Netherlands
The Journal of Medicine

MISSION STATEMENT

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

EDITORIAL INFORMATION

Editorial office
Academic Medical Centre,
Department of Medicine (E2-126)
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Tel.: +31 (0)20-566 21 71
Fax: +31 (0)20-691 96 58
E-mail: m.m.levi@amc.uva.nl
http://mc.manuscriptcentral.com/nethjmed

CITED IN

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

ISSN: 0300-2977

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

Subscription fee
The annual subscription fee within Europe is € 705, for the USA € 735 and for the rest of the world € 845. Subscriptions are accepted on a prepaid basis only and are entered on a calendar year basis.

Payment method
Please make your cheque payable to Van Zuiden Communications B.V., PO Box 2122, 2400 CC Alphen aan den Rijn, the Netherlands or you can transfer the fee to ING Bank, account number 67.89.1 0.872, Castellumstraat 1, Alphen aan den Rijn, the Netherlands, swift-code: ING BNL 2A. Do not forget to mention the complete address for delivery of the Journal.

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

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

VAN ZUIDEN communications

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

Contents

EDITORIAL

HIV testing as a normal diagnostic procedure

K. Brinkman

Department of Internal Medicine, Onze Lieve Vrouwe Gasthuis, Amsterdam, the Netherlands, e-mail: K.Brinkman@olvg.nl

Since improved antiretroviral therapy has normalised the life expectancy of HIV-infected individuals, HIV is no longer considered an important medical problem in the Netherlands. This is not only the feeling of the general public, but unfortunately also of doctors working outside the HIV field.

The cases described by Hermans *et al.* in this journal[1] are no exception: we often encounter patients with clear symptomatic clues for an HIV infection, who have been through several expensive and invasive diagnostic procedures, before an HIV test is considered. The unfortunate result is that in the Netherlands, even in 2010, 56% of patients were diagnosed as so-called late presenters: individuals either presenting for care with a CD4-cell count below 350 cells/mm^3 (normally between 800-1200 cells/mm^3) or presenting with an AIDS-defining event regardless of the CD4 count.[2] Late diagnoses are associated with poorer prognoses and increased medical costs.[3] Furthermore, when individuals are unaware of their HIV status, they cannot take preventive measures against transmitting the virus to other people.[4]

Hermans *et al.* discuss the reluctant attitude of both doctors and patients towards HIV testing in the Netherlands.[1] As a result, the percentage of persons living in the Netherlands with an HIV infection, who have not been diagnosed yet, is estimated to be around 40%, with a regional difference of 25% in the Amsterdam area and 45% in the rest of the country.[5] This percentage is one of the highest in Europe, a fact not to be proud of.

Much more effort has to be made to lower both the percentage of undiagnosed individuals as well as the number of late presenters. In the United Kingdom, a campaign was launched in 2010 to halve both numbers by 2015. This campaign was endorsed and financially supported by the central government to ensure that HIV testing became a specific priority for Public Health England and to position late diagnosis of HIV as a negative indicator in public health outcomes.[6] Also in other countries, national programs have been developed and installed to better trace and control the number of HIV-infected individuals.[7,8] Unfortunately, although our Ministry of Foreign Affairs has assigned a special ambassador for HIV/AIDS, our Ministry of Health does not yet feel the urgency to install similar programs in the Netherlands to lower the undiagnosed HIV burden.

Therefore, doctors should implement HIV testing much earlier and more routinely in their diagnostic work-up for patients with symptoms often encountered in chronic HIV infection: lymphadenopathy, thrombocytopenia, lymphoma, tuberculosis, involuntary weight loss, etc. In the HIDES-1 study, a list of indicator diseases was designed, in which HIV testing was done routinely in all patients presenting with these illnesses. The overall HIV prevalence in 3588 patients tested this way was 1.8%, almost 20 times higher than the average prevalence of HIV in the background population.[9] Classical risk factors as (former) intravenous drug use or homosexual behaviour were encountered more frequently in those patients who tested HIV positive, but these risk factors are not always asked for in routine history taking. By using this focused HIV testing routinely in patients with these frequently encountered symptoms, HIV diagnoses will be made earlier, regardless of untold or unknown risk factors.

As discussed by Hermans *et al.*[1] the informed consent and opting-in procedures in the early years formed a huge barrier for performing HIV testing in clinical practice. However, the opting-out approach is now widely accepted and should no longer be reserved for pregnancy screening or STD clinics. After informing a patient that an HIV test is included among other tests in the diagnostic work-up, our experience is that very few people will opt-out. If doctors start to act normally around performing an HIV test, the issue will be destigmatised and patients will accept it as a normal test as well. Only then will embarrassing cases as described by Hermans and others become anecdotes from the past.

REFERENCES

1. Hermans L, Wensing A, Hoepelman, A, Dutihl J, Mudrikova T, et al. Delayed HIV testing in internal medicine clinics – a missed opportunity. Neth J Med. 2012;70:69-73.
2. Stichting HIV Monitoring, Monitoring of Human Immunodeficiency Virus (HIV) infection in the Netherlands - Report 2010; www.HIV-monitoring.nl.
3. Krentz HB, Gill J. Despite CD4 cell rebound the higher initials costs of medical care for HIV-infected patients persist for 5 years after presentation with CD4 counts less than 350. AIDS. 2010;24:2750-3.
4. Pinkerton, SD, Holtgrave DR, Galletly CL. Infections prevented by increasing HIV serostatus awareness in the United States, 2001 to 2004. J Acq Immun Def Synd. 2008;47:354-7.
5. van Veen MG, Presanis AM, Conti S, et al. National estimate of HIV prevalence in the Netherlands: comparison and applicability of different estimation tools. AIDS. 2011;25:229-37.
6. Information at http://www.halveit.org.uk/.
7. Holtgrave DR. The president's fiscal year 2007 initiative for human immunodeficiency virus counseling and testing expansion in the United States: a scenario analysis of its coverage, impact, and cost-effectiveness. J Public Health Manag Pract. 2007;13:239-43.
8. Yazdanpanah Y, Sloan CE, Charlois-Ou C, et al. Routine HIV Screening in France: Clinical Impact and Cost-Effectiveness. PLoS ONE 2010;10.1371/journal.pone.0013132.
9. Sullivan AK, Reekie J, Raben D, et al. HIV Indicator Diseases across Europe Study (HIDES I): results from the pilot phase. 13th EACS conference, Belgrade 2011, abstr PS8/5.

Netherlands
The Journal of Medicine

REVIEW

Utility of desensitisation for allergy to antibiotics

H. de Groot[1*], W.M.C. Mulder[2], I. Terreehorst[3]

[1]Department of Pediatric Allergology, Diaconessenhuis Voorburg, RdGG, Delft, the Netherlands, [2]Hospital Pharmacy and [3]Department of ENT and Pediatrics, Academic Medical Center, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)70-3401243, fax: +31 (0)70-3401214, e-mail: grooth@rdgg.nl

ABSTRACT

Immediate-type allergic reactions to medication are potentially life threatening and can hamper the drug therapy of several medical conditions. If no alternative drug treatment is available, a desensitisation procedure may secure the continuation of necessary therapy by inducing a temporal state of tolerance. Desensitisation is only appropriate in case of a strong suspicion of an IgE-mediated allergic reaction. It should be performed by trained clinicians (allergy specialists) in a hospital setting where treatment of a potential anaphylactic reaction can be done without any delay. In this article, literature describing desensitisation procedures for several antibiotics is reviewed.

KEYWORDS

Antibiotics, drug allergy, diagnosis, provocation, desensitisation

INTRODUCTION AND DEFINITIONS

A drug allergy is an adverse drug reaction that results from a specific immunological response to a medication. Allergic drug reactions account for about 6 to 10% of all adverse drug reactions, but up to 10% of fatal adverse drug reactions in the adult population have an allergic origin.[1]

Adverse drug reactions can be divided into two main groups: the side effects and the hypersensitivity reactions, otherwise known as type A and type B reactions, respectively. Hypersensitivity reactions include all reactions that cannot be explained by the mechanism of the effect of the drug; as such this category contains the allergic reactions, defined as any reaction which involves the immune system but also enzyme-related reactions.

The World Allergy Organisation (WAO) has recommended dividing drug hypersensitivity reactions into immediate reactions (onset within one hour of exposure) and delayed reactions (onset after one hour), based upon the timing of the appearance of symptoms.[2] The signs and symptoms of the immediate reactions are directly attributable to the vasoactive mediators released by mast cells and basophils; the immunological route involved in this type of reaction is IgE. The most common signs and symptoms are urticaria, pruritus, flushing, angio-oedema (sometimes leading to throat tightness with stridor), wheezing, gastrointestinal symptoms, and anaphylactic shock.

The immunological mechanism involved in the delayed-type reaction is the T-cell reaction, also known as the type IV reaction. Nowadays we subdivide the type IV reactions into types IVa, IVb, IVc and IVd (*table 1*).[3]

The drugs most commonly implicated in immediate as well as delayed hypersensitivity reactions in adults are beta-lactam drugs, i.e., penicillins and cephalosporins.

Diagnostic procedures in drug allergy are usually confined to a detailed clinical history and confirmation of the immunological mechanism of the reaction, if present. The ENDA (European Network for Drug Allergy), a task force of the European Academy of Allergy and Clinical Immunology (EAACI), has set up guidelines on how to perform these tests.[4] In drug allergy, skin tests and *in vitro* laboratory tests are cumbersome; apart from penicillin determinants and amoxicillin for the IgE-mediated reactions, test reagents for skin tests are not standardised and the predictive value of the test is variable. The same is true for specific IgE laboratory tests. As for delayed type IV reactions, only skin tests (delayed reading

Table 1. *Revised type IV hypersensitivity reactions*[3]

Type of reaction	T-cell type	Immune reactant	Possible effector mechanism	Clinical symptoms (example)
IVa	Th1	IFN-γ, TNF-α	Monocyte / macrophage activation	Contact dermatitis, bullous exanthema
IVb	Th2	IL-5, IL-4, IL-13, eotaxin	T cells driving eosinophilic inflammation	Maculopapular and bullous exanthema
IVc	Cytotoxic T cells	Perforin, granzyme B	CD4+/CD8+ mediated T cell killing	Contact dermatitis; maculopapular, pustular and bullous exanthema
IVd	T cells	CXCL-8, GM-CSF	T cell leading to recruitment and activation of neutrophils	Pustular exanthema

of intracutaneous tests and patch tests) are available, although promising results are reported for the lymphocyte transformation test to evaluate T-cell mediated reactions. The lymphocyte transformation test (LTT) measures the proliferation of T cells to a drug *in vitro*, from which one concludes a previous *in vivo* reaction due to a sensitisation. This concept of the LTT has been confirmed by the generation of drug-specific T-cell clones and the finding that drugs can directly interact with the T-cell receptor, without previous metabolism or need to bind to proteins. Very few labs, however, are able to perform this LTT and this test is only investigated in a small number of drugs.

For this reason, the drug provocation test, the controlled administration of the suspected drug, is still considered to be the gold standard in order to confirm the diagnosis of drug allergy.[5]

Desensitisation aims at altering the immune response to the drug and results in temporary tolerance, allowing the patient to receive a subsequent course of the medication safely. Although this could be attractive in many patients, this procedure is only undertaken in certain predefined groups and only in type I and certain type IV reactions. Desensitisation should not be attempted in patients with a history of Stevens- Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) because even small doses of the drug may again induce severe progressive reactions. Desensitisation is also not appropriate for patients with type II or type III (IgG-mediated) hypersensitivity drug reactions such as haemolytic anaemia or nephritis. After the culprit drug is stopped, tolerance subsides in hours to days and subsequent administration should again be preceded by a desensitisation procedure.

In this review, we summarise the known literature concerning desensitisation procedures in adult patients with antibiotic hypersensitivity.

GENERAL PRINCIPLES OF DESENSITISATION

In a recent article by the Task Force Drug Desensitisation of the European Academy of Asthma, Allergy and Clinical Immunology, Cernadas *et al.* give an excellent overview of the outlines of the desensitisation procedure.[6] Besides the obvious safety measures such as an intravenous line, trained nurses and doctors and the medication to treat anaphylaxis at hand, the development of a desensitisation scheme is largely dependent of available well-tested protocols in literature and the initial reaction of the patient. Rule of thumb is that the more severe the original reaction was, the lower the starting dose. Therefore, the starting dose can vary between 1:100,000 and 1:100 of the therapeutic dose. Most schedules apply a doubling dose schedule; time between two steps can vary considerably but in the classic penicillin scheme (intravenous) the dose is doubled every 15 minutes until the full therapeutic dose is reached. Both intravenous and oral routes have been described.[7]

Whether premedication with corticosteroids and antihistamines reduces the risk of a desensitisation procedure is not known but one must be aware that by giving the patient antihistamines, the early signs of anaphylaxis during the desensitisation procedure may be masked.

INDICATIONS

Desensitisation to drugs can be considered in patients for whom there are no acceptable alternative drugs. For instance pregnant women with (latent) syphilis who are allergic to penicillin, as this antibiotic is the only treatment for syphilis that sufficiently crosses the placenta. It can also be of use when the alternatives are less effective than the culprit drug, such as cotrimoxazole in HIV patients for *Pneumocystis* prevention. A third reason could be to attempt to improve the underlying disease, i.e. aspirin desensitisation in patients with nasal polyps and severe asthma. Obviously, this last reason is not valid in the case of hypersensitivity to antibiotics.

CONTRAINDICATIONS

As stated above, desensitisation is not appropriate in serious cytotoxic reactions, vasculitis or bullous diseases, such as SJS or TEN. Other contraindications for this procedure are serious comorbidity such as pulmonary disease with an FEV1 less than 70%, uncontrolled cardiac comorbidity or haemodynamically challenged patients. In other situations, the risk must be outweighed by the

benefit such as in patients with renal disease, pregnancy or other diseases in which an anaphylactic reaction could cause severe complications. This is also true for patients who are treated with beta-adrenoreceptor antagonists or other drugs that may complicate the treatment of anaphylaxis. Preferably, these drugs are stopped before a desensitisation procedure is performed.

DESENSITISATION IN ANTIBIOTICS

Desensitisation procedures are reported to be successful in case of an IgE-mediated hypersensitivity reaction such as urticaria, angio-oedema, itch, or anaphylaxis. If reliable skin test procedures are available, such as for beta-lactam antibiotics, these should be performed first. Negative results to intradermal tests with penicilloyl poly-L-lysine and minor determinant mixture reduce the risk of hypersensitivity symptoms upon re-exposure to less than 5%. In these patients, incremental dosing may be chosen; however, studies comparing this strategy with desensitisation with regard to safety and efficacy have not been published. These strategies have been compared in HIV patients with mild to moderate hypersensitivity reactions to trimethoprim-sulfamethoxazole.[8] Success rates were 72% (18/25) for rechallenge and 79.5% (27/34) for desensitisation (not significant).

The starting dose for intravenous procedures is generally 1:1,000,000 to 1:1000 of the full therapeutic dose, but may be higher (1:100) in oral desensitisation.[6] During intravenous desensitisation the doses are infused continuously over intervals of 15 to 30 minutes, followed by intravenous administration of the full therapeutic doses. In the oral procedure, described dose intervals range from 15 minutes to 12 hours. Slow or incomplete absorption from the gastrointestinal tract should be taken into account when choosing this dose interval. An example of an oral and intravenous desensitisation protocol is presented in *tables 2A* and *2B*, respectively.[9,10]

Table 2A. *Oral penicillin desensitisation protocol*[9]

Step	Penicillin (mg/ml)	Amount (ml)	Dose (mg)	Cumulative dose (mg)
1	0.5	0.1	0.05	0.05
2	0.5	0.2	0.1	0.15
3	0.5	0.4	0.2	0.35
4	0.5	0.8	0.4	0.75
5	0.5	1.6	0.8	1.55
6	0.5	3.2	1.6	3.15
7	0.5	6.4	3.2	6.35
8	5.0	1.2	6.0	12.35
9	5.0	2.4	12.0	24.35
10	5.0	5.0	25.0	49.35
11	50.0	1.0	50.0	100.0
12	50.0	2.0	100.0	200.0
13	50.0	4.0	200.0	400.0
14	50.0	8.0	400.0	800.0

The interval between doses is 15 minutes. After the final step observe patient for 30 minutes, then give full therapeutic dose by the desired route.

Table 2B. *Intravenous penicillin desensitisation protocol using a continuous infusion pump*[10]

Step	Penicillin (mg/ml)	Flow rate (ml/h)	Dose (mg)	Cumulative dose (mg)
1	0.01	6	0.015	0.015
2	0.01	12	0.03	0.045
3	0.01	24	0.06	0.105
4	0.01	50	0.125	0.23
5	0.1	10	0.25	0.48
6	0.1	20	0.5	1.0
7	0.1	40	1.0	2.0
8	0.1	80	2.0	4.0
9	0.1	160	4.0	8.0
10	10.0	3	7.5	15.0
11	10.0	6	15.0	30.0
12	10.0	12	30.0	60.0
13	10.0	25	62.5	123.0
14	10.0	50	125.0	250.0
15	10.0	100	250.0	500.0
16	10.0	200	500.0	1000.0

The interval between doses is 15 minutes. After the final step observe patient for 30 minutes, then give full therapeutic dose by the desired route.

Premedication

Premedication can be done with (methyl)prednisolone, antihistamine, and ranitidine with or without montelukast 13, 7, and 1 hours, respectively, before start of the desensitisation procedure. However, early symptoms of anaphylaxis may be masked, while prevention of severe reactions has not been proven.

Symptoms

In almost 50% of the procedures reviewed in the paediatric literature, symptoms occurred during the procedure (reviewed by De Groot and Mulder[11]). For adults mild symptoms are reported in 30 to 80% of penicillin desensitisation procedures.[6] In general, the symptoms can be treated by antihistamines in combination with dose reduction or postponing the dose increase by repeating the symptomatic dose.

Effectivity

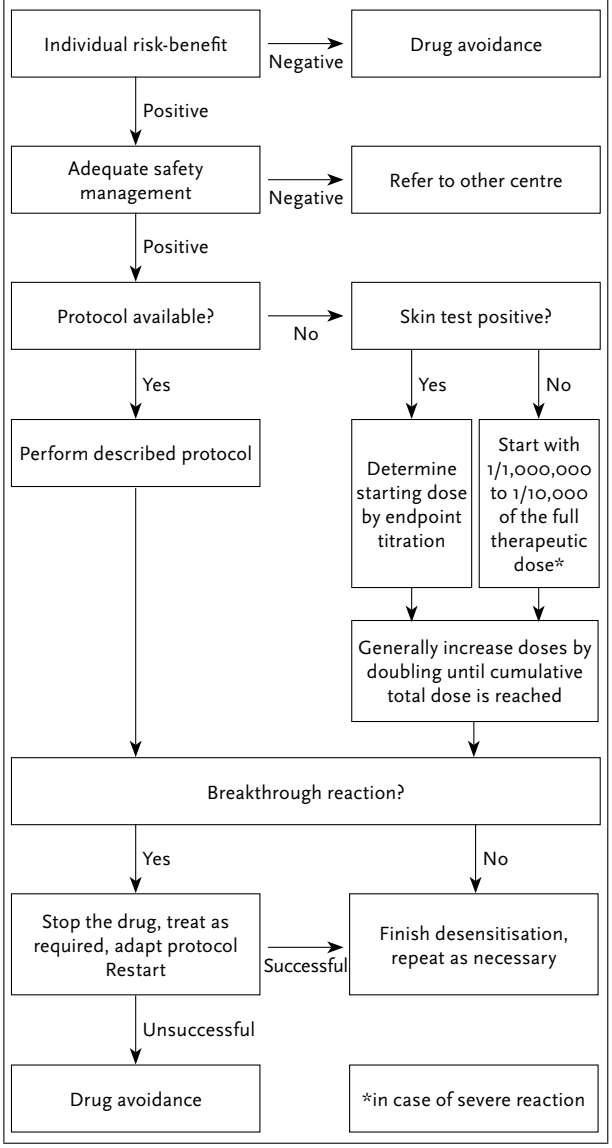
Success rates in case series of cystic fibrosis patients with a type I allergy to beta-lactam antibiotics range from 50 to 100%.[12-14] A case series of adult cystic fibrosis patients with non-immediate reactions to different classes of antibiotics

report a success rate of 55% (tazocin, 32 desensitisation procedures in 11 patients) to 88% (tobramycin, 39 procedures in 8 patients).[15]

Vancomycin can induce either IgE-mediated anaphylaxis or anaphylactoid reactions caused by direct histamine release (red man syndrome). Distinction of these two is difficult, even more so because valid skin tests for IgE-mediated vancomycin hypersensitivity are not available. A review of case reports of patients with vancomycin-induced red man syndrome that could not be managed by pretreatment with antihistamines or slowing down infusion rates showed a success rate of 100% when combining both rapid and slow desensitisation procedures.[16]

With regard to fluorquinolone hypersensitivity, some successful desensitisation procedures to ciprofloxacin in cystic fibrosis patients have been described in patients with urticaria or maculopapular exanthema.[13,17,18].

