka P, Ramadan F, Davies J. Etiology of severe pneumonia in the very elderly. Am J Respir Crit Care Med 2001;163:645-51.
14. Oosterheert JJ, Bonten MJ, Hak E, Schneider MM, Hoepelman AI. Severe community-acquired pneumonia: what's in a name? Curr Opin Infect Dis 2003;16:153-9.
15. Park DR, Sherbin VL, Goodman MS, Pacifico AD, Rubenfeld GD, Polissar NL et al. The etiology of community-acquired pneumonia at an urban public hospital: influence of human immunodeficiency virus infection and initial severity of illness. J Infect Dis 2001;184:268-77.
16. de Neeling AJ, Overbeek BP, Horrevorts AM, Ligtvoet EE, Goettsch WG. Antibiotic use and resistance of Streptococcus pneumoniae in The Netherlands during the period 1994-1999. J Antimicrob Chemother 2001;48:441-4.
17. SWAB. NethMap 2004, Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. 2004.
18. Mouton JW,.Jansz AR. The DUEL study: a multi-center in vitro evaluation of linezolid compared with other antibiotics in the Netherlands. Clin Microbiol Infect 2001;7:486-91.
19. Torres A, Dorca J, Zalacain R, Bello S, el Ebiary M, Molinos L et al. Community-acquired pneumonia in chronic obstructive pulmonary disease: a Spanish multicenter study. Am J Respir Crit Care Med 1996;154:1456-61.
20. Arancibia F, Bauer TT, Ewig S, Mensa J, Gonzalez J, Niederman MS et al. Community-acquired pneumonia due to gram-negative bacteria and pseudomonas aeruginosa: incidence, risk, and prognosis. Arch Intern Med 2002;162:1849-58.
21. Marrie TJ. Bacteraemic pneumococcal pneumonia: a continuously evolving disease. J Infect 1992;24:247-55.
22. Leroy O, Vandenbussche C, Coffinier C, Bosquet C, Georges H, Guery B et al. Community-acquired aspiration pneumonia in intensive care units.