Figure 1. *General algorithm for drug desensitisation*[6]



Several case series of desensitisation to trimethoprim-sulfamethoxazole in HIV-positive immune compromised patients report success rates varying from 50 to 80%.[8,19-23] Success rates seem to be lower in patients who experienced an urticarial rash compared with those with other rashes. Individual reports of desensitisation to clarithromycin,[24,25] clindamycin,[26] rifampicin,[27] ticarcillin[28] and tobramycin[13,29] have been reported. Most desensitisations reported were successful, but a selection bias towards more successful cases is probable.

Setting

Drug desensitisation should only be performed by clinicians trained in the technique (usually allergy specialists), in a hospital setting (or outpatient setting under close observation), with intravenous access and necessary medications and equipment to treat anaphylaxis. Pharmacy staff may be consulted prior to the procedure to assist with preparation of the required drug dilutions.

Conclusion and practical proposal

An algorithm taking into account all important decisions concerning the antibiotic-allergic patient for whom desensitisation is considered is described in the EAACI position paper on rapid drug desensitisation (*figure 1*).[6] The balance of risks and benefit for each particular individual and the possibility to guarantee patient safety in a particular setting will direct the management of the individual patients. On the other hand, withholding optimal antibiotic therapy because of unfamiliarity with desensitisation protocols and procedures is not in the best interest of patients. Referral to a centre where desensitisation is performed should be aimed at in these particular cases.

REFERENCES

1. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA. 1998;279:1200-5.
2. Johansson SGO, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. J Allergy Clin Immunol. 2004;113:832-6.
3. Posadas SJ, Pichler WJ. Delayed drug hypersensitivity reactions – new concepts. Clin Exp Allergy. 2007;37:989-99.
4. Brockow K, Romano A, Blanca M, et al. General considerations for skin test procedures in the diagnosis of drug hypersensitivity. Allergy. 2002;57:45-51.
5. Aberer W, Bircher A, Romano A, et al. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy. 2003;58:854-63.
6. Cernadas JR, Brockow K, Romano A, Aberer W, Torres MJ, Bircher A, Campi P, Sanz ML, Castells M, Demoly P, Pichler WJ for the European Network of Drug Allergy and the EAACI interest group on drug hypersensitivity. General considerations on rapid desensitization for drug hypersensitivity – a consensus statement. Allergy. 2010;65:1357-66.

7. Stark BJ, Earl HS, Gross GN, et al. Acute and chronic desensitization of penicillin-allergic patients using oral penicillin. J Allerg Clin Immunol. 1987;79:523–32.

8. Bonfanti P, Pusterla L, Parazzini F, Libanore M, Cagni AE, Franzetti M, Faggion I, Landonio S. Quirino T. The effectiveness of desensitization versus rechallenge treatment in HIV-positive patients with previous hypersensitivity to TMP-SMX : a randomized multicentric study. Biomedicine & Pharmacotherapy. 2000;54:45-9.

9. Sullivan TJ. Allergy, Principles and Practice. In: Middleton E Jr, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, editors. Drug allergy. St. Louis: Mosby Co 1993:1726-46.

10. Solensky R. Drug desensitization. Immunol Allergy Clin North Am. 2004;24:425-43.

11. De Groot H, Mulder MWM. Drug desensitisation in children. Eur J Pediatr. 2010;169:1305-9.

12. Burrows JA, Toon M, Bell SC. Antibiotic desensitization in adults with cystic fibrosis. Respirology. 2003;8:359-64

13. Legere HJ III, Palis RI, Rodriguez Bouaza T, Uluer AZ, Castells MC. A safe protocol for rapid desensitization in patients with cystic fibrosis and antibiotic hypersensitivity. J Cyst Fibrosis. 2009;8:418-24.

14. Turvey SE, Cronin B, Arnold AD, Dioun AF. Antibiotic desensitization for the allergic patient: 5 years of experience and practice. Ann Allergy Asthma Immunol. 2004; 92:426-32.

15. Whitaker P, Shaw N, Gooi J, Etherington C, Conway S, Peckham D. Rapid desensitization for non-immediate reactions in patients with cystic fibrosis. J Cyst Fibrosis. 2011;10:282-5.

16. Wazny LD, Daghigh B. Desensitization protocols for vancomycin hypersensitivity. Ann Pharmacother. 2001;35:1458-64.

17. Bircher AJ, Ritishauser M. Oral desensitization of maculopapular exanthema from ciprofloxacin. Allergy. 1997;52:1246-8.

18. Lantner RR. Ciprofloxacin desensitization in a patient with cystic fibrosis. J Allergy Clin Immunol. 1995;96:1001-2.

19. Kalanadhabhatta V, Muppidi D, Sahni H, Robles A, Kramer M. Successful oral desensitization to trimethoprim-sulfamethoxazole in acquired immune deficiency syndrome. Ann Allergy Asthma Immunol. 1996;77:394-400.

20. Kletzel M, Beck S, Elser J, et al. Trimethoprim-sulfamethoxazole oral desensitization in hemophiliacs infected with human immunodeficiency virus with a history of hypersensitivity reactions. AJDC. 1991;145:1428-9.

21. Palusci VJ, Kaul A, Lawrence RM, Haines KA, Kwittken PL. Rapid oral desensitization to trimethoprim-sulfamethoxazole in infants and children. Pediatr Infect Dis J. 1996;15:456-60.

22. Theodore CM, Holmes D, Rodgers M, McLean KA. Co-trimoxazole desensitization in HIV-seropositive patients. Int J STD AIDS. 1998;9:158-61.

23. Yoshizawa S, Yasuoka A, Kikuchi Y, Honda M, Gatanaga H. Tchikawa N et al. A 5-day course of oral desensitization to trimethoprim/sulfamethoxazole (T/S) in patients with human immunodeficiency virus type-1 infections who were previously intolerant to T/S. Ann Allergy Asthma Immunol. 2000;95:241-4.

24. Holmes NE, Hodgkinson M, Dendle C, Korman TM. Report of oral clarithromycin desensitization. BJCP. 2008;66:323-4.

25. Swamy N, Laurie SA, Ruiz-Huidobro E, Kahm DA. Successful clarithromycin desensitization in a multiple macrolide-allergic patient. Ann Astma Allergy. 2010;105:489-90.

26. Marcos C, Sopeña B, Luna I, González R, de la Fuente J, Mártinez-Vásquez C. Clindamycin desensitization in an AIDS patient. AIDS. 1995;9:1201-2.

27. Buergin S, ,Scherer K, Häusermann P, Bircher AJ. Immediate Hypersensitivity to Rifampicin in 3 Patients: Diagnostic Procedures and Induction of Clinical Tolerance Int Arch Allergy Immunol. 2006;140:20-6.

28. Brown LA, Goldberg ND, Shearer WT. Long-term ticarcillin desensitization by the continuous oral administration of penicillin. J Allergy Clin Immunol. 1982;69:51-4.

29. Spigarelli MG, Hurwitz ME, Nasr SZ. Hypersensitivity to inhaled Tobi® following reaction to gentamycin. Pediatr Pulmonol. 2002;33:311-4.

Netherlands
The Journal of Medicine

REVIEW

Systemic vasculitis in myelodysplastic syndromes

R. Oostvogels[1*], E.J. Petersen[1], M.L. Chauffaille[2], A.C. Abrahams[3]

[1] Department of Haematology, University Medical Center Utrecht, the Netherlands, [2] Department of Haematology, Universidade Federal de São Paulo, Brazil, [3] Department of Nephrology and Hypertension, University Medical Center Utrecht, the Netherlands, [*]corresponding author: tel.: +31 (0)88-7557587, fax: +31 (0)88-7555418, e-mail: R.Oostvogels@umcutrecht.nl

ABSTRACT

The development of immunological abnormalities in various neoplasms is a rather common phenomenon. The prevalence of life-threatening systemic vasculitis in malignancy, however, is much lower. Nonetheless we found an unexpected frequency of several autoimmune manifestations, including systemic vasculitis, in certain myelodysplastic syndromes.

We illustrate this finding with the case of a 43-year-old man with signs of polyarteritis nodosa-like systemic vasculitis during progression of chronic myelomonocytic leukaemia. Subsequently, we review the literature on the combination of myelodysplastic syndromes and systemic vasculitis and discuss the prognostic consequences, considerations for treatment and possible pathophysiological mechanisms.

KEYWORDS

Chronic myelomonocytic leukaemia, myelodysplastic syndromes, polyarteritis nodosa, vasculitis

INTRODUCTION

The myelodysplastic syndromes (MDS) comprise a heterogeneous group of haematological diseases, characterised by cytopenia and the presence of dysplastic blood cells. According to the World Health Organisation (WHO) classification of the myeloid neoplasms, chronic myelomonocytic leukaemia (CMML) is classified as an overlap syndrome of myelodysplastic syndromes and myeloproliferative neoplasms (MDS/MPN), since it can present with both myelodysplastic symptoms such as cytopenia and proliferative features such as remarkable leucocytosis and splenomegaly.[1,2] CMML is a relatively rare disease with an annual incidence rate of 0.3 to 0.5 per 100,000 persons in all ages, and around 3 per 100,000 in persons of 60 years and older. Median survival is poor with 18 to 40 months, which justifies the use of aggressive therapy such as stem cell transplantation in selected patients.[3-5]

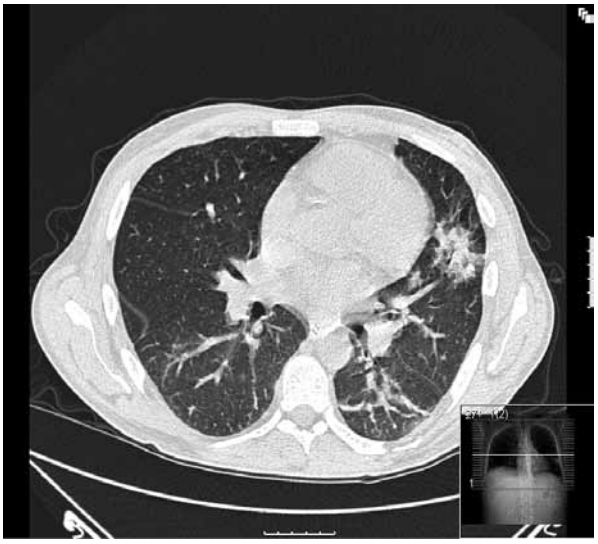
The combination of MDS with autoimmune manifestations has been described before in a number of case reports. In 1997 Pirayesh *et al.* published a review of the literature on the combination of MDS and vasculitis, of which the majority had leucocytoclastic cutaneous vasculitis.[6] Other reports also state that the autoimmune manifestations seen in MDS largely concern cases of mild rheumatological symptoms or cutaneous vasculitis.[7,8] In this article, we present a case of a 43-year-old man who developed severe symptoms consistent with systemic vasculitis in the same period he was diagnosed with progressive CMML. In addition we review the literature on the combination of systemic vasculitis with MDS or MDS/MPN, describing previously reported cases with their treatment and outcomes, and discuss possible pathophysiological mechanisms.

CASE REPORT

A 43-year-old male with progressive CMML presented with pain in the left upper abdomen and fever. Abdominal CT scanning showed splenomegaly with areas of both infarction and haemorrhage, which caused rupture of the splenic capsule. After emergency splenectomy he developed respiratory failure due to pleural and pericardial fluids, containing 42% monocytic cells, and high-resolution CT scanning showed multiple intrapulmonary abnormalities (*figure 1*). Furthermore,

he developed hypovolaemic shock, which turned out to be caused by massive bleeding from multiple microaneurysms in both kidneys (*figure 2*).
A medium-sized vessel vasculitis was suspected because of these characteristic abnormalities at imaging. Also, no alternative explanation for this combination of symptoms was found. He qualified for a diagnosis of polyarteritis nodosa (PAN) as he fulfilled five out of ten American College of Rheumatology (ACR) 1990 criteria for PAN.[9]
High-dose corticosteroids were started, but the recurring severe haemoptysis remained. It was then decided to treat the underlying malignancy more vigorously and induction chemotherapy (idarubicin and cytarabine) was started, leading to a complete remission. Remarkably, all symptoms of vasculitis then quickly diminished and the pulmonary CT scan normalised completely within three months.

Figure 1. *Infiltrative and nodular abnormalities with ground glass aspect on the pulmonary CT scan, suggestive of polyarteritis nodosa*



DISCUSSION

In our case a clinical diagnosis of PAN was made using the 1990 criteria of the ACR. We attain the same diagnosis when applying the clinical algorithm for differentiation between types of vasculitis of Kallenberg *et al.*[10] A histological diagnosis could not be obtained because renal biopsy was considered to be too dangerous in the presence of aneurysms in the kidneys, prolonged coagulation and low platelet count. Unfortunately, histological examination of the spleen could not confirm the presence of vasculitis because of extensive localisation of CMML and haemorrhage.
Like other haematological malignancies, MDS is associated with extrahaematological manifestations, mainly immunological features. However, these appear to occur more often in MDS and especially CMML than in other haematological neoplasms, with a reported prevalence of autoimmune manifestations of 10 to 18% in CMML.[8,11] A wide spectrum of autoimmune abnormalities has been reported in patients with MDS (*table 1*).[7] In the literature several types of vasculitis have been associated with MDS: most frequently cutaneous leucocytoclastic vasculitis, but also various types of systemic vasculitis.[12-14] In this review we will focus on systemic vasculitis, since

Figure 2. *Typical microaneurysms in medium-sized arteries of the kidney (left), bleeding from these microaneurysms led to enormous bilateral haematomas (right) and haemorrhagic shock*

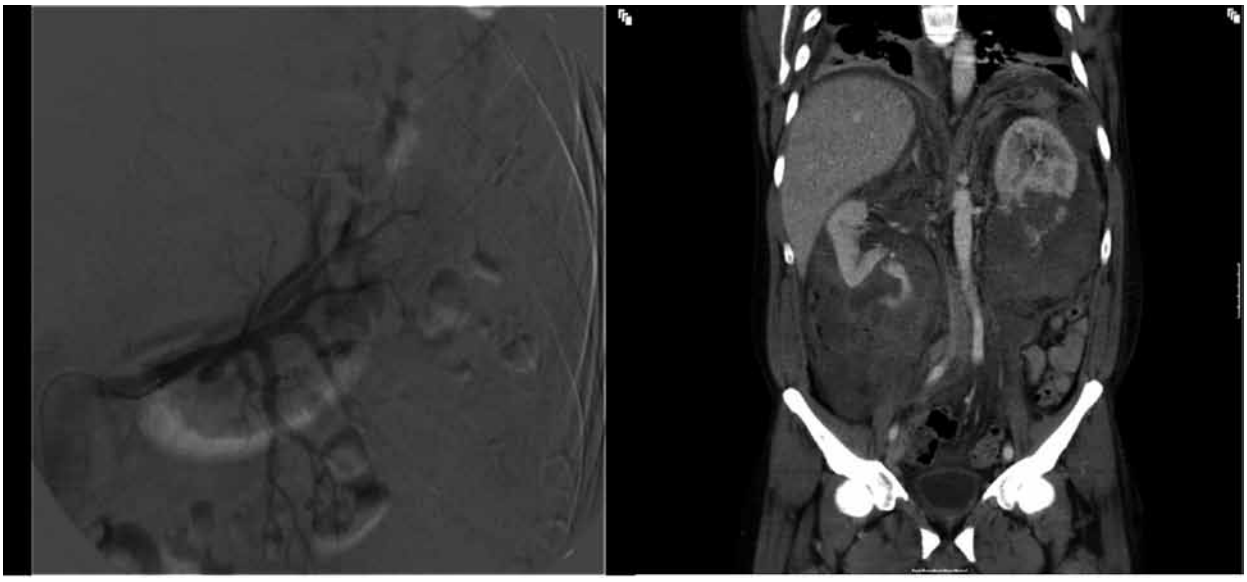


Table 1. *Autoimmune manifestations in MDS*

Type of autoimmune manifestation in MDS	Examples
Systemic vasculitis	Giant-cell arteritis Aortitis Medium- and small-sized vessel vasculitis
Isolated autoimmune disorders	Cutaneous vasculitis Polyarthritis Polyneuropathy
Classical connective tissue disorders	Systemic lupus erythematosus Raynaud's disease Polymyalgia rheumatica
Autoimmune haematological disorders	Autoimmune haemolytic anaemia Immune thrombocytopenia
Asymptomatic immunological serological abnormalities	Positive antineutrophil antibody Positive rheumatoid factor

this autoimmune phenomenon will probably most affect prognosis and treatment choices.

MDS and systemic vasculitis

We reviewed all English, Spanish, French, German and Dutch literature on the combination of MDS with systemic vasculitis. We found 23 publications with 55 cases in total (*table 2*). The prevalence of PAN in the normal population is around 3 per 100,000, hence the chance of one individual accidentally having both CMML and PAN would normally be very small.[15-18] Nonetheless, our review includes 26 cases of MDS in combination with PAN, of which 17 cases of CMML with PAN.

Remarkably, in four of the described cases spontaneous bilateral perirenal haemorrhage from microaneurysms occurred, as was seen in our patient.[14,19,20] The kidney is the most affected organ in PAN with or without associated myelodysplasia, with involvement in 70 to 80% of patients, but this most frequently leads to renal insufficiency, hypertension, proteinuria and sometimes modest haematuria. Spontaneous renal haemorrhages from microaneurysms in isolated PAN are rare and when it occurs it is usually unilateral.[19,21] Spontaneous bilateral perirenal haemorrhage thus seems to develop more often in patients with underlying MDS. In addition to perirenal haemorrhages, also haemorrhage from other organs such as the gastrointestinal tract or the lungs is reported in the described patients. Probably this is caused by the simultaneous presence of thrombocytopenia and other coagulation disorders in combination with (micro) aneurysms and other vessel abnormalities.

Prognosis and treatment considerations

Previous reports about the prognosis of patients with haematological malignancies in combination with autoimmune disorders have shown conflicting results. In some retrospective reports a worse outcome in MDS patients with immunological manifestations or with systemic vasculitis was demonstrated when compared with other MDS patients.[8,22] However, in a prospective study by Giannouli *et al.* in 13 patients no influence on median survival was reported, when corrected for the International Prognostic Scoring System (IPSS) score. Moreover, they did not find any association between IPSS score and risk of development of autoimmune abnormalities. But in this study patients with various types of autoimmune manifestations of variable severity were included; only two of the studied patients had a systemic vasculitis.[23]

In contrast, when we analyse the outcome in our reviewed cases of systemic vasculitis in MDS, we find that nine of 55 patients died from possible vasculitis-related causes such as haemorrhage and embolism. Another nine patients died from infection during treatment with immunosuppressive agents and one died due to an unspecified cause shortly after the diagnosis of vasculitis. Five patients developed steroid dependence, in six patients the MDS transformed into acute leukaemia and only three patients had long-term stable MDS without signs of active vasculitis and no need for treatment. From the other 22 patients the outcome could not be deduced. Taken together, this suggests that the development of a systemic vasculitis is associated with worse outcome in MDS patients. Treatment of the vasculitis itself with immunosuppressive medication can indeed improve symptoms, but also seems to be associated with an increased risk of fatal infections in the long term. Of course, publication bias in these reviewed cases cannot be excluded.

Our case illustrates that successful treatment of the underlying MDS can cure secondary vasculitis. This underscores the importance of a rapid diagnosis of MDS-related vasculitis and immediate treatment of MDS in the case of severe accompanying immunological manifestations.

Pathogenesis

The pathogenesis of vasculitis in MDS is still largely unknown. In patients with CMML, high numbers of circulating monocytes and related cytokines are found which may lead to vascular inflammation. At the same time phagocytic clearance is impaired, leading to prolonged circulation of immune complexes with subsequent activation of inflammatory mediators. This is assumed to be the result of gammopathies, abnormally functioning B and T lymphocytes, reduced CD4 count, immature natural killer cells and impaired function of monocytes and dendritic cells with abnormal antigen presentation. These features may result from abnormal stimulation by dysplastic bone marrow stem cells.[12,19,24,25] Furthermore the presence of interferon regulatory factor-1 (IRF-1) has been associated with the development

Table 2. *Overview of reported cases of MDS in combination with systemic vasculitis*

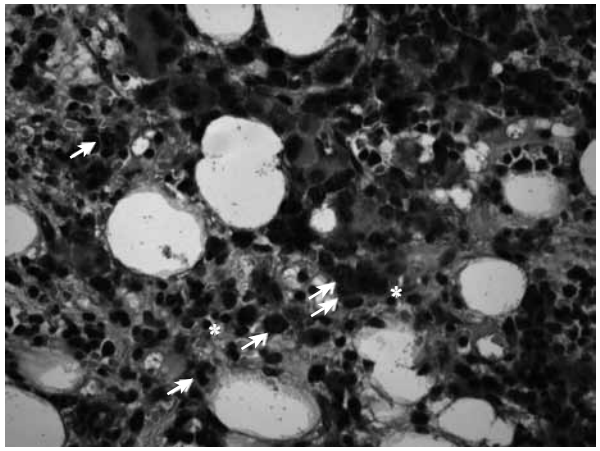
Reference	Sex	Age	MDS type	Vasculitis type	Diagnosis vasculitis	Treatment	Outcome
Saif[7]	Female	59	CMML	Systemic, ns	Histology (lung, skin, bowel)	CS	Death from gastrointestinal haemorrhage
Lopez[12]	Female	52	RAEB-t	Aortitis	CT-scan / MRI scan	CS, ASCT	Death from infection
Espinosa[13]	Female	75	RAEB	Giant-cell arteriitis	Histology (art. temp)	CS	Death from infection
	Male	79	CMML	Giant-cell arteriitis	Clinical criteria	CS	Steroid dependence
Hamidou[14]	Male	58	CMML	PAN	Histology (lung)	CS, CP	Death from infection
	Female	57	CMML	PAN	Angiography	CS, CP, ET	Death from myocarditis and encephalitis
	Female	67	CMML	PAN	Histology (stomach)	CS	Death from gastrointestinal haemorrhage
	Male	58	CMML	PAN, perirenal haematoma	Angiography	CS, CP	Stable MDS without active vasculitis
	Male	72	CMML	PAN	Histology (skin)	CS	Death from myocardial infarction
	Male	73	CMML	PAN	Histology (art. temp)	CS, MTX	Death from possible CNS vasculitis
	Male	76	CMML	PAN	Angiography	CS, CP	Death from infection
	Male	66	CMML	PAN	Clinical criteria	CS, MTX	Death from infection
Fain[17]	6 patients, ns		CMML	PAN	Histology	ns	ns
	3 patients, ns		ns	PAN	Histology	ns	ns
	1 patient, ns		ns	Wegener	Histology	ns	ns
	3 patients, ns		ns	MPA	Histology	ns	ns
Aslangul[19]	Male	61	CMML	PAN, perirenal haematoma	Histology (gall bladder)	CS	Death from bowel perforation
	Female	73	CMML	PAN, perirenal haematoma	Angiography	CS, CP	Death from gastrointestinal haemorrhage
Brickner[20]	Female	63	RAEB-t	PAN, perirenal haematoma	Angiography	CS	Stable MDS without active vasculitis
Giannouli[23, 29]	Male	67	RAEB	Systemic, small-vessel	Histology (lung)	CS	Stable MDS without active vasculitis
	Male	59	RAEB-t	MPA	Histology (skin, nerve)	CS, CY	Death from pulmonary haemorrhage
Incalzi[30]	Female	78	RCMD	Systemic, small-vessel	Histology (autopsy)	CS, AZ	Death from pulmonary embolism
Belizna[31]	Male	71	RAEB	Systemic, ns	Clinical criteria	CS	Steroid dependence
Steurer[32]	Male	67	RAEB	Systemic, large-vessel	CT scan	CS	Steroid dependence
	Male	60	RAEB	Aortitis	CT scan	CS	Transformation to leukaemia
Warren[33]	Male	72	RAEB	Systemic, small-vessel	Histology (skin)	ns	ns
Leung[34]	Male	67	CMML	PAN	Histology (kidney)	CS, CP	Death from infection
van Rijn[35]	Male	66	RAEB	MPA	Histology (skin)	CS	Transformation to leukaemia
Philippe[36]	Male	68	RAEB	PAN	Histology (skin, testis)	CS	Steroid-dependence
	Male	27	RA	Systemic, small-vessel	Histology (lung)	CS	ns
Smail[37]	Female	68	CMML	Wegener	Histology (sinus)	CS, CP	Transformation to leukaemia
Taillan[38]	Male	75	RA	Wegener	Histology (sinus)	CS	Transformation to leukaemia
Constans[39]	Male	75	RA	PAN	Histology (nerve)	CS	ns
	Male	77	RA	PAN	Histology (skin)	CS	ns
Fernandez[40]	Male	57	RA	PAN	Histology (skin, nerve)	CS, CP	Steroid-dependence
	Female	58	RARS	Systemic, ns	Histology (skin)	CS	Death from infection
Roy-Peaud[41]	Male	75	RA	PAN	ns	CS, CP	Death from unspecified cause
	Male	79	RARS	Systemic, ns	ns	CS, CP, MTX	Death from infection
	Male	59	RA	Systemic, ns	ns	CS, AZ	Death from infection
Berthier[42]	5 patients, ns		RAEB/ RARS/ RA	Giant-cell arteritis/ PAN	ns	ns	ns
Park[43]	Female	54	CMML	Systemic, large-vessel	CT scan	CS, CP	Transformation to leukaemia
Cohen[44]	Male	62	RCMD	Takayasu	ns	CS, MTX	Transformation to leukaemia

Art. temp = temporal artery; ASCT = allogeneic stem cell transplantation; AZ = azathioprine; CMML = chronic myelomonocytic leukaemia; CP = cyclophosphamide; CS = corticosteroids; CY = cyclosporine; ET = etoposide; MTX = methotrexate, MPA = microscopic polyangiitis; ns = not specified; PAN = polyarteritis nodosa; RA = refractory anaemia, RAEB = refractory anaemia with excess blasts; RAEB-t = refractory anaemia with excess blasts in transformation; RARS = Refractory anemia with ringed sideroblasts; RCMD= refractory cytopenia with multilineage dysplasia.

of autoimmune deregulation in MDS. The IRFs are transcriptional factors, known to be involved in both cell growth control and tumour suppression. In myeloproliferative diseases a decrease in IRF can lead to weakened tumour suppression and this is associated with progressive disease and drug resistance.[26] IRF-1 also plays a role in the induction of immune responses. IRF-1 is usually low in MDS patients when compared with healthy individuals. This decrease probably plays a role in the pathogenesis of MDS and in the transformation to acute leukaemia.[27,28] However, in an observational study of 14 patients with MDS, increased levels of IRF-1 were seen in the seven MDS patients with accompanying autoimmune manifestations when compared with the other seven MDS patients without autoimmune manifestations. In this small group it could not be demonstrated that this increased level of IRF-1 was associated with a lower rate of transformation to leukaemia.[29] In our patient with both CMML and PAN, we evaluated the IRF-1 immunoexpression level in bone marrow and indeed found an increased level (*figure 3*).
Other previously stated hypotheses for the development of autoimmunity in MDS are the existence of one common trigger predisposing for both myeloid and lymphoid disorders or the presence of an immune deregulation preceding and possibly causing the development of MDS. These hypotheses could, however, not be confirmed by experimental studies.[7,30]

In conclusion, systemic vasculitis is more prevalent in patients with MDS and in particular CMML, in comparison with the general population, with a particular risk of bilateral renal haemorrhage. The pathogenesis is incompletely understood and seems multifactorial, but IRF-1 appears to be one factor that plays a role in the development of immunological manifestations in MDS. According to our review the prognosis of MDS patients with systemic vasculitis is worse than similar patients without vasculitis, because of the risk of both vasculitis-related and treatment-related complications. Therefore we recommend to treat the underlying haematological disease as soon and effectively as possible, when an associated vasculitis is diagnosed.

Figure 3. *High IRF-1 expression in mature (arrows) and precursor (arrow heads) myeloid cells in bone marrow from the described patient; erythroid cells are low in IRF-1 (asterisks)*



REFERENCES

1. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 2002; 100(7): 2292-302.
2. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009; 114(5): 937-51.
3. Tefferi A, Elliott MA, Pardanani A. Atypical myeloproliferative disorders: diagnosis and management. Mayo Clin Proc. 2006;81(4):553-63.
4. Orazi A, Germing U. The myelodysplastic/ myeloproliferative neoplasms: myeloproliferative diseases with dysplastic features. Leukemia. 2008;22:1308-19.
5. Rollison DE, Howlader N, Smith MT, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008;112(1):45-52.
6. Pirayesh A, Verbunt RJAM, Kluin PM, et al. Myelodysplastic syndrome with vasculitic manifestations. J Int Med. 1997;242:425-31.
7. Saif MW, Hopkins JL, Gore SD. Autoimmune phenomena in patients with myelodysplastic syndromes and chronic myelomonocytic leukemia. Leuk Lymphoma. 2002;43(11):2083-92.
8. Enright H, Jacob HS, Vercelotti G, et al. Paraneoplastic autoimmune phenomena in patients with myelodysplastic syndromes: response to immunosuppressive therapy. Br J Haematology. 1995;91(2):403-8.
9. Lightfoot RW, Michel BA Jr, Bloch DA, et al. The American College of Rheumatology 1990 criteria for the classification of polyarteritis nodosa. Arthritis Rheum. 1990;33:1088-93.
10. Kallenberg CG. The last classification of vasculitis. Clin Rev Allergy Immunol. 2008;35: 5-10.
11. Hamidou MA, Derenne S, Audrain MAP, et al. Prevalence of rheumatic manifestations and antineutrophil cytoplasmic antibodies in haematological malignancies. A prospective study. Rheumatology. 2000;39:417-20.
12. Lopez FF, Vaidyan PB, Mega AE, Schiffman FJ. Aortitis as a manifestation of myelodysplastic syndrome. Postgrad Med J. 2001;77(904):116-8.
13. Espinosa G, Font J, Muñoz-Rodríguez FJ, et al. Myelodysplastic and myeloproliferative syndromes associated with giant cell arteritis and polymyalgia rheumatica: a coincidental coexistence or a causal relationship? Clin Rheumatol. 2002;21(4):309-13.
14. Hamidou MA, Boumalassa A, Larroche C, et al. Systemic medium-sized vessel vasculitis associated with chronic myelomonocytic leukemia. Semin Arthritis Rheum. 2001;31:119-26.
15. Mahr A, Guillevin L, Poissonnet M, et al. Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. Arthritis Rheum. 2004;51(1):92-9.
16. Mohammad AJ, Jacobsson LT, Mahr AD, Sturfeld G, Segelmark M. Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg-Strauss syndrome within a defined population in southern Sweden. Rheumatology 2007;46(8):1329-37

17. Fain O, Hamidou M, Cacoub P, Godeau B, Wechsler B, Pariès J, et al. Vasculitides associated with malignancies: analysis of sixty patients. Arthritis Rheum. 2007 Dec 15;57(8):1473-80.

18. Rosen AM, Haines K 3rd, Tallman MS, Hakimian D, Ramsey-Goldman R. Rapidly progressive cutaneous vasculitis in a patient with chronic myelomonocytic leukemia. Am J Hematol. 1995 Dec; 50(4):310-2.

19. Aslangul-Castier E, Papo T, Amoura Z, Baud O, Leblond V, Charlotte F, et al. Systemic vasculitis with bilateral perirenal haemorrhage in chronic myelomonocytic leukaemia. Ann Rheum Dis. 2000 May;59(5):390-3.

20. Brickner LA, Scannell KA. Medium-vessel PAN-type vasculitis associated with myelodysplastic syndrome and presenting as bilateral perinephric hematomas. Clin Exp Rheumatol. 1997;15(2):221-2.

21. Presti JC, Carroll PR. Bilateral spontaneous renal haemorrhage due to polyarteritis nodosa. West J Med. 1991;155:527-8.

22. Hollanda A, Beucher A, Henrion D, et al. Systemic and immune manifestations in myelodysplasia. A multicenter retrospective study. Arthritis Care Res. 2011;63(8):1188-94.

23. Giannouli S, Voulgarelis M, Zintzaras E, et al. Autoimmune phenomena in myelodysplastic syndromes: a 4-yr prospective study. Rheumatology. 2004;43:626-32.

24. Shetty V, Allampallam K, Raza A. Increased macrophages, high serum M-CSF and low serum cholesterol in myelodysplasia and Kawasaki disease. Br J Haematol. 1999;106:1068-70.

25. Voulgarelis M, Giannouli S, Ritis K, Tzioufas AG. Myelodysplasia-associated autoimmunity: clinical and pathophysiologic concepts. Eur J Clin Inv. 2004;34:690-700.

26. Thielen N, Ossenkoppele G, Schuurhuis GJ, Janssen J. New insights in the pathogenesis of chronic myeloid leukemia: towards a path to cure. Neth J Med. 2011;10:430-40.

27. Pinheiro RF, Metze K, Silva MRR, Chauffaille ML. The ambiguous role of interferon regulatory factor-1 (IRF-1) immunoexpression in myelodysplastic syndrome. Leuk Res. 2009;33:1308-12.

28. Liebermann DA, Hoffman B. Good and bad IRF-1: Role in tumor suppression versus autoimmune disease. Leuk Res. 2009; 33:1301-2.

29. Giannouli S, Tzoanopoulos D, Ritis K, et al. Autoimmune manifestations in human myelodysplasia: a positive correlation with interferon regulatory factor-1 (IRF-1) expression. Ann Rheum Dis. 2004;63(5):578-82.

30. Incalzi RA, Arena V, Capelli A, Gambassi G. Isolated PACNS-like presentation of a systemic vasculitis complicating a myelodysplastic syndrome. J Intern Med. 2004;255(6):674-9.

31. Belizna CC, Kerleau JM, Heron F, et al. Vasculitis and myelodysplasia. Isr Med Assoc J. 2008;10(2):156-7.

32. Steurer M, Fritsche G, Tzankov A, et al. Large-vessel arteritis and myelodysplastic syndrome: report of two cases. Eur J Haematol. 2004;73(2):128-33.

33. Warren AJ, Hegde UM, Nathwani A, Reilly IAG. Systemic vasculitis and myelodysplasia. Br J Haematol. 1990;75:627-9.

34. Leung AC, McLay A, Boulton-Jones JM. Polyarteritis nodosa and monocytic leukaemia. Postgrad Med J 1986;62(723):35-7.

35. van Rijn RS, Wittebol S, Graafland AD, Kramer MH. Immunologic phenomena as the first sign of myelodysplastic syndrome. Ned Tijdschr Geneeskd. 2001;145(32):1529-33.

36. Philippe B, Couderc LJ, Droz D, et al. Systemic vasculitis and myelodysplastic syndromes. A report of two cases. Arthritis Rheum. 1997;40(1):179-82.

37. Smail A, Ducroix JP, Sahir R, et al. Wegener's disease and myelomonocytic leukemia: a fortuitous association? Rev Med Interne. 1989;10(5):463-5.

38. Taillan B, Ferrari E, Garnier G, et al. Association of Wegener's disease and myelodysplastic syndrome. Ann Med Interne (Paris). 1992;143(7):478-9.

39. Constans J, Vidal E, Conri C, et al. Periarteritis nodosa and preleukemic states. Ann Med Interne (Paris). 1992;143(7):477-8.

40. Fernández-Miranda C, García-Marcilla A, Martín M, et al. Vasculitis associated with a myelodysplastic syndrome: a report of 5 cases. Med Clin (Barc). 1994 Oct 29;103(14):539-42.

41. Roy-Peaud F, Paccalin M, Le Moal G, et al. Association of systemic diseases and myelodysplastic syndromes. A retrospective study of 14 cases. Presse Med. 2003 Mar 29;32(12):538-43.

42. Berthier S, Magy N, Gil H, et al. Myelodysplasias and systemic diseases. A non-fortuitous association. Rev Med Interne. 2001 May;22(5):428-32.

43. Park JK, Gelber AC, Zheng G, et al. Large vessel vasculitis as an early manifestation of chronic myelomonocytic leukemia. J Clin Oncol. 2011; 29(20):e601-3.

44. Cohen MJ, Shyman A, Klein M, et al. Large vessel (Takayasu's) arteritis in a patient with myelodysplastic syndrome: is there a common pathogenesis? Clin Lymphoma Myeloma Leuk. 2011;11(1):60-3.

Netherlands
The Journal of Medicine

REVIEW

Delayed HIV testing in internal medicine clinics – a missed opportunity

L. Hermans[1,2], A. Wensing[1], A. Hoepelman[2], J. Dutilh[2], T. Mudrikova[2*]

Departments of [1]Virology, Medical Microbiology, [2]Internal Medicine & Infectious Diseases, University Medical Center Utrecht, the Netherlands, [*]corresponding author: tel.: +31 (0)88-755 55 55, fax: +31 (0)88-755 55 63, e-mail: t.mudrikova@umcutrecht.nl

ABSTRACT

As HIV infection may be non-symptomatic for many years, many HIV-infected individuals are not aware of their infection. At a certain point in time non-specific symptoms may occur for which patients are likely be referred to internal medicine outpatient clinics. In the absence of systematic screening for HIV and in particular in patients who do not have classical risk factors for HIV, the diagnosis of HIV infection may easily be overlooked. In this manuscript it is illustrated that this diagnostic and therapeutic delay can lead to increased morbidity and mortality. Moreover, undiagnosed individuals are on average more likely to transmit HIV than diagnosed individuals. It is important for public health to identify people harbouring HIV infection, as this is expected to reduce the number of new infections. HIV infection should be considered a possible cause of unexplained symptoms in an early stage of the diagnostic process, in particular in patients with symptoms such as unexplained fever, lymphadenopathy or weight loss or in the presence of conditions suggestive of possible immune deficiency, regardless of the absence of risk factors.