Epidemiological and prognosis data. Am J Respir Crit Care Med 1997;156:1922-9.
23. Mier L, Dreyfuss D, Darchy B, Lanore JJ, Djedaini K, Weber P et al. Is penicillin G an adequate initial treatment for aspiration pneumonia? A prospective evaluation using a protected specimen brush and quantitative cultures. Intensive Care Med 1993;19:279-84.
24. Bohte R, Hermans J, van den Broek PJ. Early recognition of Streptococcus pneumoniae in patients with community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 1996;15:201-5.
25. Farr BM, Kaiser DL, Harrison BD, Connolly CK. Prediction of microbial aetiology at admission to hospital for pneumonia from the presenting clinical features. British Thoracic Society Pneumonia Research Subcommittee. Thorax 1989;44:1031-5.
26. Sopena N, Sabria-Leal M, Pedro-Botet ML, Padilla E, Dominguez J, Morera J et al. Comparative study of the clinical presentation of Legionella pneumonia and other community-acquired pneumonias. Chest 1998;113:1195-200.
27. Riquelme R, Torres A, el Ebiary M, de la Bellacasa JP, Estruch R, Mensa J et al. Community-acquired pneumonia in the elderly: A multivariate analysis of risk and prognostic factors. Am J Respir Crit Care Med 1996;154:1450-5.
28. Metlay JP, Schulz R, Li YH, Singer DE, Marrie TJ, Coley CM et al. Influence of age on symptoms at presentation in patients with community-acquired pneumonia. Arch Intern Med 1997;157:1453-9.
29. Marrie TJ. Pneumonia in the elderly. Curr Opin Pulm Med 1996;2:192-7.
30. Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Town GI et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax 2003;58:377-82.
31. Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997;336:243-50.
32. Kamath A, Pasteur MC, Slade MG, Harrison BD. Recognising severe pneumonia with simple clinical and biochemical measurements. Clin Med 2003;3:54-6.
33. Aujesky D, Auble TE, Yealy DM, Stone RA, Obrosky DS, Meehan TP et al. Prospective comparison of three validated prediction rules for prognosis in community-acquired pneumonia. Am J Med 2005;118:384-92.
34. Gleason PP, Meehan TP, Fine JM, Galusha DH, Fine MJ. Associations between initial antimicrobial therapy and medical outcomes for hospitalized elderly patients with pneumonia. Arch Intern Med 1999;159:2562-72.
35. Dudas V, Hopefl A, Jacobs R, Guglielmo BJ. Antimicrobial selection for hospitalized patients with presumed community-acquired pneumonia: a survey of nonteaching US community hospitals. Ann Pharmacother 2000;34:446-52.
36. Houck PM, MacLehose RF, Niederman MS, Lowery JK. Empiric antibiotic therapy and mortality among medicare pneumonia inpatients in 10 western states : 1993, 1995, and 1997. Chest 2001;119:1420-6.
37. Stahl JE, Barza M, DesJardin J, Martin R, Eckman MH. Effect of macrolides as part of initial empiric therapy on length of stay in patients hospitalized with community-acquired pneumonia. Arch Intern Med 1999;159:2576-80.
38. Martinez JA, Horcajada JP, Almela M, Marco F, Soriano A, Garcia E et al. Addition of a macrolide to a beta-lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia. Clin Infect Dis 2003;36:389-95.
39. Mufson MA, Stanek RJ. Bacteremic pneumococcal pneumonia in one American City: a 20-year longitudinal study, 1978-1997. Am J Med 1999;107:34S-43S.