KEYWORDS

HIV, late diagnosis, screening, opt-out, internal medicine, AIDS

INTRODUCTION

Human immunodeficiency virus (HIV) infection causes progressive destruction of the host immune system. Early diagnosis and initiation of antiretroviral treatment dramatically improves the prognosis of patients. As HIV infection can present with a broad variety of non-specific symptoms, a certain degree of awareness is necessary. However, in some cases clinicians disregard the possibility of HIV infection in patients who display symptoms which could be related to the disease, but who are not known to have any HIV-associated risk factors.[1] As a consequence, a broad array invasive investigations may precede serological testing for HIV.

In this article we present three patients with symptoms known to be associated with HIV infection, which remained undiagnosed or unexplained for a considerable time. None of them had apparent risk factors for an infection with HIV. All patients underwent multiple diagnostic and therapeutic interventions before an HIV test was performed. In the review section, we discuss factors that might contribute to diagnostic delay in HIV infections. In the Netherlands, as well as in many other industrialised countries, opt-in testing policies may feed general reluctance towards HIV testing and thus contribute to the high incidence of late HIV diagnosis.

Patient A, a 45-year-old female, was admitted to the department of internal medicine of a regional hospital on several occasions over a two-year period for analysis of fever and macrocytic anaemia which persisted despite vitamin B12 substitution. She also complained of fatigue which had been present since an episode of thoracic herpes zoster. The patient had a medical history of premature coronary artery disease with an episode of an acute coronary syndrome, hypertension and non-specific colitis. Laboratory investigation performed on her last admission showed anaemia (haemoglobin 4.1 mmol/l), atypical lymphocytosis, high erythrocyte sedimentation rate (ESR) and polyclonal hypergammaglobulinaemia. Temporal arteritis was suspected and a biopsy of the

temporal artery was performed, showing no abnormalities. Extensive evaluation with CT and PET scans showed a generalised lymphadenopathy. To exclude a lymphoproliferative disorder, an excision biopsy of a cervical lymphatic node was taken showing a reactive dysplasia. As a sampling error was suspected, a bronchoscopic biopsy of a mediastinal lymphatic node was performed, which confirmed the findings of the first biopsy. As an infectious cause seemed likely, treating physicians conducted serological screening aimed at several possible infectious causes. An HIV test was positive and the patient was referred to our centre. The CD4 cell count was 236/mm^3 upon referral. Shortly after transfer *Candida* oesophagitis was diagnosed and successfully treated with fluconazole. The patient received highly active antiretroviral therapy (HAART), consisting of tenofovir, emtricitabine and atazanavir boosted with ritonavir. The haemoglobin concentration normalised within a few months. The HIV RNA viral load became undetectable (<50 copies/ml) and the CD4 cell count increased to 450/mm^3 after six months of treatment.

Patient B, a 40-year-old heterosexual male, with a medical history of chronic hepatitis B was referred to an ophthalmologist because of pain and redness of his left eye, with decreased visual acuity. Toxoplasmic uveitis was diagnosed and treated with pyrimethamine and azithromycin with folic acid suppletion. The patient had a medical history of chronic hepatitis B, diagnosed five years earlier, and had been treated with entecavir for 18 months. In the previous year, the patient had experienced an episode of thoracic herpes zoster and pneumonia with prolonged recovery, and also reported weight loss of 10 kg. Because screening for HIV had never been performed, the ophthalmologist requested serological testing, and test results returned positive. A CD4 cell count of 53/mm^3 indicated advanced infection. An MRI of the brain showed a lesion in the right occipital lobe which could be a result of cerebral toxoplasmosis. Antitoxoplasmic treatment was adjusted (azithromycin was substituted by sulphadiazine). Antiretroviral treatment with tenofovir, emtricitabine and atazanavir boosted with ritonavir was prescribed. Entecavir was discontinued as tenofovir and emtricitabine, which also have an anti-HBV efficacy, were initiated. Use of entecavir as monotherapy in HIV/HBV-co-infected patients has been associated with the development HIV drug resistance compromising antiretroviral treatment options.[2] Fortunately, no resistance-related HIV mutations were detected in this case.

Patient C was a 64-year-old Dutch female who had been suffering from various unexplained symptoms for 12 years. Initially, she developed fatigue and myalgia, accompanied by a high ESR and thrombocytopenia. Her treating physicians suspected Sjögren's disease and idiopathic thrombocytopenic purpura. Treatment with prednisone was started but did not relieve her symptoms. During the following years, the patient experienced peripheral facial palsy, pneumonia, sensory polyneuropathy, mild cognitive impairment, deep venous thrombosis, septic shock of unknown origin, *Candida* oesophagitis and recurrent episodes of diarrhoea. In 2010, she was referred to our hospital for a second opinion due to dysarthria, dysphagia, apathy and sensory loss in both legs, rendering her unable to walk. HIV testing was performed and the results were positive. The CD4 cell count was 141 cells/mm^3. An MRI scan of the brain showed extensive leukoencephalopathy. Examination of the cerebrospinal fluid did not point to an opportunistic infection. Antiretroviral treatment was prescribed (tenofovir, emtricitabine, atazanavir boosted with ritonavir). During the following months, the patient was readmitted twice, first due to complicated urinary tract infection, and later due to bloody diarrhoea with stool samples positive for *Clostridium difficile* toxin. Her neurological condition remained unchanged after initiation of HAART. During the third readmission, three months after the HIV diagnosis, the patient suffered from pseudomembranous colitis with severe metabolic dysregulation due to dehydration, renal insufficiency and respiratory insufficiency, and died despite intensive treatment.

DISCUSSION AND REVIEW OF THE LITERATURE

Consequences of late diagnosis

The case histories presented above concern patients with symptoms that remained unexplained or undiagnosed for a considerable period of time, and ultimately appeared to result from infection with HIV. Although all patients had symptoms suggestive of cellular immune deficiency, HIV testing was not performed until considerable delay had occurred. Ensuingly, complications occurred that might have been avoided or possibly more adequately treated if HIV testing and treatment had been initiated in an earlier stage.

Late recognition of HIV infection has a number of important consequences regarding prognosis, transmission of infection and healthcare costs. It has been shown that testing positive in a late phase of the HIV infection, when a severe immunodeficiency is present, worsens the prognosis compared with early diagnosis.[3-5] Patients who started HAART in an advanced stage of the infection, prior to the development of AIDS, were shown to have a significantly greater risk of progression to AIDS and a higher mortality rate. [6] It is well known that if advanced immune deficiency is present, the risk of acquiring

an opportunistic infection or a malignancy is greatly increased. Moreover, antiretroviral treatment started in the setting of a profoundly impaired immune system often results in a slow and incomplete immunological recovery. From a public health perspective, early diagnosis is also beneficial as untreated patients with uncontrolled viral replication are more likely to transmit the virus to their sexual partners.[7] High morbidity in patients presenting late results in higher treatment costs.[8,9] Early diagnosis of HIV infection enables screening for other infections more frequently present in HIV patients, such as hepatitis C, which also has a better prognosis if treated early.[10]

European data indicate that in 33% of cases, the diagnosis of HIV infection is made in a late stage of the disease, at CD4 cell counts ≤350 cells/mm^3 or after the occurrence of an AIDS-defining event.[11] Until now, the presence of one of several well-known risk factors for HIV infection has been the main argument to perform an HIV test in Dutch clinical practice.[12] This could explain the large proportion of Dutch patients presenting late. According to the Dutch Athena cohort more than 50% of the heterosexual men and women and 40% of men who have sex with men (MSM) are late presenters.[13] Recent studies show that risk factors appear to be more often absent in patients who are diagnosed in a late stage of the infection. These patients have more frequently acquired HIV through heterosexual contact and belong to the older age groups.[14-18] These patient characteristics correspond with the case histories described above, in which the mean age was 47 years (range 40-64 years) and where heterosexual contact was the presumed transmission route.

An important cause of late diagnosis is patient delay. However, as the presented cases have shown, doctor's delay, due to postponing or not considering testing for HIV, also contributes to late testing. This has been recognised in other reports as well.[1,19-21] Several factors can be responsible for this delay. First, the diagnosis is often regarded as uncommon and as such is overlooked in the differential diagnosis of unexplained symptoms. Symptoms tend to be unspecific; complaints of weight loss, night sweats and fatigue are often present in advanced infection. If unexplained, such general symptoms as well as signs of immune deficiency should prompt HIV testing. Second, many physicians only expect the disease to affect patients involved in risk behaviour, such as male homosexual contact or intravenous drug use, and overlook cases of HIV infection when obvious risk factors are absent.[1,19] Last, doctors may fear that suggesting HIV testing could give the patient the impression of being suspected of risk behaviour, thus compromising the doctor-patient relationship.

HIV testing in the Netherlands

In the Netherlands, an opt-in testing policy for HIV infection is maintained in most clinical settings, requiring physicians to ask for patient consent for HIV testing. HIV infection is the only infectious disease to which this policy applies. This practice, combined with the tendency to only test patients if risk factors are clearly present, fuels the reluctance of doctors to perform an HIV test when risk factors are not apparent or when symptoms are ambiguous.

The reluctant attitude towards HIV testing in the Netherlands has its roots in the earlier days of the HIV epidemic.[22] Poor prognosis in the absence of effective treatment and social stigma associated with HIV infection implicated possible negative social consequences. The general feeling was that diagnosing the disease in an early stage only meant doing additional harm rather than being of benefit to the patient. This consideration led to repeated advice by the Dutch Health Council not to use the HIV test as a screening tool.[12] The introduction of HAART in the mid 1990s dramatically improved the prognosis of HIV-infected patients, prompting the Dutch Health Council to advise a more active HIV-testing policy in some situations. Still, in 2007 the percentage of Dutch people who had undergone HIV testing at least once in their life was lower than in other industrialised countries. Up to that year, only 70 to 80% of MSM in Amsterdam had undergone HIV testing at least once in their life, compared with 95% in Sydney and San Francisco.[23] Currently, all pregnant women are screened for HIV infection in an opt-out fashion and outpatient STD clinics have initiated opt-out screening for HIV infection in all patients.[24]

In 2005, the total number of HIV-infected individuals living in the Netherlands was estimated to be about 18,500, of which only two thirds were diagnosed. The remaining third, approximately 6000 individuals, were unaware of their seropositive status.[24] In 2010 the total number of HIV-infected individuals was expected to have risen to 21,500, and the number of unidentified cases to 8600.[25] In order to be able to identify at least some individuals who are unaware of their infection, it has been proposed to implement an opt-out policy towards HIV testing in the Netherlands regarding screening for HIV infection in pregnant women.[26] American research, published in 2005, has granted plausibility to the assumption that screening of all patients presenting in healthcare settings will be comparable in cost-effectiveness with common public health initiatives such as screening of blood transfusion products and vaccination, even if the nationwide prevalence of HIV-infected individuals is only 0.1%.[27] In line with this,

in 2006 the American Centre for Disease Control and Prevention (CDC) recommended nationwide screening for HIV infection in all patients between the ages of 13 and 64 presenting to all healthcare settings.[28] In the United Kingdom, a new policy calling for a low clinical threshold towards testing for HIV infection has been in use since 2008.[29] A pilot study performed in 2009 at the accident and emergency units of two London hospitals, in which all patients between 16 and 65 years of age were offered an HIV test, showed that routine HIV testing is accepted by most patients.[30]

In spite of this, in the majority of European countries, as well as in Australia and Canada, opt-in policies towards HIV testing have remained largely unchanged.

CONCLUSION

In industrialised countries, approximately one-third of HIV-infected individuals are unaware of their HIV status. As HIV infection often presents with non-specific symptoms, many undiagnosed individuals will likely be referred to internal medicine outpatient clinics. In European internal medicine practice, screening for HIV infection is uncommon, and the decision to test in patients with atypical symptoms is primarily prompted by the presence of risk factors for this infection. This policy causes HIV infection to be overlooked as a possible cause of unexplained symptoms in individuals without risk factors. The presented cases and literature illustrate that this diagnostic and therapeutic delay can lead to increased morbidity and mortality. Moreover, undiagnosed individuals are on average more likely to transmit HIV than diagnosed individuals. It is imperative to public health to identify people harbouring HIV infection, as this is expected to reduce the number of new infections. As the absence of risk factors is not sufficient to rule out the diagnosis, HIV infection should be considered as a possible cause of unexplained symptoms in an early stage of the diagnostic process. Furthermore, all conditions suggestive of possible immune deficiency, community acquired pneumonia, tuberculosis, viral hepatitis and symptoms such as unexplained fever, lymphadenopathy or weight loss should warrant an early HIV test, regardless of the absence of risk factors.

REFERENCES

1. van den Berk GE, Jarbandhan AA, Regez RM, van Bergen JE, Brinkman K. [No HIV test: a chance missed]. Ned Tijdschr Geneeskd. 2007 Dec 1;151(48):2648-51.
2. McMahon MA, Jilek BL, Brennan TP, Shen L, Zhou Y, Wind-Rotolo M, Xing S, Bhat S, Hale B, Hegarty R, Chong CR, Liu JO, Siliciano RF, Thio CL. The HBV drug entecavir – effects on HIV-1 replication and resistance. N Engl J Med. 2007 Jun 21;356(25):2614-21.
3. Sabin CA, Smith CJ, Youle M, Lampe FC, Bell DR, Puradiredja D, Lipman MC, Bhagani S, Phillips AN, Johnson MA. Deaths in the era of highly active anti retroviral therapy: contribution of late presentation, treatment exposure, resistance and abnormal laboratory markers. AIDS. 2006 2;20(1):67-71.
4. Chadborn TR, Baster K, Delpech VC, Sabin CA, Sinka K, Rice BD, Evans BG. No time to wait: how many HIV-infected homosexual men are diagnosed late and consequently die? (England and Wales, 1993 – 2002). AIDS. 2005;19:513-20.
5. Korenromp EL, Williams BG, Schmid GP, Dye C. Clinical prognostic value of RNA viral load and CD4 cell counts during untreated HIV-1 infection--a quantitative review. PLoS One. 2009 Jun 17;4(6):e5950.
6. When To Start Consortium, Sterne JA, May M, Costagliola D, et al. Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies. Lancet. 2009 Apr 18;373(9672):1352-63.
7. Marks G, Crepaz N, Senterfitt JW. Meta-analysis of high-risk sexual behavior in persons aware and unaware they are infected with HIV in the United States: implications for HIV prevention programs. J Acquir Immune Defic Syndr. 2005 Aug 1;39(4):446-53.
8. Krentz HB, Auld MC, Gill MJ. The high cost of medical care for patients who present late (CD4 <200 cells/microL) with HIV infection. HIV Med. 2004 Mar;5(2):93-8.
9. Read P, Armstrong-James D, Tong CY, Fox J. Missed opportunities for HIV testing--a costly oversight. QJM. 2010 Dec 5.
10. Arends JE, Lambers FA, van der Meer JT, Schreij G, Richter C, Brinkman K, Hoepelman AI, The Netherlands Society for AIDS Physicians-NVAB Treatment of acute hepatitis C virus infection in HIV+ patients: Dutch recommendations for management. Neth J Med. 2011 Jan;69(1):43-9.
11. Antinori A, Coenen T, Costagiola D, Dedes N, Ellefson M, Gatell J, Girardi E, Johnson M, Kirk O, Lundgren J, Mocroft A, D'Arminio Monforte A, Phillips A, Raben D, Rockstroh JK, Sabin C, Sönnerborg A, De Wolf F; European Late Presenter Consensus Working Group. Late presentation of HIV infection: a consensus definition. HIV Med. 2010 Jun 17.
12. Gezondheidsraad. Herziening van het HIV-testbeleid. Den Haag: Gezondheidsraad, 1999; publicatie nr. 1999/02.
13. Stichting HIV monitoring. Report 2010: Monitoring of human immunodeficiency-virus (HIV) infection in the Netherlands. Amsterdam: Stichting HIV Monitoring; 2010.
14. Delpierre C, Cuzin L, Lauwers-Cances V, Marchou B, Lang T. High-risk groups for late diagnosis of HIV infection: a need for rethinking testing policy in the general population. AIDS Patient Care STDS. 2006 Dec;20(12):838-47.
15. Manavi K, McMillan A, Ogilvie M, Scott G. Heterosexual men and women with HIV test positive at a later stage of infection than homo- or bisexual men. Int J STD AIDS. 2004 Dec;15(12):811-4.
16. Borghi V, Girardi E, Bellelli S, Angeletti C, Mussini C, Porter K, Esposito R. Late presenters in an HIV surveillance system in Italy during the period 1992-2006. J Acquir Immune Defic Syndr. 2008 Nov 1;49(3):282-6.
17. Sabin CA, Smith CJ, Gumley H, Murphy G, Lampe FC, Phillips AN, Prinz B, Youle M, Johnson MA. Late presenters in the era of highly active anti retroviral therapy: uptake of and responses to antiretroviral therapy. AIDS. 2004 Nov 5;18(16):2145-51.
18. Althoff KN, Gebo KA, Gange SJ, et al. North American AIDS Cohort Collaboration on Research and Design. CD4 count at presentation for HIV care in the United States and Canada: Are those over 50 years more likely to have a delayed presentation? AIDS Res Ther. 2010 Dec 15;7(1):45.
19. Paull SA, Waring J, Post JJ. Late diagnosis of human immunodeficiency virus (HIV) in two patients previously treated for pulmonary tuberculosis--a missed opportunity for an earlier HIV diagnosis? Intern Med J. 2010 Dec;40(12):e2-3.

20. Khorvash F, Naeini AE, Behjati M, Jalali M. HIV associated thrombocytopenia, misdiagnosed as thrombotic thrombocytopenic purpura: a case report. Cases J. 2009 Oct 29;2:175.

21. Kovari H, Ebnöther C, Schweiger A, Berther N, Kuster H, Günthard HF. Pulmonary toxoplasmosis, a rare but severe manifestation of a common opportunistic infection in late HIV presenters: report of two cases. Infection. 2010 Apr;38(2):141-4.

22. Gezondheidsraad. Permanente Commissie AIDS. AIDS-problematiek in Nederland. Richtlijnen voor groepsonderzoek en adviezen voor preventie. Den Haag: Gezondheidsraad, 1986; publicatie nr. 1986/22.

23. Dukers-Muijrers NH, Heijman RL, van Leent EJM, Coutinho RA, Thiesbrummel HFJ, Fennema JSA. Hoog tijd voor brede toepassing van 'opting-out'-strategie bij hiv-tests. Ned Tijdschr Geneeskd. 2007 Dec 1;151(48):2661-5

24. Van der Bij AK, Dukers NH, Coutinho RA, Fennema HS. Low HIV-testing rates and awareness of HIV infection among high-risk heterosexual STI clinic attendees in The Netherlands. Eur J Public Health. 2008 Aug;18(4):376-9. Epub 2008 Mar 31.

25. Dukers-Muijrers, NHTM, Koedijk, F, Hoebe, CJPA. Standaard op hiv testen bij soacentra; een evaluatie van deze opt-out strategie in Zuid Limburg. Infectieziektenbulletin. 2010;21 (7):237-42.

26. Gezondheidsraad. Wet bevolkingsonderzoek: screening op hiv-infectie. Den Haag: Gezondheidsraad, 2000; publicatie nr. 2000/03wbo.

27. Sanders GD, Bayoumi AM, Sundaram V, et al. Cost-effectiveness of screening for HIV in the era of highly active antiretroviral therapy. N Engl J Med. 2005 Feb 10;352(6):570-85.

28. Branson BM, Handsfield HH, Lampe MA, et al. Centers for Disease Control and Prevention (CDC). Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings. MMWR September 22, 2006 / 55(RR14);1-17.

29. Palfreeman A, Fisher M, Ong E. Testing for HIV: concise guidance. Clin Med. 2009, Vol 9, No 5:471-6.

30. London A&E patients offered HIV tests. BBC News London [Internet]. 2011 Jun 8 [cited 2011 Sep 9]; available from: (http://www.bbc.co.uk/news/uk-england-london-13695756)

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Risk of cardiovascular events in patients with polycystic ovary syndrome

S. Iftikhar[1*], M.L. Collazo-Clavell[2], V.L. Roger[3], J. St. Sauver[4], R.D. Brown Jr[5], S. Cha[6], D.J. Rhodes[7]

Divisions of [1]General Internal Medicine, [2]Endocrinology, Diabetes, Metabolism, and Nutrition, [3]Cardiovascular Diseases, [4]Epidemiology, [5]the Department of Neurology, Divisions of [6]Biomedical Statistics and Informatics, [7]Preventive, Occupational, and Aerospace Medicine, Mayo Clinic, Rochester, Minnesota, [*]corresponding author: e-mail: iftikhar.salma@mayo.edu

ABSTRACT

Women with polycystic ovary syndrome (PCOS) have increased prevalence of cardiovascular (CV) risk factors. However, data on the incidence of CV events are lacking in this population.

Using Rochester Epidemiology Project resources, we conducted a retrospective cohort study comparing CV events in women with PCOS with those of women without PCOS in Olmsted County, Minnesota.

Between 1966 and 1988, 309 women with PCOS and 343 without PCOS were identified. Mean (SD) age at PCOS diagnosis was 25.0 (5.3) years; mean age at last follow-up was 46.7 years. Mean (SD) follow-up was 23.7 (13.7) years. Women with PCOS had a higher body mass index (29.4 kg/m^2 vs 28.3 kg/m^2; p=.01). Prevalence of type 2 diabetes mellitus and hypertension and levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides were similar in the two groups. We observed no increase in CV events, including myocardial infarction (adjusted hazard ratio [HR] 0.74; 95% confidence interval [CI] 0.32 to 1.72; p=.48); coronary artery bypass graft surgery (adjusted HR 1.52; 95% CI 0.42 to 5.48; p=.52); death (adjusted HR 1.03; 95% CI, 0.29 to 3.71; p=.96); death due to CV disease (adjusted HR 5.67; 95% CI 0.51 to 63.7; p=.16); or stroke (adjusted HR 1.05; 95% CI 0.28 to 3.92; p=.94).

Although women with PCOS weighed more than controls, there was no increased prevalence of other CV risk factors. Furthermore, we found no increase in CV events. While prospective studies are needed to confirm these findings, women with PCOS do not appear to have adverse CV outcomes in midlife.

KEYWORDS

Cardiovascular disease; polycystic ovary syndrome; Rochester Epidemiology Project

INTRODUCTION

Stein and Leventhal[1] first described polycystic ovary syndrome (PCOS) in 1935 on the basis of case reports of seven women sharing a constellation of signs and symptoms. Today, PCOS is the most common endocrinopathy in women during their childbearing years, with a reported prevalence ranging from 4 to 12%.[2] It is likely that this range significantly under-represents the true prevalence because many cases are unrecognised.[2] Several studies have reported that the prevalence of cardiovascular (CV) risk factors is higher in women with PCOS compared with age-matched controls, including low high-density lipoprotein (HDL) cholesterol, elevated triglycerides, elevated low-density lipoprotein (LDL) cholesterol, elevated homocysteine, and endothelial dysfunction.[3] Patients with PCOS are more likely to be overweight, insulin resistant, and hypertensive.[4] Additionally, Christian *et al.*[5] found an increased prevalence of coronary artery calcification, a surrogate marker for CV disease (CVD), in women with PCOS compared with controls. Other investigators have documented coronary atherosclerosis and increased carotid intimal media thickness among PCOS patients.[6,7]

It has been assumed that PCOS patients are at increased risk for CVD and CV events; Dahlgren and Janson[8] predicted a sevenfold increased risk for myocardial infarction (MI) in women with PCOS. However, few studies have examined actual CV events among

women with PCOS. Pierpoint *et al.*[9] conducted a large, retrospective cohort study among women in the United Kingdom, quoting an increased risk of stroke but no difference in coronary heart disease (CHD) events. The same group did not find an increase in CVD-related deaths, despite the increased prevalence of CV risk factors in women with PCOS.[4,10]

Given the relative lack of data on CVD risk in women with PCOS, it is difficult to provide evidence-based CV management guidelines.[11] We conducted a community-based retrospective cohort study in women with PCOS in Olmsted County, Minnesota, and compared CV risk factors and incidence of CV events with those in women without PCOS.

METHODS

Setting and participants

By using the Rochester Epidemiology Project resources,[12] a unique system that links and indexes the records of virtually all medical providers in Olmsted County, investigators can electronically identify and review records for all patients who received a particular diagnosis during a defined time period. Previous studies have shown that about 89 to 96% of all care within the county is delivered at one of the participating sites, allowing for population-based studies. This study was approved by the Mayo Clinic and the Olmsted Medical Center Institutional Review Boards. The study cohort was identified between 1966 and 1988 and medical records were abstracted for events through 2005. A follow-up survey was done to update their CV health status through 2007.

Identification of PCOS Cases

Cases were defined as patients aged 18 to 40 years, residing in Olmsted County, who were diagnosed with PCOS between 1966 and 1988, using Hospital International Classification of Diseases Adapted code 02568, International Classification of Diseases 9 code 256.4, and Berkson code 027904. The keywords used were polycystic ovaries, Stein-Leventhal syndrome, and sclerocystic ovaries.

Once other causes (listed below) had been excluded, we used the current Rotterdam consensus criteria[13,14] for diagnosis. PCOS was defined as meeting two of the following three criteria: presence of oligo-ovulation or anovulation, clinical or biochemical signs of hyperandrogenism (not due to pituitary, adrenal, or tumour-related causes), and presence of polycystic ovaries by ultrasound. Chronic anovulation was defined as amenorrhoea of 3 months' duration or oligomenorrhoea (i.e. intermenstrual intervals >35 days). Excluded were women with active thyroid disease, prolactin elevation, adrenal or ovarian tumours, or late-onset 21-hydroxylase deficiency (as shown by either a basal serum 17-hydroxyprogesterone >2.0 ng/ml or an elevated one-hour adrenocorticotropic hormone stimulation test). Follow-up for more than five years without evidence for another disorder contributing to the clinical characteristics was accepted as evidence for inclusion.

Identification of women without PCOS

Ten control subjects for each case were first identified using an established computerised matching algorithm,[15] where age and calendar year during their clinic visit plus three years were matching factors. The matching scheme ensured that both groups received medical care in Olmsted County during the same time period. However, for 115 cases, none of the control subjects had available data for this study. For the other cases, one to three control subjects had available survey data.

Outcome measures

Information on CV events and deaths (due to CVD or another cause) was collected according to the following definitions.

MI, percutaneous coronary intervention, coronary artery bypass grafting

Standard epidemiological criteria were applied to assign a diagnosis of MI on the basis of cardiac pain, biomarker elevation,[16] and Minnesota Code for Electrocardiograms. Information on percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass grafting (CABG) was also collected.

Stroke and transient ischaemic attack

Strokes and transient ischaemic attacks (TIAs) were identified by criteria described previously.[17] Briefly, stroke was defined as the acute onset, over minutes to hours, of a focal neurological deficit persisting for longer than 24 hours, with or without computed tomographic or magnetic resonance imaging documentation. TIA included an episode of focal neurological symptoms with abrupt onset and rapid resolution lasting less than 24 hours. Cases were confirmed by the study neurologist (R.D.B.).

CV death

CV death was identified as the primary or secondary cause of death on the death certificates.

Risk factor identification

Hypertension and type 2 diabetes mellitus

Hypertension and type 2 diabetes mellitus (T2DM) were defined by the most current physician-identified diagnosis, prescription of medication for hypertension or T2DM, or self-report of either diagnosis on patient survey.

Body mass index

Body mass index (BMI) was calculated using the most recent weight (kilograms) documented in the medical record divided by the height (metres squared).

Framingham Risk Score

The Framingham Risk Score is the risk assessment tool that predicts a person's chance of having a heart attack in the next 10 years. This tool was designed for adults aged 20 years or older without heart disease or T2DM. We incorporated gender and most recent complete data on age, total cholesterol, HDL cholesterol, smoking status, systolic blood pressure, and use of antihypertensive medications into a risk score calculator.[18] In the absence of records on systolic blood pressure readings on all patients, we used physician diagnosis of hypertension or use of antihypertensive medications as evidence of hypertension. For hypertension, T2DM, BMI and Framingham risk score, we used the last documented measure for analysis. If multiple measures were recorded during the period of analysis, we used only the most recent measure during the study period.

Follow-up procedures

Passive

The cohort was gathered from 1966 to 1988. Retrospective chart reviews were performed through 2005.

Active

In December 2006, a survey was mailed to the last documented address for study subjects requesting updated clinical information on CV risk factors and outcomes pertinent to this study. Surveys were sent to all cases unless there was documentation that the patient had died. Surveys were sent to all control subjects for whom adequate documentation through to at least the year 2000 was not available. Survey recipients received a follow-up phone call if the survey had not been returned within three months.

Quality control

All outcomes of interest were identified using standardised methods.[16] Furthermore, previous work from our centre indicated that this case-finding approach yielded results similar to those of a cohort approach, confirming the robustness of our method of ascertainment.[19]

Statistical method

Demographic and cardiovascular disease risk factor data were summarised using standard descriptive measures. For continuous variables, two-sample t tests or Wilcoxon rank-sum tests were used to compare cases and controls. For categorical variables, χ^2 tests or Fisher's exact tests were used to compare the two groups. Overall survival and CVD event-free survival were compared using log-rank tests. Multivariate Cox proportional hazards analyses were used to assess the effects of potential confounders in the survival models. Assessed confounders included age; BMI; smoking status; presence of T2DM, hypertension or hyperlipidaemia; and family history of CVD, hypertension or T2DM. Due to low event rates, especially in women without PCOS, p values were derived from the likelihood ratio test.[20] Our sample size provided 80% power at α=.05 to detect a hazard ratio of 1.2. Thus, 287 patients were needed per group. Although a smaller effect might have been missed, the study was powered to detect effects of clinical and public health importance.[21]

RESULTS

We identified 400 potential PCOS cases diagnosed between 1966 and 1988 (*figure 1*). Ninety were excluded; 44 did not meet the Rotterdam consensus criteria; 39 were not residents of Olmsted County; and seven did not provide research authorisation. One patient was lost to follow-up. The resultant study cohort numbered 309 cases. We identified 343 women without PCOS to serve as controls. Following data abstraction, 426 surveys were sent to 298 cases with 149 returned (50%) and 128 controls with 27 returned (21%). Thus, data on CV risk factors and events were updated at least through the year 2000 from data abstraction, survey results, or a combination of both.

The mean follow-up time for both groups was 23.7 years. The mean (SD) age at diagnosis for women with PCOS was 25.0 (5.3) years (*table 1*). Mean age at the end of study was 46.7 years.

Women with PCOS weighed more than women without PCOS (74.4 kg *vs* 71.2 kg; p=.05) and had a higher BMI (29.4 kg/m^2 *vs* 28.3 kg/m^2; p=.01). More women with PCOS were obese compared with women without PCOS (36 *vs* 30%, with BMI >30 kg/m^2; p=.19) (*table 2*), but this difference was not statistically significant. We found no significant differences in total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, or fasting blood

Figure 1. *Flow chart of cases and controls. PCOS indicates polycystic ovary syndrome.*

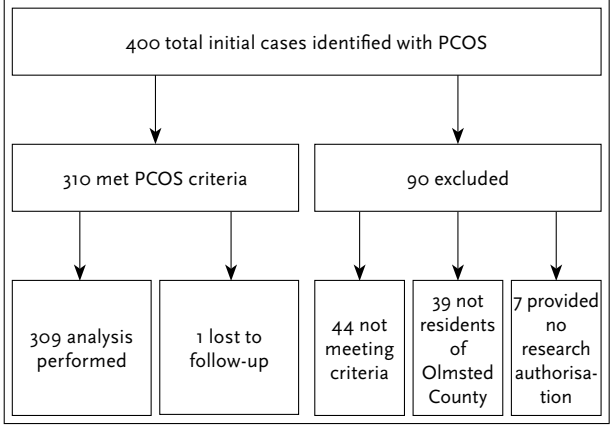


Table 1. *Baseline characteristics of patients with and without PCOS*

Variable	No. used	Overall (n=652)	No. used	No PCOS (n=343)	No. used	PCOS (n=309)	p value
Age at diagnosis, mean (SD), y	309	25.0 (5.3)	...	...	309	25.0 (5.3)	...
Age at last FU, mean (SD), y	652	46.7 (11.8)	343	48.8 (10.2)	309	44.4 (12.9)	<.001[a]
Length of FU, mean (SD), y	652	23.7 (13.7)	343	23.7 (12.3)	309	23.7 (22.7)	.98[a]
Weight, median (range), kg	525	72.5 (37-184)	294	71.2 (43-184)	231	74.4 (37-179)	.05[b]
Height, median (range), cm	525	164.0 (123-249)	294	164.0 (132-183)	231	164.0 (123-249)	.66[b]
No. of pregnancies, median (range)	499	2 (0-10)	295	2 (0-6)	204	2 (0-10)	<.001[b]
No. of births, median (range)	486	2 (0-7)	291	2 (0-6)	195	2 (0-7)	<.001[b]
Infertility treatment, no. (%)	652	91 (14)	343	10 (3)	309	81 (26)	<.001[c]

PCOS = polycystic ovary syndrome; [a]two-sample *t* test; [b]Wilcoxon rank sum test; [c]Fisher's exact test.

Table 2. *Distribution of cardiovascular disease risk factors in patients with and without PCOS*

Variable	No. used	Overall (n=652)	No. used	No PCOS (n=343)	No. used	PCOS (n=309)	p value
TC, median (range), mg/dl	482	198 (0-369)	286	199 (0-369)	196	197 (92-330)	.82[a]
HDL, median (range), mg/dl	464	58 (23-129)	283	59 (30-129)	181	57 (23-106)	.16[a]
TG, median (range), mg/dl	478	109 (29-473)	285	110 (33-431)	193	107 (29-473)	.75[a]
LDL, median (range), mg/dl	442	110 (26-225)	278	111 (38-211)	164	108 (26-225)	.74[a]
Fasting blood glucose, median (range), mg/dl	455	94 (39-338)	276	94 (68-208)	179	94 (39-338)	.51[a]
History of diabetes	652	11 (1.7)	343	9 (2.6)	309	2 (0.7)	.07[b]
Framingham risk score, median (range)	464	4 (−9-19)	283	4 (−4-17)	181	4 (−9-19)	.76[a]
BMI, mean (SD)	519	28.8 (7.61)	291	28.3 (7.47)	228	29.4 (7.77)	.01[c]
BMI categories	519		291		228		.19[b]
≤30 kg/m²		351 (68)		204 (70)		147 (64)	
>30 kg/m²		168 (32)		87 (30)		81 (36)	
Oestrogen/progesterone treatment	652	451 (69)	343	200 (58)	309	251 (81)	<.001[b]
Duration of oestrogen /progesterone treatment	440		197		243		.28[d]
0-1 y		115 (26)		43 (22)		72 (30)	
1-3 y		93 (21)		45 (23)		48 (20)	
3-5 y		66 (15)		33 (17)		33 (14)	
>5 y		166 (38)		76 (39)		90 (37)	
Hypertension	652	133 (20)	343	73 (21)	309	80 (26)	.20[b]
Antihypertensive treatment	652	130 (20)	343	72 (21)	309	73 (24)	.45[b]
Smoking	547		312		235		.67[d]
Never		314 (57)		182 (58)		132 (56)	
Ex-smoker		153 (28)		88 (28)		65 (28)	
Current smoker		80 (15)		42 (13)		38 (16)	
Postmenopausal hormonal treatment	652		343		309		.01[d]
None		502 (77)		249 (73)		253 (82)	
Current user		71 (11)		47 (14)		24 (8)	
Past user		79 (12)		47 (14)		32 (10)	
Family history of heart disease	542	444 (82)	302	249 (82)	240	195 (81)	.74[b]
Family history of hypertension	541	399 (74)	300	209 (70)	241	190 (79)	.02[b]
Family history of diabetes	532	297 (56)	300	155 (52)	232	142 (61)	.03[b]

The data are presented as numbers (percentages) unless otherwise stated. BMI = body mass index; HDL = high density lipoprotein; PCOS = polycystic ovary syndrome; TC = total cholesterol; TG = triglycerides; [a]Wilcoxon rank sum test; [b]Fisher's exact test; [c]Two-sample *t* test; [d]Pearson χ^2 p value.

glucose levels or the presence of T2DM and hypertension between women with and without PCOS. Parameters of insulin resistance were not available. Women with PCOS were more likely than those without PCOS to have a family history of hypertension (79 vs 70%; p=.02) and T2DM (61 vs 52%; p=.03). Use of statins, antihypertensive medications, and anti-T2DM medications was similar, as was smoking status. The Framingham coronary disease risk scores were similar for both groups (4 and 4; p=.76). The only significantly different variables were BMI, family history of hypertension, family history of T2DM, oestrogen use, and postmenopausal hormone therapy.

There was no increased risk of CV events in the women with PCOS as defined by MI (adjusted hazard ratio [HR] 0.74; 95% confidence interval [CI] 0.32 to 1.72; p=.48); CABG (adjusted HR 1.52; 95% CI 0.42 to 5.48; p=.52); overall deaths (adjusted HR 1.03; 95% CI 0.29 to 3.71; p=.96); and death due to CVD (adjusted HR 5.67; 95% CI 0.51 to 63.7; p=.16). Incidence of stroke too was not different (adjusted HR 1.05; 95% CI 0.28 to 3.92; p=.94) (*table 3*). After adjusting for age, BMI, infertility, and history of hypertension no significant difference in CV events remained. All CV events were identified from the medical index database, and when compared with survey results, no additional CV events were identified.

DISCUSSION

In our retrospective cohort study of 309 women with PCOS and 343 women without PCOS, we found few differences in CV risk factors and no overall difference in CV events (MI, unstable angina, stroke, TIA, CABG, or PTCA or death due to CVD) during a mean follow-up of 23 years (through the ages of 45 to 50 years). Relative to women without PCOS, women with PCOS had a higher BMI, but were not significantly different in total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, or fasting blood glucose measurements. We are unable to comment on the prevalence of insulin resistance in the cohort because parameters for insulin resistance were not routinely measured as part of clinical practice. There was no significant difference in the prevalence of T2DM and hypertension between the two groups, although women with PCOS were more likely than those without PCOS to have a family history of T2DM and hypertension.

Our findings challenge conclusions of previous studies. For example, the sevenfold increased risk of CHD suggested by Dahlgren and Janson[8] on the basis of calculations from their model may represent an overestimation of CHD risk among women with PCOS. Review of that study must take into consideration the limitations imposed by the small cohort of only 34 women with PCOS and 132 controls. Actual CV events were rare (one MI in each group, stroke in two women with PCOS and three without PCOS) and were not statistically significant. Yet, when CHD risk factors, triglycerides, waist-to-hip ratio, T2DM, and hypertension were applied to a risk factor assessment model, the estimated risk ratio for MI was 4.2 in women with PCOS aged 40 to 49 years and 11 for those aged 50 to 61 years. This risk factor model remains unvalidated and does not include the many other recognised risk factors for CHD. Cases relied on ovarian biopsies for definition, likely including women with cystic ovaries but not PCOS and excluding women who had PCOS but did not have ovarian biopsy.