40. Waterer GW. Combination antibiotic therapy with macrolides in community-acquired pneumonia: more smoke but is there any fire? Chest 2003;123:1328-9.
41. Baddour LM, YU VL, Klugman KP, Feldman C, Ortqvist A, Rello J et al. Combination antibiotic therapy lowers mortality among severely ill patients with pneumococcal bacteremia. Am J Respir Crit Care Med 2004;170:440-4.
42. Oosterheert JJ, Bonten MJ, Hak E, Schneider MM, Hoepelman IM. How good is the evidence for the recommended empirical antimicrobial treatment of patients hospitalized because of community-acquired pneumonia? A systematic review. J Antimicrob Chemother 2003;52:555-63.
43. File TM, Jr., Segreti J, Dunbar L, Player R, Kohler R, Williams RR et al. A multicenter, randomized study comparing the efficacy and safety of intravenous and/or oral levofloxacin versus ceftriaxone and/or cefuroxime axetil in treatment of adults with community-acquired pneumonia. Antimicrob Agents Chemother 1997;41:1965-72.
44. Finch R, Schurmann D, Collins O, Kubin R, McGivern J, Bobbaers H et al. Randomized controlled trial of sequential intravenous (i.v.) and oral moxifloxacin compared with sequential i.v. and oral co-amoxiclav with or without clarithromycin in patients with community-acquired pneumonia requiring initial parenteral treatment. Antimicrob Agents Chemother 2002;46:1746-54.
45. Norrby SR, Petermann W, Willcox PA, Vetter N, Salewski E. A comparative study of levofloxacin and ceftriaxone in the treatment of hospitalized patients with pneumonia. Scand J Infect Dis 1998;30:397-404.
46. Petitpretz P, Arvis P, Marel M, Moita J, Urueta J. Oral moxifloxacin vs high-dosage amoxicillin in the treatment of mild-to-moderate, community-acquired, suspected pneumococcal pneumonia in adults. Chest 2001;119:185-95.
47. Aubier M, Verster R, Regamey C, Geslin P, Vercken JB. Once-daily sparfloxacin versus high-dosage amoxicillin in the treatment of community-acquired, suspected pneumococcal pneumonia in adults. Sparfloxacin European Study Group. Clin Infect Dis 1998;26:1312-20.
48. Frank E, Liu J, Kinasewitz G, Moran GJ, Oross MP, Olson WH et al. A multicenter, open-label, randomized comparison of levofloxacin and azithromycin plus ceftriaxone in hospitalized adults with moderate to severe community-acquired pneumonia. Clin Ther 2002;24:1292-308.
49. Mills GD, Oehley MR, Arrol B. Effectiveness of beta lactam antibiotics compared with antibiotics active against atypical pathogens in non-severe community acquired pneumonia: meta-analysis. BMJ 2005;330:456.
50. Shefet D, Robenshtock E, Paul M, Leibovici L. Empiric antibiotic coverage of atypical pathogens for community acquired pneumonia in hospitalized adults. Cochrane Database Syst Rev 2005;CD004418.
51. Bohte R, van't Wout JW, Lobatto S, Blusse van Oud AA, Boekhout M, Nauta EH et al. Efficacy and safety of azithromycin versus benzylpenicillin or erythromycin in community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 1995;14:182-7.
52. Ailani RK, Agastya G, Ailani RK, Mukunda BN, Shekar R. Doxycycline is a cost-effective therapy for hospitalized patients with community-acquired pneumonia. Arch Intern Med 1999;159:266-70.
53. Ragnar NS. Atypical pneumonia in the Nordic countries: aetiology and clinical results of a trial comparing fleroxacin and doxycycline. Nordic Atypical Pneumonia Study Group. J Antimicrob Chemother 1997;39:499-508.