In contrast, our study is a population-based study with consistent and validated case-finding criteria based on International Classification of Diseases and Hospital International Classification of Diseases Adapted coding, clear definitions for women with PCOS, event verification by the study's principal investigator (S.I.) and endocrinologist (M.L.C.-C.), use of a validated risk

Table 3. *Cardiovascular events, overall deaths, and deaths due to CVD*

Events	Total No. (%) (n=652)	PCOS No. (%) (n=309)	No PCOS No. (%) (n=343)	Hazard ratio (95% CI)	p value[a]	Adjusted hazard ratio[b] (95% CI)	p value[a]
Myocardial infarction	31 (4.8)	15 (4.9)	16 (4.7)	0.82 (0.39-1.69)	.58	0.74 (0.32-1.72)	.48
Unstable angina	16 (2.5)	10 (3.2)	6 (1.8)	1.44 (0.51-4.07)	.49	1.32 (0.42-4.13)	.63
Stroke	13 (2.0)	6 (1.9)	7 (2.0)	0.75 (0.25-2.27)	.61	1.05 (0.28-3.92)	.94
CABG	12 (1.8)	7 (2.3)	5 (1.5)	0.99 (0.31-3.19)	.99	1.52 (0.42-5.48)	.52
At least 1 CV event	54 (8.3)	26 (8.4)	28 (8.2)	0.87 (0.51-1.50)	.62	0.82 (0.44-1.54)	.54
Overall deaths	19 (2.9)	11 (3.6)	8 (2.3)	1.16 (0.46-2.90)	.76	1.03 (0.29-3.71)	.96
Deaths due to CVD	6 (0.9)	4 (1.3)	2 (0.6)	1.57 (0.28-8.75)	.61	5.67 (0.51-63.7)	.16

BMI = body mass index; CABG = coronary artery bypass grafting; CI = confidence interval; CV = cardiovascular; CVD = cardiovascular disease; PCOS = polycystic ovary syndrome; [a]*P* values came from the likelihood ratio test; [b]Adjusted for age at last follow-up, BMI, infertility treatment, postmenopausal hormone therapy, and family history of hypertension.

assessment tool (the Framingham Risk Assessment Tool), and a long follow-up period.

Our findings agree with those of a previous retrospective study by Pierpoint *et al.*,[9] who reported prevalence of CHD risk factors and CHD mortality in 760 women followed for more than 30 years in a predominantly white population in the United Kingdom. Based on review of death records, they reported an increased prevalence of CV risk factors, including T2DM and insulin resistance, but no excess risk of CHD mortality. Our study population was similar (predominantly white); we believe that the strict requirement for PCOS diagnosis (based on laparotomy, laparoscopy, and wedge resection) used in their study may have excluded the less severe cases.

Using the same cohort in the United Kingdom, Wild *et al.*[10,22] conducted a follow-up study on 319 PCOS cases looking for cardiac events and reported an increased risk of stroke but no difference in coronary events. This study was limited by a large dropout rate, reporting on less than 50% of the study subjects, which may limit conclusions.

We found no difference in the use of antihypertensive or anti-T2DM agents or statins between the two groups, suggesting that the women with PCOS were not more diligently managed compared with women without PCOS, despite elevated BMI. We cannot address the possibility that women with PCOS received and followed lifestyle change recommendations (exercise and diet) more aggressively potentially impacting insulin resistance and the prevalence of CV risk factors.

Compared with women without PCOS, women with PCOS used hormone preparations more frequently before menopause but less frequently after menopause. The possibility that these differences in hormone therapy could have provided some protective benefit to women with PCOS is interesting but impossible to conclude from our study.

Another consideration for the findings observed in our study is that PCOS may actually offer some protective benefits that lower the risk of CV events in an unrecognised way. This was also suggested by Wild *et al.*[10] and Pierpoint *et al.*[9] Additionally, the majority of the women in this study have not yet reached ages at which CV events are common. Therefore, we cannot rule out the possibility that the incidence of CV events will increase in women with PCOS at older ages. However, our data do suggest that women with PCOS are not at an increased risk of CV events compared with the general population, at least through midlife. These findings are consistent with the recent Androgen Excess and PCOS Society statement[23] and the Dallas Heart Study (a cross-sectional analysis of a US obese PCOS cohort in the Dallas Heart Study),[24] which reported no difference in subclinical markers of coronary artery disease or abdominal atherosclerosis between PCOS patients and controls, thus supporting our findings.

Limitations and strengths

The primary limitation of this study is the retrospective design. However, this allowed us to follow individuals over an extended time (nearly 24 years). Additionally, for individuals who were lost to follow-up, we attempted contact via a survey to ascertain their outcomes. The survey response rates differed between cases and controls. However, updated medical information was available from the medical records for more than 63% of controls, with an additional 21% updated from the survey, thus yielding updated data on 84% of the control subjects, similar to the rate for the case patients. No additional CV events were discovered, but we must acknowledge potential bias introduced by unrecognised differences between responders and nonresponders. The likelihood of this is low, as Rochester Epidemiology Project records are updated at least with death data even if the subject moved away.

Additionally, as the calculated Framingham Risk scores did not differ between women with and without PCOS, our data also suggest that ten-year risks of CV events will be similar in both groups. Finally, based on the 1990 census, until recently the population of Rochester has been predominantly white, hence generalisability may be limited to white women aged between 18 and 50 years.

Strengths included a population-based cohort study in a defined geographic area, limiting the biases that can occur in studying referral populations. Additionally, we were able to take advantage of the extensive patient information available through the Rochester Epidemiology Project and supplemented these data with active follow-up of study participants who had relocated from Olmsted County, Minnesota. This retrospective cohort study design also represented the best and most feasible method to answer our study question. A prospective study is conceivable but would take decades and substantial funding to complete. Additionally, using the survey to update our data mitigated the bias from subjects being lost to follow-up and gave us information on the recent health status of the cohort.

Conclusions

In this community-based cohort, women with PCOS were significantly more likely than controls to be overweight. However, there were no statistically significant differences in other CV risk factors between cases and controls, including total cholesterol, HDL cholesterol, triglyceride, LDL cholesterol, and fasting blood glucose levels or the presence of T2DM and hypertension. This result contradicts those of other studies, which have reported an increased prevalence of T2DM, dyslipidaemia, and hypertension in women with PCOS. Furthermore, there was no observed increase in CV events in this cohort through midlife. Prospective community-based studies are needed to confirm this lower-than-anticipated prevalence of

CV risk factors and events and to determine whether this prevalence persists in later decades of life.

ACKNOWLEDGMENTS

We thank the Survey Research Centre for help with formatting, collection, and compilation of the surveys and Kathryn Trana for help with manuscript preparation.
Grant support: This study was funded by Solvay Pharmaceuticals through Women's Health Fellowship (S.I.) funds and was made possible by the Rochester Epidemiology Project (grant AR30582 from the National Institute of Arthritis and Musculoskeletal and Skin Diseases).

REFERENCES

1. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol. 1935;29:181-91.
2. Broekmans FJ, Knauff EA, Valkenburg O, Laven JS, Eijkemans MJ, Fauser BC. PCOS according to the Rotterdam consensus criteria: change in prevalence among WHO-II anovulation and association with metabolic factors. BJOG. 2006 Oct;113(10):1210-7.
3. Talbott EO, Zborowski JV, Sutton-Tyrrell K, McHugh-Pemu KP, Guzick DS. Cardiovascular risk in women with polycystic ovary syndrome. Obstet Gynecol Clin North Am. 2001 Mar;28(1):111-33.
4. Wild RA. Long-term health consequences of PCOS. Hum Reprod Update. 2002 May-Jun;8(3):231-41.
5. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF 2nd, Fitzpatrick LA. Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab. 2003 Jun;88(6):2562-8.
6. Guzick DS, Talbott EO, Sutton-Tyrrell K, Herzog HC, Kuller LH, Wolfson SK Jr. Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. Am J Obstet Gynecol. 1996 Apr;174(4):1224-9.
7. Talbott EO, Guzick DS, Sutton-Tyrrell K, et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thromb Vasc Biol. 2000 Nov;20(11):2414-21.
8. Dahlgren E, Janson PO. Polycystic ovary syndrome: long-term metabolic consequences. Int J Gynaecol Obstet. 1994 Jan;44(1):3-8.
9. Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS. Mortality of women with polycystic ovary syndrome at long-term follow-up. J Clin Epidemiol. 1998 Jul;51(7):581-6.
10. Wild S, Pierpoint T, McKeigue P, Jacobs H. Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf). 2000 May;52(5):595-600.
11. American College of Obstetricians and Gynecologists. ACOG practice bulletin. Polycycstic ovary syndrome. Number 41, December 2002. Int J Gynaecol Obstet. 2003 Mar;80(3):335-48.
12. Melton LJ 3rd. History of the Rochester Epidemiology Project. Mayo Clin Proc. 1996 Mar;71(3):266-74.
13. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004 Jan;19(1):41-7.
14. Dumesic DA, Nielsen MF, Abbott DH, Eisner JR, Nair KS, Rizza RA. Insulin action during variable hyperglycemic-hyperinsulinemic infusions in hyperandrogenic anovulatory patients and healthy women. Fertil Steril. 1999 Sep;72(3):458-66.
15. Bergstralh EJ, Kosanke JL. Computerized matching of cases to controls. Technical Report Series/Section of Medical Research Statistics. Mayo Clinic. 1995 Apr; No. 56.
16. Roger VL, Jacobsen SJ, Weston SA, et al. Trends in the incidence and survival of patients with hospitalized myocardial infarction, Olmsted County, Minnesota, 1979 to 1994. Ann Intern Med. 2002 Mar 5;136(5):341-8.
17. Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Stroke incidence, prevalence, and survival: secular trends in Rochester, Minnesota, through 1989. Stroke. 1996 Mar;27(3):373-80.
18. National Cholesterol Education Program. Risk assessment tool for estimating your 10-year risk of having a heart attack. http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=pub%20 (accessed%203/23/2010). Accessed March 23, 2010.
19. Whisnant JP, Melton LJ 3rd, Davis PH, O'Fallon WM, Nishimura K, Schoenberg BS. Comparison of case ascertainment by medical record linkage and cohort follow-up to determine incidence rates for transient ischemic attacks and stroke. J Clin Epidemiol. 1990;43(8):791-7.
20. Wei LJ. The Robust inference for Cox proportional hazards. J Am Stat Assoc. 1989;84(408):1074-8.
21. Arciero TJ, Jacobsen SJ, Reeder GS, et al. Temporal trends in the incidence of coronary disease. Am J Med. 2004 Aug 15;117(4):228-33.
22. Wild S, Pierpoint T, Jacobs H, McKeigue P. Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. Hum Fertil (Camb). 2000;3(2):101-5.
23. Wild RA, Carmina E, Diamanti-Kandarakis E, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab. 2010 May;95(5):2038-2049. Epub 2010 Apr 7.
24. Chang AY, Ayers C, Minhajuddin A, et al. Polycystic ovarian syndrome and subclinical atherosclerosis among women of reproductive age in the Dallas heart study. Clin Endocrinol (Oxf). 2011 Jan;74(1):89-96.

CASE REPORT

Vasculitis revealed by posterior stroke

A.M.S. Goedhart-de Haan[1], S.JA. Pans[1], K.D.F. Lensen[2], M.R. Meijerink[3], E.F.I. Comans[4], Y.M. Smulders[2*]

[1]Institute for Medical Education, [2]Department of Internal Medicine and the Institute for Cardiovascular Research, ICaR-VU, Departments of [3]Radiology, [4]Nuclear Medicine, VU University Medical Center, Amsterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)20-4444307, fax: +31 (0)20-4444313, e-mail: y.smulders@vumc.nl

ABSTRACT

Posterior ischaemic stroke is relatively uncommon, and its occurrence should alert clinicians to possible uncommon underlying disease.
We report a patient with occipital brain infarction. The combination of age, gender, general malaise and elevated erythrocyte sedimentation rate led to the clinical suspicion of giant cell arteritis. Vertebral artery vasculitis was confirmed by 18-FDG positron emission tomography, combined with CT angiography, and immediate immunosuppressive therapy was started.
Symptoms of stroke should, in a particular clinical context, raise suspicion of giant cell arteritis.

What was known on this topic?
Posterior stroke is a known, but rare, complication of giant cell arteritis.

What does this add?
Combining clinical pattern recognition, i.e. typical signs and symptoms in a particular context, with modern imaging techniques, i.e. 18-FDG PET, can lead to early diagnosis and treatment of large artery vasculitis.

KEYWORDS

CT angiography, giant cell arteritis, positron emission tomography, vertebral artery insufficiency

INTRODUCTION

Giant cell arteritis (GCA) is a vasculitis of predominantly large- and medium-sized arteries, characterised by granulomatous inflammation in the vessel wall.[1] Symptomatic vessel inflammation usually involves cranial branches of arteries originating from the aortic arch, including the superficial temporal artery, the ophthalmic artery and the posterior ciliary arteries.[1,2] The incidence of GCA increases after the age of 50 and peaks between 70 and 80 years of age. Two thirds are women. The disease is associated with polymyalgia rheumatica.[1]
The clinical phenotype of temporal artery involvement in GCA can be quite typical and often includes unilateral headache, jaw claudication and visual loss due to ischaemic optic neuropathy. In more than half of GCA cases, however, arteries other than the temporal artery are involved.[1] In such patients, symptoms are often atypical and may involve arm claudication, signs of regional or global cerebral hypoperfusion, low-grade fever and general malaise. Stroke associated with extracranial involvement in GCA occurs in 3 to 7% of GCA patients.[3,4] Previous studies suggested that 10% of early deaths in GCA were caused by stroke.[5]
In this report we present a patient in whom GCA was revealed by stroke caused by vertebral artery inflammation. The latter was confirmed by positron emission tomography (PET) scintigraphy combined with computer tomography (CT) angiography. Although rare, the combination of cerebral ischaemia with the specific signs and symptoms should raise suspicion of GCA.

CASE PRESENTATION

A 76-year-old woman presented with a fortnight's history of disturbed vision. She also reported complaints of proximal myalgia, general malaise, and headache above the

left eye for two to three months. Neurological examination revealed globally decreased muscle strength. Visual field examination revealed loss of vision on the left side. Laboratory results showed an erythrocyte sedimentation rate (ESR) of 117 mm/h, C-reactive protein (CRP) 137 mg/l, normocytic anaemia (haemoglobin 6.2 mmol/l) and thrombocytosis (630 x 10^9/l).

Brain CT showed abnormalities in the right occipital cortex, compatible with semi-recent infarction and with the characteristics of her visual impairment. The neurologist prescribed aspirin, ended his consultation and requested the internist to analyse potential causes for her seemingly unrelated general symptoms and acute-phase response. The neurological findings, combined with other symptoms, age, gender and sharply elevated ESR, raised our suspicion of GCA causing posterior cerebral ischaemia. However, further questioning revealed no complaints of jaw claudication or scalp tenderness. Physical examination of both temporal arteries showed no abnormalities. Subsequently, an 18-fluorodeoxyglucose (FDG) PET scan was performed which showed clearly enhanced FDG uptake in the vertebrobasillary region (*figures 1* and *2*), as well as some enhanced uptake in part of the aorta and the aortic arch. The other large arteries displayed a normal uptake pattern. CT angiography showed irregular wall thickening and stenoses along the course of both vertebral arteries (*figures 2* and *3*).

To protect the posterior circulation from further ischaemia, treatment with intravenous pulses of methylprednisolone (1 g/day for three days) was commenced, followed by rapid improvement of general complaints and no further deterioration of the neurological deficit. She was discharged on oral prednisone 60 mg/day. At follow-up, her myalgia and malaise had disappeared, and ESR and haemoglobin normalised.

Figure 1. *F6: Coronal 18F-FDG PET slice showing much more intense 18-FDG uptake in both vertebral arteries (red arrows) compared to the ascending aorta (green arrow). 18-FDG uptake in myocardium and brain is physiological*

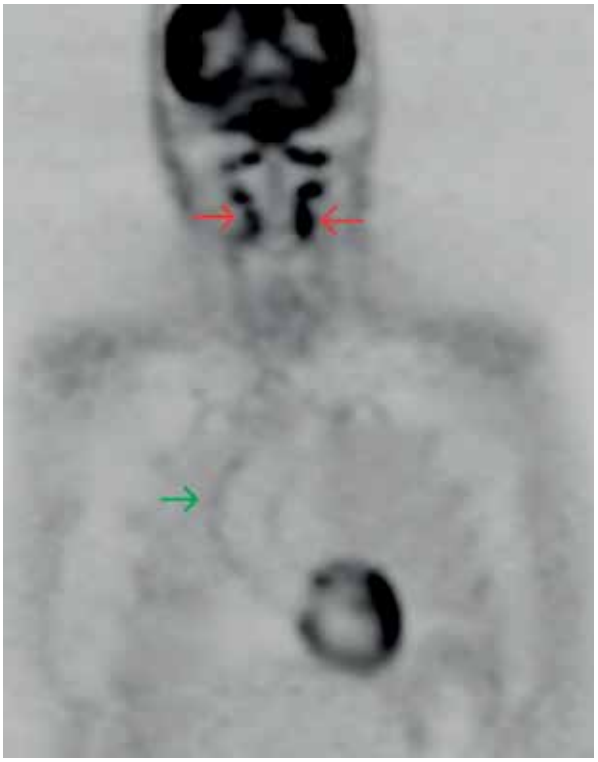


Figure 2. *Transaxial 18-FDG PET (left) showing increased 18-FDG uptake in both vertebral arteries (red arrowheads) with a more intense uptake in the right artery. At the same level CT-angiography (right) shows luminal stenosis and vessel wall thickening (red arrowhead)*

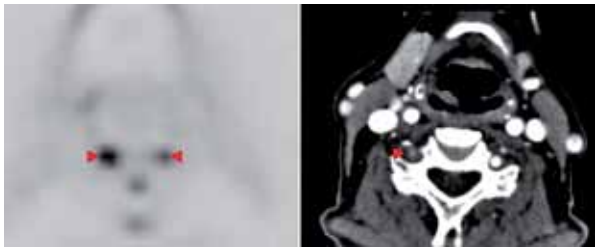
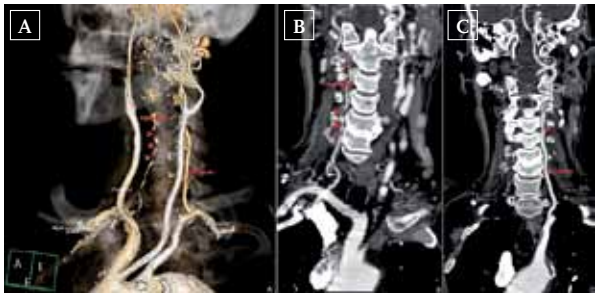


Figure 3. *A: 3D VRT (Volume Rendering Technique)-reconstruction of CT-angiography showing multiple stenosis (red arrowheads) along the course of the right vertebral artery. B and C: oblique-coronal views of curved MPR's (multiplanar reformatting) showing multiple stenoses (red arrowheads) along the course of the right vertebral artery (B) and a mild stenosis (red arrowhead) of the left vertebral artery (C)*



DISCUSSION

The central message of this case report is that pattern recognition of GCA symptoms is important when cerebral ischaemia presents in a non-typical fashion, or when other symptoms are present. Also, 18-FDG-PET images of

vertebral involvement in GCA have appeared only rarely in the medical literature.[6]

Vertebral artery involvement in GCA is usually limited to the extracranial sections, except for the first 5 mm after passing the dura mater.[7] Arteritis of the vertebrobasilar system may result in the full spectrum of associated posterior cerebral ischaemic symptoms and signs.[4] Symptomatic bilateral vertebral artery involvement in GCA is found in only one to two of 1000 patients with ischaemic stroke.[7]

Why did we not perform temporal artery biopsy to support the diagnosis of GCA? Our motivation was that such biopsies, although specific, lack sensitivity. Even if typical signs of temporal artery involvement are present, sensitivity for an abnormal biopsy ranges from 66 to 90%, depending on which signs are present (highest when headache, jaw claudication and scalp tenderness are all present).[8] Limited sensitivity is explained by segmental involvement of the vessel wall.[1] In patients with GCA not associated with specific signs of temporal artery involvement, such as our case, sensitivity drops to less than 50%.[8,9] The generic dilemma here is whether, in the fact of the possibility of having to start long-term, high-risk treatment, one should perform an additional test which is specific, but not sensitive. Obviously, a positive specific test result is reassuring in terms of not exposing the patient to such therapy for no good reason. Admittedly, some patients (and physicians) may benefit from the additional motivation provided by a positive biopsy but, overall, this benefit may be lost by loss of impetus from a negative test result, however irrational this may seem, and however well-explained in advance to patients. In our opinion, the key issue is whether a negative result would change the therapeutic decision. If no alternative additional confirmatory tests are available, the answer to the latter question will often be 'no'. This being the case, we decided that temporal artery biopsy would not have changed diagnosis or management in our case. We do not suggest that performing a biopsy would have been wrong, but rather point to the risks of misinterpretation of a negative test. Unfortunately, we have seen several dramatic cases of GCA with a previously refuted diagnosis based on a negative temporal artery biopsy. Often, as in our case, arteries suspected to be affected by large artery GCA are inaccessible for biopsy, and the impossibility of obtaining histopathological confirmation is thus common.[9]

Large artery GCA is often diagnosed on clinical grounds, complemented with laboratory tests and imaging. The role of 18-FDG-PET in establishing the diagnosis seems essential, and calls for reconsideration of formal diagnostic criteria for GCA.[9,10]

In general, GCA is treated with oral corticosteroids.[1] There are no evidence-based guidelines for treatment of GCA associated with vertebrobasillary ischaemia. As we feared progression of posterior cerebral ischaemia, we instituted immediate high-dose immunosuppression, i.e. intravenous methylprednisolone, followed by oral prednisone.[11]

In conclusion, in patients with cerebral ischaemia, specific symptoms, combined with an elevated ESR, should raise the suspicion of GCA. Clinical judgement (i.e. pattern recognition) and modern imaging (i.e. 18-FDG-PET) go hand in hand, as they should.

REFERENCES

1. Salvarani C, Cantini F, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. Lancet. 2008;372:234-45.
2. Wilkinson IM, Russell RW. Arteries of the head and neck in giant cell arteritis. A pathological study to show the pattern of arterial involvement. Arch Neurol. 1972;27:378-91.
3. Caselli RJ, Hunder GG, Whisnant JP. Neurologic disease in biopsy-proven giant cell (temporal) arteritis. Neurology. 1988;38:352-9.
4. Gonzalez-Gay MA, Vazquez-Rodriguez TR, et al. Strokes at time of disease diagnosis in a series of 287 patients with biopsy-proven giant cell arteritis. Medicine (Baltimore). 2009;88:227-35.
5. Nordborg E, Bengtsson BA. Death rates and causes of death in 284 consecutive patients with giant cell arteritis confirmed by biopsy. BMJ. 1989;299:549-50.
6. Pfadenhauer K, Weinerth J, Hrdina C. Vertebral arteries: A target for FDG-PET imaging in giant cell arteritis? Clinical, ultrasonographic and PET study in 46 patients. Nuklearmedizin. 2010;50:1.
7. Ruegg S, Engelter S, Jeanneret C, et al. Bilateral vertebral artery occlusion resulting from giant cell arteritis: report of 3 cases and review of the literature. Medicine (Baltimore). 2003;82:1-12.
8. Younge BR, Cook BE, Jr., Bartley GB, Hodge DO, Hunder GG. Initiation of glucocorticoid therapy: before or after temporal artery biopsy? Mayo Clin Proc. 2004;79:483-91.
9. Janssen SP, Comans EH, Voskuyl AE, Wisselink W, Smulders YM. Giant cell arteritis: heterogeneity in clinical presentation and imaging results. J Vasc Surg. 2008;48:1025-31.
10. Schafer VS, Warrington KJ, Williamson EE, Kermani TA. Delayed diagnosis of biopsy-negative giant cell arteritis presenting as fever of unknown origin. J Gen Intern Med. 2009;24:532-6.
11. Sutter R, Renaud S, Bonati L, Lyrer P, Tolnay M, Wetzel S, Ruegg S, Engelter S. Bilateral vertebral giant cell arteritis--favourable outcome in two cases. J Neurol. 2008;255:133-4.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Crystalluria

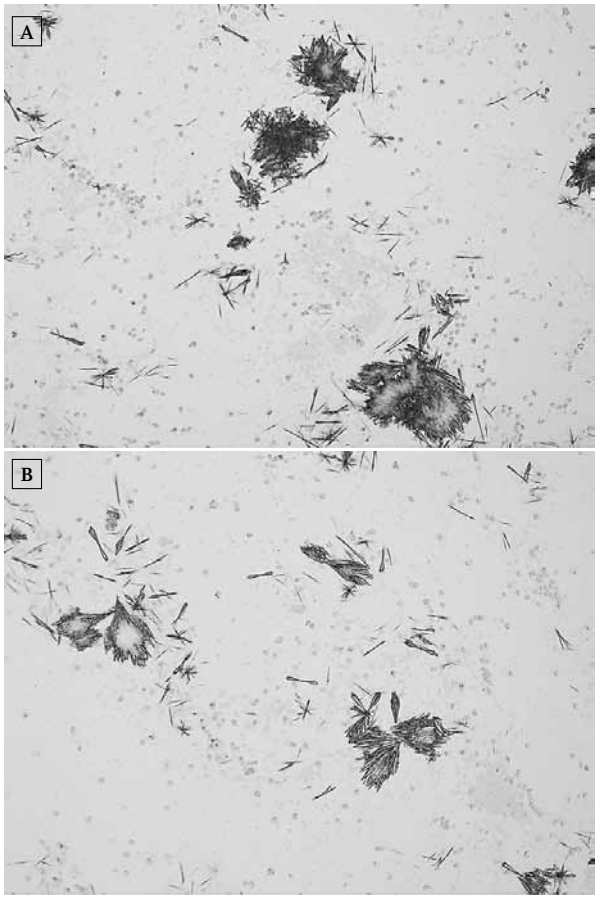
C. van Noord[1*], R.W. Wulkan[2], M.A. van den Dorpel[1]

Departments of [1]Internal Medicine, [2]Clinical Chemistry, Maasstad Hospital, Rotterdam, the Netherlands, [*]corresponding author: tel.: +031 (0)10-2912867, e-mail: NoordC@Maasstadziekenhuis.nl

CASE REPORT

A 68-year-old male was admitted to the hospital with a community-acquired pneumonia. He had a previous history of Parkinson's disease and benign prostatic hyperplasia; his medication consisted of levodopa/carbidopa (8 x 100/25 mg/day) and tamsulosin (0.4 mg/day). At admission, he had a normal renal function (GFR >90 ml/min) and a normal urine sample with a pH of 6. Treatment with intravenous amoxicillin/clavulanic acid (4 x 1000/200 mg/day) was started. During treatment the patient developed macroscopic haematuria. Repeated urine examination, three days after admission, showed the presence of erythrocytes, some leucocytes, a pH of 6 and multiple crystals (*figures 1A* and *B*) with a negative urine culture.

Figure 1 (A and B). *Crystalluria*



WHAT IS YOUR DIAGNOSIS?

See page 87 for the answer to the photo quiz.

PHOTO QUIZ

A leg with an ulcer

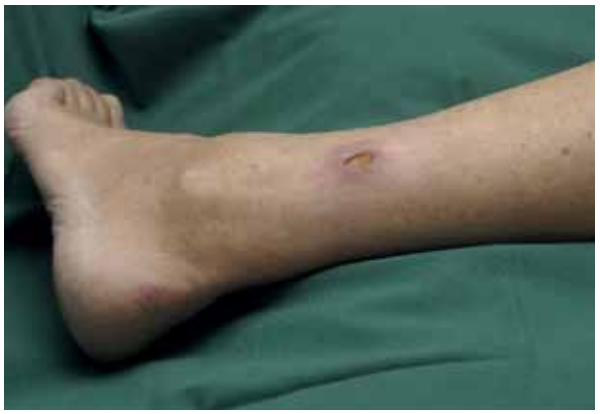
P.A.M. Kracht[1*], V. Sigurdsson[2], M. Hoogewerf[3], J.E. Arends[1]

Departments of [1]Internal Medicine and Infectious Diseases, [2]Dermatology, University Medical Center Utrecht, Utrecht, the Netherlands, [3]Department of Medical Microbiology, VU University Medical Center, Amsterdam, the Netherlands, [*]corresponding author: tel: +31 (0)6-28713875, e-mail: p.a.m.kracht@students.uu.nl

CASE REPORT

An 87-year-old man came to the Dermatology Outpatient Clinic with a painless ulcer on his left lower leg, which had slowly progressed over eight months. It started with a papule which subsequently erupted, slowly increased in size and became an ulcer. His medical history revealed a 'calcified primary complex' on a chest X-ray made in 1945 and rheumatoid arthritis (RA). For the past 2.5 years he had been treated for his RA with prednisone and methotrexate (MTX) during which the ulcer had appeared. On dermatological examination there was an undermined ulcer measuring 1.5 x 2 cm with yellow purulent exudate (*figure 1*). There were no signs of venous insufficiency and duplex ultrasound showed normal vascular function.
He was referred to the Internal Medicine Department for further analysis and treatment. He had no complaints. Physical examination revealed no abnormalities. Laboratory tests were normal besides an erythrocyte sedimentation rate (ESR) of 23 mm/h. A human immunodeficiency virus (HIV) test was negative. A chest X-ray showed linear fibrotic markings in both lungs. An MRI of the left lower leg showed a lesion ventrolateral from the distal fibula with continuation to the overlying skin and without signs of osteomyelitis.

Figure 1. *An undermined ulcer on the lateral side of the left lower leg measuring 1.5 x 2 cm with yellow purulent exudate*



WHAT IS YOUR DIAGNOSIS?

See page 89 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

An Aruban man with fever, abdominal mass and eosinophilia

G.J. Westland[1], T. Peterson[2], J.A. van Raalte[4], R.M.H.G. Huits[3*]

[1]VU University, Amsterdam, the Netherlands, Departments of [2]Surgery, [3]Internal Medicine, Dr. Horacio E. Oduber Hospitaal,[4]Department of Pathology, Landslaboratorium, [*]corresponding author: tel.: +29 7 5824749, fax: +29 7 5826163, e-mail: ralph.huits@gmail.com

CASE REPORT

A 30-year-old Aruban man with a history of diabetes presented with abdominal pain and fever, six weeks after a laparoscopic appendectomy for acute appendicitis. Ultrasound of the abdomen showed abscess formation in the right lower quadrant. A drain was percutaneously inserted into the mass and intravenous antibiotic treatment with piperacillin-tazobactam was initiated. The following day, computed tomography of the abdomen (*figure 1A*) showed a thick-walled cavity, filled with air and fluid and with unclear anatomical relation to the ascending colon. The patient did not improve: inflammatory parameters persisted and marked eosinophilia was present (7000 cells/μl). A fistula developed to the appendectomy scar. Ten days after admission a laparotomy was performed and the infiltrate, which involved the coecum and part of the ascending colon, was removed by right hemicolectomy.

Figure 1 (A and B). *A) Computed tomography showing a thick-walled infiltrate in the right lower abdomen after insertion of a drain. The anatomical relation to the ascending colon is not clear. B) Hemicolectomy specimen, opened in longitudinal direction viewing coecum and ascending colon. On the left side the terminal ileum.*

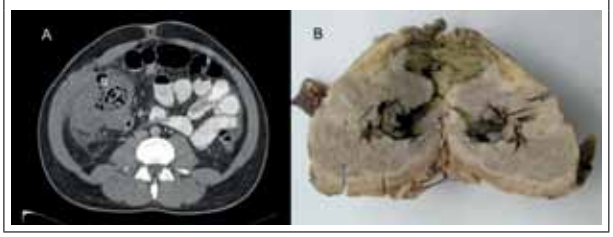
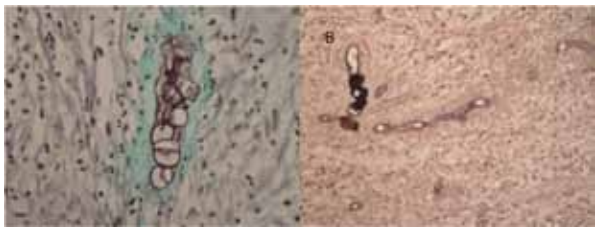


Figure 2 (A and B). *Grocott's methenamine silver stain. A) A cluster of zygospores. The surrounding eosinophilic material (Splendore-Hoeppli phenomenon) stains green. B) Spores germinating into hyphae and a branching hyphen.*



On macroscopic examination the coecum and ascending colon showed a 15 x 9 x 7 cm solid white lesion surrounding the colon (*figure 1B*). Histopathological examination showed fibrous tissue with necrosis extending towards the intestinal lumen. Using Grocott's methenamine silver stain, thin-walled, broad septate hyphae were found in this tissue. The hyphae surrounded by an eosinophilic sheath constitute the so-called Splendore-Hoeppli phenomenon. Large clusters of zygospores were also present (*figure 2A* and *2B*). In retrospect, a sporadic zygospore was present in the removed appendix.

WHAT IS YOUR DIAGNOSIS?

See page 88 for the answer to the photo quiz

ANSWER TO PHOTO QUIZ (PAGE 84)

CRYSTALLURIA

DIAGNOSIS

The crystals were different from the crystals commonly seen, such as uric acid and calcium oxalate. The crystals appeared as needles, 'shocks of wheat', 'broom bush-like' and one sea urchin-shaped crystal. These atypical shaped crystals can be seen in patients using amoxicillin or ampicillin.[1] After replacing amoxicillin/ clavulanic acid by cefuroxim, crystals were no longer present in the urine sample from five days later, nor were erythrocytes and leucocytes present. The renal function remained normal (GFR >90 ml/min) during treatment with amoxicillin/ clavulanic acid.

Several drugs can cause transient crystalluria, including sulfadiazine, amoxicillin and ciproxin. Risk factors are drug overdose, dehydration, hypoalbuminaemia (which increases the fraction of unbound drug), and low (<4) or high (>7) urine pH, due to U-shaped pH solubility curves.[1,2] Amoxicillin is excreted by the kidneys, 90% by the proximal tubules and 10% by glomerular filtration,[2] and can cause reversible asymptomatic crystalluria without renal damage, crystalluria with macroscopic haematuria or crystalluria with acute renal failure.[1] It is hypothesised that haematuria and renal failure are due to tubular damage and medullary congestion caused by intratubular precipitation of crystals; however, this has never been confirmed with renal biopsy.[1] After discontinuation, crystalluria usually disappears within 24 hours, haematuria within three days and acute renal failure in 3 to 17 days.[2]

Amoxicillin crystalluria was first described in 1985 in a 26-year-old healthy volunteer, who received an overdose of amoxicillin to investigate renal excretion. Three hours after termination of the infusion, crystals could no longer be found in the sediment. The crystalluria in this volunteer was probably due to the urinary concentration exceeding the urinary solubility of the drug in combination with a low urinary pH of 5.[2]

Recently, it was shown that crystalluria was present in 8.2% of almost 10,000 regular urine samples. In 8.1% of these samples 'typical' crystals were identified, mainly calcium oxalate and uric acid. Three out of 14 'atypical' crystals were due to use of a drug, one due to amoxicillin.[3]

In conclusion, our patient experienced reversible amoxicillin crystalluria with macroscopic haematuria without acute renal failure, possibly due to a relatively low urine pH, mild dehydration and urine retention due to benign prostatic hyperplasia. Crystalluria is a rare adverse event associated with use of amoxicillin.

REFERENCES

1. Fogazzi GB. Chapter 3. Changes of urinary sediment caused by drugs. The urinary sediment. An integrated view. Third edition. 2010:159-69.
2. Sjovall J, Westerlund D, Alvan G. Renal excretion of intravenously infused amoxycillin and ampicillin. Br J Clin Pharmacol. 1985 Feb;19(2):191-201.
3. Verdesca S, Fogazzi GB, Garigali G, Messa P, Daudon M. Crystalluria: prevalence, different types of crystals and the role of infrared spectroscopy. Clin Chem Lab Med. 2011 Mar;49(3):515-20.

ANSWER TO PHOTO QUIZ (PAGE 86)

AN ARUBAN MAN WITH FEVER, ABDOMINAL MASS AND EOSINOPHILIA

DIAGNOSIS

A diagnosis of intestinal *Basidiobolus ranarum* infection was made. Although this fungus can be cultured, the diagnosis of intestinal basidiobolomycosis is frequently established histologically by the presence of its thin-walled broad hyphae and large zygospores. These characteristic fungal elements are surrounded by eosinophilic material, the morphologically unique Splendore-Hoeppli phenomenon.[1]

Basidiobolus ranarum is a fungus of the class *Zygomycetes*, order *Entomophthorales*, which is encountered worldwide. It is frequently isolated from faecal material of amphibians and reptiles. As a human pathogen, it is known for its association with subcutaneous fat tissue infections, which may occur after traumatic inoculation.[2] Over the last decades, cases of intestinal basidiobolomycosis are reported with increasing frequency in both children and adults.[3] An association with consumption of reptile meat is postulated; in our case, the patient had eaten poached iguana.

Clinically, intestinal basidiobolomycosis often mimics inflammatory bowel disease or colon carcinoma. It rarely presents with an acute condition such as appendicitis. Unfamiliarity with this fungal infection leads to diagnostic delay and failure to include it in the differential diagnosis of fever, abdominal mass and marked eosinophilia. The outcome of intestinal basidiobolomycosis is often fatal.[4] Our patient made an uneventful recovery after surgery and treatment with ketoconazole 200 mg once daily for six weeks. He remains without relapse during 12 months of follow-up.

REFERENCES

1. Geramizadeh B, Modjalal M, Nabai S, Banani A, Forootan HR, Hooshdaran F, et al. Gastrointestinal zygomycosis: a report of three cases. Mycopathologia. 2007;164(1):35-8.
2. Gugnani HC. A review of zygomycosis due to Basidiobolus ranarum. Eur J Epidemiol. 1999; 15(10):923-9.
3. El-Shaba MH, Kamal NM. Gastrointestinal basidiobolomycosis in children: an overlooked emerging infection? J Med Microbiol. 2011;60(7):871-0.
4. van den Berk GE, Noorduyn LA, van Ketel RJ, van Leeuwen J, Bemelman WA, Prins JM. A fatal pseudo-tumour: disseminated basidiobolomycosis. BMC Infect Dis. 2006;6:140.