54. Rello J, Catalan M, Diaz E, Bodi M, Alvarez B. Associations between empirical antimicrobial therapy at the hospital and mortality in patients with severe community-acquired pneumonia. Intensive Care Med 2002;28:1030-5.
55. Lode H, File TM, Jr., Mandell L, Ball P, Pypstra R, Thomas M. Oral gemifloxacin versus sequential therapy with intravenous ceftriaxone/oral cefuroxime with or without a macrolide in the treatment of patients hospitalized with community-acquired pneumonia: a randomized, open-label, multicenter study of clinical efficacy and tolerability. Clin Ther 2002;24:1915-36.
56. Gaillat J, Bru JP, Sedallian A. Penicillin G/ofloxacin versus erythromycin/amoxicillin-clavulanate in the treatment of severe community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 1994;13:639-44.
57. Chen DK, McGeer A, de Azavedo JC, Low DE. Decreased susceptibility of Streptococcus pneumoniae to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. N Engl J Med 1999;341:233-9.
58. Davidson R, Cavalcanti R, Brunton JL, Bast DJ, de Azavedo JC, Kibsey P et al. Resistance to levofloxacin and failure of treatment of pneumococcal pneumonia. N Engl J Med 2002;346:747-50.
59. Pestova E, Millichap JJ, Noskin GA, Peterson LR. Intracellular targets of moxifloxacin: a comparison with other fluoroquinolones. J Antimicrob Chemother 2000;45:583-90.
60. Thomas JK, Forrest A, Bhavnani SM, Hyatt JM, Cheng A, Ballow CH et al. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. Antimicrob Agents Chemother 1998;42:521-7.
61. Blondeau JM, Zhao X, Hansen G, Drlica K. Mutant prevention concentrations of fluoroquinolones for clinical isolates of Streptococcus pneumoniae. Antimicrob Agents Chemother 2001;45:433-8.
62. Soman A, Honeybourne D, Andrews J, Jevons G, Wise R. Concentrations of moxifloxacin in serum and pulmonary compartments following a single 400 mg oral dose in patients undergoing fibre-optic bronchoscopy. J Antimicrob Chemother 1999;44:835-8.
63. Florea NR, Tessier PR, Zhang C, Nightingale CH, Nicolau DP. Pharmacodynamics of moxifloxacin and levofloxacin at simulated epithelial lining fluid drug concentrations against Streptococcus pneumoniae. Antimicrob Agents Chemother 2004;48:1215-21.
64. Capitano B, Mattoes HM, Shore E, O'Brien A, Braman S, Sutherland C et al. Steady-state intrapulmonary concentrations of moxifloxacin, levofloxacin, and azithromycin in older adults. Chest 2004;125:965-73.
65. Carbon C. Comparison of side effects of levofloxacin versus other fluoroquinolones. Chemotherapy 2001;47 Suppl 3:9-14.
66. Musher DM, Montoya R, Wanahita A. Diagnostic value of microscopic examination of Gram-stained sputum and sputum cultures in patients with bacteremic pneumococcal pneumonia. Clin Infect Dis 2004;39:165-9.
67. Kazandjian D, Chiew R, Gilbert GL. Rapid diagnosis of Legionella pneumophila serogroup 1 infection with the Binax enzyme immunoassay urinary antigen test. J Clin Microbiol 1997;35:954-6.
68. Dominguez JA, Gali N, Pedroso P, Fargas A, Padilla E, Manterola JM et al. Comparison of the Binax Legionella urinary antigen enzyme immunoassay (EIA) with the Biotest Legionella Urin antigen EIA for detection of Legionella antigen in both concentrated and nonconcentrated urine samples. J Clin Microbiol 1998;36:2718-22.
69. Roig J, Rello J. Legionnaires' disease: a rational approach to therapy. J Antimicrob Chemother 2003;51:1119-29.
70. Murdoch DR, Laing RT, Mills GD, Karalus NC, Town GI, Mirrett S et al. Evaluation of a rapid immunochromatographic test for detection of Streptococcus pneumoniae antigen in urine samples from adults with community-acquired pneumonia. J Clin Microbiol 2001;39:3495-8.
71. Farina C, Arosio M, Vailati F, Moioli F, Goglio A. Urinary detection of Streptococcus pneumoniae antigen for diagnosis of pneumonia. New Microbiol 2002;25:259-63.
72. Dominguez J, Gali N, Blanco S, Pedroso P, Prat C, Matas L et al. Detection of Streptococcus pneumoniae antigen by a rapid immunochromatographic assay in urine samples. Chest 2001;119:243-9.
73. Burel E, Dufour P, Gauduchon V, Jarraud S, Etienne J. Evaluation of a rapid immunochromatographic assay for detection of Streptococcus pneumoniae antigen in urine samples. Eur J Clin Microbiol Infect Dis 2001;20:840-1.
74. Oosterheert. Diagnosis and treatment of community-acquired lower respiratory tract infections. 151-170. 2005.
75. Ramirez JA, Vargas S, Ritter GW, Brier ME, Wright A, Smith S et al. Early switch from intravenous to oral antibiotics and early hospital discharge: a prospective observational study of 200 consecutive patients with community-acquired pneumonia. Arch Intern Med 1999;159:2449-54.
76. Rhew DC, Tu GS, Ofman J, Henning JM, Richards MS, Weingarten SR. Early switch and early discharge strategies in patients with community-acquired pneumonia: a meta-analysis. Arch Intern Med 2001;161:722-7.
77. Polk R. Optimal use of modern antibiotics: emerging trends. Clin Infect Dis 1999;29:264-74.
78. Boersma WG, Lowenberg A, Holloway Y, Kuttschrutter H, Snijder JA, Koeter GH. Pneumococcal capsular antigen detection and pneumococcal serology in patients with community acquired pneumonia. Thorax 1991;46:902-6.
79. Bohte R, van Furth R, van den Broek PJ. Aetiology of community-acquired pneumonia: a prospective study among adults requiring admission to hospital. Thorax 1995;50:543-7.
80. Van der Eerden, M. M., Vlaspolder, F., de Graaff, C. S., Groot, T., Bronsveld, W., Jansen, H. M., and Boersma, W. G. A prospective comparison of pathogen-directed antibiotic therapy and empirical tharapy in patents with community-acquired pneumonia. Abstract ATS Toronto 2002. 2005.
81. Braun JJ, de Graaff CS, de Goey J, Zwinderman AH, Petit PL. Community-acquired pneumonia: pathogens and course in patients admitted to a general hospital. Ned Tijdschr Geneeskd 2004;148:836-40.
82. Vegelin AL, Bissumbhar P, Joore JC, Lammers JW, Hoepelman IM. Guidelines for severe community-acquired pneumonia in the western world. Neth J Med 1999;55:110-7.
83. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis 2000;31:347-82.
84. De Vries PA, van der Werf TS, Manson WL, Zijlstra JG. Choice of antimicrobial therapy for Legionnella infection. Ned Tijdschr Geneeskd 2005;149:452-7.

ANSWER TO PHOTO QUIZ (ON PAGE 322)

PNEUMOTHORAX?

DIAGNOSIS

The second radiograph (*figure 2*) shows a gas configuration reflecting insufflation of the reconstructed gastrointestinal continuity ten years earlier. If this had been a pneumothorax, one would expect mass effects deviating the mediastinal structures toward the right side, or a downward shift of the left hemi-diaphragm, or both. Moreover, the gas configuration had its medial margin at the vertebral column suggesting that it is located posteriorily.
The noninvasive ventilatory strategy combined perhaps with opiates facilitated some gas insufflation into the upper digestive tract with subsequent inflation of the gastric tube.

Noninvasive ventilatory support is a major goal in the management of respiratory failure in immunosuppressed haematological patients, as it has been shown to improve outcome.[1]

Our patient developed respiratory failure some six hours after the bronchoalveolar lavage (BAL). BAL may induce an immediate drop in oxygenation,[2] but also an inflammatory response some three to six hours later.[3,4]

The concern with intensive treatment in haematological malignancies should be to select those patients who will truly benefit from this intervention, as patients with ongoing, progressive respiratory failure with increasing ventilatory pressure demands may not be good candidates for noninvasive mechanical ventilation. Patients with haematological diseases who develop multiple organ failure may have a negligible chance of surviving the acute illness[5,6] and may therefore not benefit from further intensive treatment in the ICU.

Our patient did well and recovered from this acute episode, and could be transferred back to the ward. Eventually, his condition deteriorated, and as there were no plans to treat him actively, further intensive treatment was withheld, and he died after palliative treatment was started.

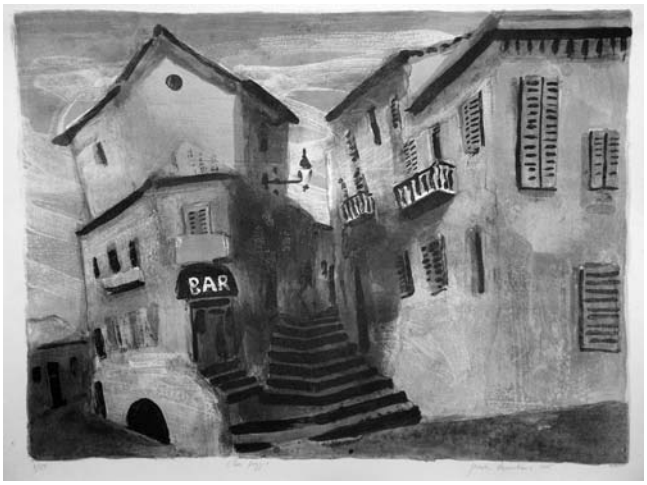
REFERENCES

1. Hilbert G, Gruson D, Vargas F, Valentino R, Gbikpi-Benissan G, Dupon M, Reiffers J, Cardinaud JP. Noninvasive ventilation in immunosuppressed patients with pulmonary infiltrates, fever, and acute respiratory failure. N Engl J Med 2001;344:481-7.
2. Montravers P, Gauzit R, Dombret MC, Blanchet F, Desmonts JM. Cardiopulmonary effects of bronchoalveolar lavage in critically ill patients. Chest 1993;104:1541-7.
3. Pugin J, Suter PM. Diagnostic bronchoalveolar lavage in patients with pneumonia produces sepsis-like systemic effects. Intensive Care Med 1992;18:6-10.
4. Bauer TT, Torres A, Ewig S, Hernandez C, Sanchez-Nieto JM, Xaubet A, Agusti C, Rodriguez-Roisin R. Effects of bronchoalveolar lavage volume on arterial oxygenation in mechanically ventilated patients with pneumonia. Intensive Care Med 2001;27:384-93.
5. Rubenfeld GD, Crawford SW. Withdrawing life support from mechanically ventilated recipients of bone marrow transplants: a case for evidence-based guidelines. Ann Intern Med 1996;125:625-33.
6. Bach PB, Schrag D, Nierman DM, Horak D, White P Jr, Young JW, Groeger JS. Identification of poor prognostic features among patients requiring mechanical ventilation after hematopoietic stem cell transplantation. Blood 2001;98:3234-40.

ABOUT THE COVER

ELBA

Jeroen Hermkens



This month's cover is a lithography entitled ELBA, made by Jeroen Hermkens.

Jeroen Hermkens (St. Anthonis, 1960) is an artist who follows his own path, without being bothered by any tendency what so ever. At the Academy in Utrecht he developed an interest in lithographic art. While working in Paris he turned out to be a real master in this field. In the 1990s he started working with oil paint. Working outdoor in all kinds of weather and close to nature he accomplished a fascinating oeuvre in this technique.

Jeroen's work, which has a surprising persuasiveness, varies from streets, buildings, squares, industrial inheritance, statues and dreamy little houses near a ferryboat, to cosmopolitan business.

An original print of this lithography (56 x 76 cm) is available at a price of € 300 and can be ordered from Galerie Unita, Rijkkstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl or www.galerie-unita.com.

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

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

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

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

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

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

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Keywords: Include three to five keywords.

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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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