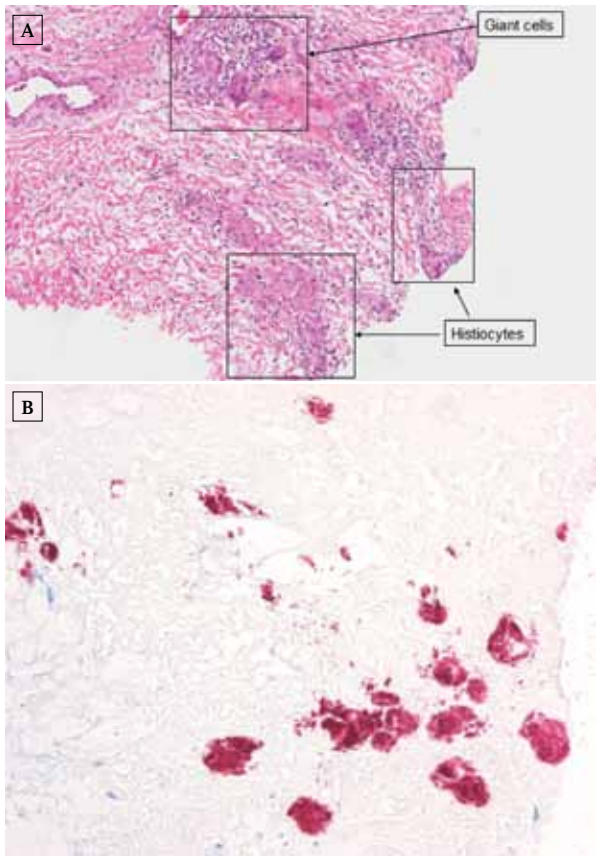
ANSWER TO PHOTO QUIZ (PAGE 85)

A LEG WITH AN ULCER

DIAGNOSIS

This report describes a rare presentation of cutaneous tuberculosis (TB) localised on the left lower leg. A skin biopsy showed granulomatous inflammation in the dermis and on Wade-Fite staining (modified Ziehl-Nielsen) many mycobacteria were seen (*figure 2*). Both nucleic acid amplification and tissue culture were positive for *Mycobacterium tuberculosis* sensitive to all antituberculous drugs. There was no active pulmonary tuberculosis. The TB infection in our patient had been latent for a period estimated to be more than 65 years. Reactivation was most likely due to the immunosuppressive therapy in combination with his increasing age. Standard quadruple therapy was initiated consisting of pyrazinamide, isoniazid, ethambutol and rifampicin daily for two months followed by four months of continuation therapy with isoniazid and rifampicin. Treatment was intensified with ethambutol and moxifloxacin for another six months due to the formation of an underlying abscess which was shown on another MRI scan. This resulted in a complete cure 15 months after start of the treatment.

Figure 2. *Biopsy from the bottom of the ulcer with (A) granulomatous inflammation with giant cells and histiocytes (200x magnification) and (B) a Wade-Fite staining (modified Ziehl-Nielsen) showing many mycobacteria (200x magnification)*



Cutaneous TB accounts for 1 to 2% of all TB cases.[1] This can occur after primary infection or, possibly after many years, by reactivation of latent infection. There are various clinical presentations such as scrofuloderma, lupus vulgaris, tuberculosis verrucosa cutis and tuberculous gumma. Our patient had a tuberculous gumma which compromises about 5% of all cases of skin TB.[2]

Tuberculosis gumma or metastatic tuberculous abscesses develop as a result of haematogenous metastasis. It can be distinguished from the resembling scrofuloderma because there is no underlying tuberculous focus such as a bone or lymph node. It mainly manifests in immunocompromised patients; however, several cases have been described in immunocompetent persons[3] whereas others have reported gummas at the site of previous sterile trauma.[4]

This cases emphasises the importance of considering cutaneous TB in patients with unexplained dermatological lesions, especially when the person is immunocompromised.

REFERENCES

1. Umapathy KC, Begum R, Ravichandran G, Rahman F, Paramasivan CN, Ramanathan VD. Comprehensive findings on clinical, bacteriological, histopathological and therapeutic aspects of cutaneous tuberculosis. Trop Med Int Health. 2006 Oct;11(10):1521-8.
2. Kumar B, Muralidhar S. Cutaneous tuberculosis: a twenty-year prospective study. Int J Tuberc Lung Dis. 1999 Jun;3(6):494-500.
3. Almagro M, Del Pozo J, Rodriguez-Lozano J, Silva JG, Yebra-Pimentel MT, Fonseca E. Metastatic tuberculous abscesses in an immunocompetent patient. Clin Exp Dermatol. 2005 May;30(3):247-9.
4. Vidal D, Barnadas M, Perez M, Coll P, Alomar A. Tuberculous gumma following venepuncture. Br J Dermatol 2001 Mar;144(3):601-3.

SPECIAL ARTICLE

SWAB/NVALT (Dutch Working Party on Antibiotic Policy and Dutch Association of Chest Physicians) Guidelines on the Management of Community-Acquired Pneumonia in Adults

W.J. Wiersinga[1*], M.J. Bonten[2], W.G. Boersma[3], R.E. Jonkers[4], R.M. Aleva[5], B.J. Kullberg[6], J.A. Schouten[7], J.E. Degener[8], R. Janknegt[9], T.J. Verheij[10], A.P.E. Sachs[10], J.M. Prins[1]

[1]Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, [2]Department of Medical Microbiology, University Medical Center, Utrecht, [3]Department of Pulmonary Diseases, Medical Center Alkmaar, Alkmaar, [4]Department of Respiratory Medicine, Academic Medical Center, Amsterdam, [5]Department of Pulmonary Diseases, Máxima Medisch Centrum, Eindhoven, [6]Nijmegen University Center for Infectious Diseases (NUCI) and Department of General Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, [7]Department of Intensive Care, Canisius Wilhelmina Hospital, Nijmegen, [8]Department of Medical Microbiology, University Medical Center, Groningen, [9]Department of Clinical Pharmacy, Orbis Medisch Centrum, Sittard-Geleen, [10]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht,
[*]corresponding author: tel.: +31 (0)20-5664380, fax: +31 (0)20-6972286, e-mail: w.j.wiersinga@amc.uva.nl

ABSTRACT

The Dutch Working Party on Antibiotic Policy (SWAB) and the Dutch Association of Chest Physicians (NVALT) convened a joint committee to develop evidence-based guidelines on the diagnosis and treatment of community-acquired pneumonia (CAP). The guidelines are intended for adult patients with CAP who present at the hospital and are treated as outpatients as well as for hospitalised patients up to 72 hours after admission. Areas covered include current patterns of epidemiology and antibiotic resistance of causative agents of CAP in the Netherlands, the possibility to predict the causative agent of CAP on the basis of clinical data at first presentation, risk factors associated with specific pathogens, the importance of the severity of disease upon presentation for choice of initial treatment, the role of rapid diagnostic tests in treatment decisions, the optimal initial empiric treatment and treatment when a specific pathogen has been identified, the timeframe in which the first dose of antibiotics should be given, optimal duration of antibiotic treatment and antibiotic switch from the intravenous to the oral route. Additional recommendations are made on the role of radiological investigations in the diagnostic work-up of patients with a clinical suspicion of CAP, on the potential benefit of adjunctive immunotherapy, and on the policy for patients with parapneumonic effusions.

KEYWORDS

Antimicrobial therapy, community-acquired pneumonia, guidelines

INTRODUCTION

Community-acquired pneumonia (CAP) is defined as an acute symptomatic infection of the lower respiratory tract which in general develops outside a hospital or nursing home, whereby a new infiltrate is demonstrated. CAP is a common condition that carries a high burden of mortality and morbidity, particularly in the elderly.[1] The estimated annual incidence of CAP in the Western world is 5 to 11 cases per 1000 adult population.[1,2] CAP is the number one cause of death due to an infection in the developed world.[1,2]

The Dutch Working Party on Antibiotic Policy (SWAB; Stichting Werkgroep Antibiotica Beleid), established by the Dutch Society for Infectious Diseases (VIZ), the Dutch Society for Medical Microbiology (NVMM) and the Dutch Society for Hospital Pharmacists (NVZA), coordinates activities in the Netherlands aimed at optimalisation of antibiotic use and containment of the development of antimicrobial resistance. SWAB and the Dutch Association of Chest Physicians (Nederlandse Vereniging van Artsen

voor Longziekten en Tuberculose, NVALT) decided to make their revisions of previously published guidelines[3,4] a combined effort, and to publish a joint guideline on the management of CAP.

The Dutch guidelines presented here describe the most relevant aspects of the antibiotic and non-antibiotic treatment of CAP. This guideline is meant for the treatment of adult patients who present at the hospital, and are treated as outpatients, as well as for hospitalised patients up to 72 hours after admission, and is in full accordance with the 2011 Dutch College of General Practitioners (NHG) practice guidelines for GPs.[5] The recommendations given are applicable to adult patients with CAP 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.

METHODS AND SYSTEMIC LITERATURE REVIEW

This guideline was drawn up according to the EBRO (Evidence Based Richtlijn-Ontwikkeling) and AGREE (Appraisal of Guidelines Research and Evaluation) recommendations for the development of guidelines.[6] A review of existing (inter)national guidelines[2-5,7-12] was performed in addition to a literature search in the PubMed database, Cochrane Register of Controlled Trials (CENTRAL), EMBASE, BMJ's Best Practice® and in Sumsearch® engine. Furthermore, InforMatrix on "Antibiotic in CAP" (Digitalis Mx bv) was used. For resistance surveillance data we utilised NethMap 2010.[13] Preparation of the guideline text was carried out by a multidisciplinary committee consisting of experts delegated from the professional societies for infectious diseases (VIZ), medical microbiology (NVMM), hospital pharmacists (NVZA), pulmonary diseases (NVALT), intensive care (NVIC) and general practice (NHG). After consultation with the members of the involved professional societies, the definitive guidelines were drawn up by the delegates and approved by the boards of SWAB and NVALT. Full guideline text and literature review are available at www.swab.nl.

CAUSATIVE BACTERIAL SPECIES OF CAP IN THE NETHERLANDS AND ANTIBIOTIC SUSCEPTIBILITY

S. pneumoniae is the most commonly isolated bacterial cause of CAP in the Netherlands and should therefore always be covered in the empirical treatment. In patients with severe CAP or in patients who must be admitted to the intensive care unit, *Legionella* spp. and *S. aureus* infection are encountered more frequently in comparison with patients with mild to moderately severe CAP (*table 1*).[2,14,15] It has to be noted that in up to 50% of CAP episodes no causative microorganism can be identified.[16-21] Infection with *Coxiella burnetii* has to be considered to be an occupational and environmental hazard in endemic areas, but after the Dutch epidemic in 2007-2010, the number of new cases now seems to have again returned to the pre-epidemic level (http://www.rivm.nl/Onderwerpen/Ziekten_Aandoeningen/Q/Q_koorts).

Regarding antibiotic susceptibility, resistance of *S. pneumoniae* is highest against ciprofloxacin (up to 37%), followed by erythromycin and clarithromycin (10%), co-trimoxazole (6-14%) and doxycycline (7-12%), which limits the use of these agents for empirical treatment of CAP. Resistance of *S. pneumoniae* against penicillins is low (1-3%), of which 50% is intermediately susceptible. Resistance to levofloxacin and moxifloxacin is very uncommon (NethMap 2010[13]). In the Netherlands, it is not recommended that penicillin-resistant *S. pneumoniae* be covered by empirical therapy, except for patients who have recently returned from a country with known high prevalence of penicillin-resistant *S. pneumoniae*. Of note, 17% of *H. influenzae* strains are resistant to the combination of amoxicillin with a beta-lactamase inhibitor.[13]

Table 1. *Most common aetiologies of community-acquired pneumonia in the Netherlands*

	Patient type		
	Community	**Hospital**	**Intensive care unit**
	1 study[99*]	7 studies[16,18-20,74,78,100]	1 study[15]
S. pneumoniae	6%	25-59%	35%
H. influenzae	9%	2-15%	11%
Legionella spp.	0%	0-8%	5%
S. aureus	0%	0-5%	7%
M. catharalis	0%	2-6%	0%
Enterobacteriaceae	-	0-4%	11%
M. pneumoniae	9%	0-24%	0%
Chlamydophila spp.	2%	1-6%	-
C. burnetii	-	0-1%	-
Viral (e.g. influenza)	37%	0-22%	-
Other	2%	3-14%	10%
No pathogen identified	33%	13-51%	34%

Data derived from most recent studies and categorised per patient type. *This study included patients with a lower respiratory tract infection in general practice, no standard X-ray was performed for the diagnosis of CAP.

GUIDANCE BY SPECIFIC SYMPTOMS AND COMORBIDITY IN THE CHOICE OF INITIAL ANTIBIOTIC THERAPY

The signs and symptoms of CAP at initial presentation should not be used to predict the cause of CAP or to guide pathogen-specific empirical antimicrobial therapy for CAP. Prognostic factors such as age, co-morbidity and specific exposure are only of modest importance for the choice of initial antibiotic treatment.[22,23] There is no convincing evidence that *H. influenzae* and *M. catarrhalis* are more common causes of CAP among patients with COPD.[22,24] Therefore, it is not recommended to cover *H. influenzae* and *M. catarrhalis* in the initial treatment of CAP in patients with COPD. An exception is bronchopneumonia, in which case it is advised to cover *H. influenzae* by empirical antibiotic therapy. CAP in patients with serious structural lung disease is more frequently caused by *P. aeruginosa* when compared with patients without an underlying lung disease.[25] In the case of aspiration, anaerobes and *Enterobacteriaceae* are more often identified.[26] Prospective studies are needed to address the question whether or not it is of clinical benefit to cover anaerobes in the case of aspiration pneumonia. In the meantime, it is recommended that in those patients anaerobes and *Enterobacteriaceae* are covered by initial antibiotic therapy. CAP caused by *S. aureus* is often preceded by influenza virus infection; however the incidence of *S. aureus* pneumonia is very low in patients with non-severe CAP. In non-severe CAP it is therefore not recommended that *S. aureus* be covered by the empiric antibiotic regimen. *Legionella* infection should be considered in patients with CAP who have recently travelled abroad.[27] Penicillin resistance of *S. pneumoniae* should be considered in patients with CAP and recent stay in countries with a high prevalence of penicillin-resistant pneumoccoci. Infection with *Coxiella burnetii* should be considered in patients with CAP living in endemic areas of *C. burnetii* infection.[28,29]

SEVERITY OF DISEASE ON PRESENTATION IMPORTANT FOR CHOICE OF INITIAL TREATMENT

Patients with CAP may be classified according to severity: mild, moderate-severe and severe CAP. Selection of empiric antibiotic therapy should be guided by the severity of the disease at presentation. Three validated scoring systems are in use: the Pneumonia Severity Index (PSI or Fine score), the CURB-65 score and the CRB-65 score (*table 2*).[30-32] PSI, CURB-65 and CRB-65 are equally reliable in predicting 30-day mortality in patients hospitalised with CAP.[33-35] Alternatively, a pragmatic classification (treatment at home, admission to a general medical ward, and admission to an intensive care unit) can be used. The committee does not recommend any of the scoring systems over the others; however, we recommend that each hospital consistently uses only one of these scoring systems in daily practice.

Table 2. *Validated scoring systems to measure the severity of disease in patients with community-acquired pneumonia: the CURB-65 and Pneumonia Severity Index*[30,31]

CURB-65

CURB-65 criteria

- **C**onfusion: defined as a new disorientation in person, place or time
- **U**rea >7 mmol/l
- **R**espiratory Rate ≥30/min
- **B**lood pressure: Systolic blood pressure <90 mmHg or diastolic blood pressure ≤60 mmHg
- Age ≥ 65

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

Pneumonia Severity Index (PSI or Fine score)

Step 1. Patient with community-acquired pneumonia

If presence of any of the following proceed to step 2, if all are absent assign to risk class I:
Over 50 years of age; altered mental status; pulse ≥125/min; respiratory rate >30/min; systolic blood pressure <90 mmHg; temperature <35°C or ≥40°C and/or a history of neoplastic disease, congestive heart failure, cerebrovascular disease, renal disease, liver disease

Step 2. Point scoring system (Characteristic and points assigned)

Age: Age in years (male); Age in years –10 (female)

Coexisting conditions: 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 + 30; glucose ≥14.0 mmol/l + 10; haematocrit <30% + 10; partial oxygen pressure <60 mmHg + 10; pleural effusion + 10

Step 3. Calculation of 30-day mortality

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%

Please visit www.jniv.nl for easy calculation tools.

RADIOLOGICAL INVESTIGATIONS IN THE DIAGNOSTIC WORK-UP OF PATIENTS SUSPECTED FOR CAP

The chest X-ray does not allow prediction of the causative microorganism in CAP.[21,36,37] In patients with a clinical suspicion of CAP the sensitivity of the initial chest X-ray compared with high-resolution computed tomography as the reference test ranges from approximately 60% in the primary care setting to 70% in hospital care settings.[38-40] However, it is not recommended that CT scanning be performed routinely in the diagnostic workup of patients with CAP. In patients with clinical features of CAP but without signs of infection on the initial chest X-ray, an additional chest X-ray within 48 hours may help to establish the diagnosis of CAP.[41]

MICROBIOLOGICAL INVESTIGATIONS AND RAPID DIAGNOSTIC TESTS

Although interpretation of Gram stains of sputum may allow early identification of the bacteriological cause of CAP, it is not recommended for guiding initial treatment. However, before starting antimicrobial therapy, blood and (if possible) sputum specimens should be obtained for culture because this can enable streamlining of antibiotic therapy once a specific pathogen has been isolated. In addition, isolating pathogens associated with CAP from blood and/or sputum allows susceptibility testing, which is important for monitoring longitudinal trends in antibiotic susceptibilities.[42] A urinary antigen test for *Legionella* spp. should be performed in all patients with severe CAP.[2,14,43,44] One should be aware that in the early stages of the disease the *Legionella* urinary antigen test may be falsely negative, especially in patients with mild pneumonia.

The pneumococcal urinary antigen test can be performed easily and quickly (<15 minutes). Reported sensitivities of this test have ranged from 65 to 92% in adult patients with definite pneumococcal pneumonia (mostly with bacteraemia), and from 27 to 74% in patients with probable pneumococcal infection (based on positive sputum results only).[45-49] In most studies the specificity of the test was determined in pneumonia caused by another pathogen and ranged around 90%.[45-49] It has to be noted that urinary pneumococcal antigens may be detectable in adult patients with exacerbations of COPD and pneumococcal carriage without pneumonia.[50] The question is whether and how to use this test in patients with (suspected) CAP. Empiric therapy for CAP should always cover pneumococci, independent of a negative or positive urinary test. On the other hand, also when the initial pneumococcal urinary antigen test is positive, one should not withhold empirical antibiotic coverage for atypical pathogens in patients with severe CAP, as the test specificity is not 100%. In the opinion of the committee, the use of the pneumococcal urinary antigen test has no direct consequences for initial antibiotic therapy in patients with non-severe CAP, but in patients with severe CAP a urinary antigen test should be performed, as a positive test – when no other pathogen is detected – can help to streamline antibiotic treatment to penicillin or amoxicillin once clinical stability (often within 48 hours) has been reached.

For the diagnosis of Q fever during the first two to three weeks after onset of illness, the preferred tests are polymerase chain reaction (PCR) on serum or plasma.[51] For the diagnosis of Q fever >3 weeks after disease onset, or when the PCR is negative, serology (emzyme-linked immunosorbent assay, immunoglobulin M, indirect immunofluorescence and CF) is the recommended test. Seroconversion or a fourfold rise in antibody titre are diagnostic of Q fever.[51] PCR results from nasopharyngeal swabs are considered the most reliable indicator for influenza virus replication in the human body.[52-54] Validated PCR tests for respiratory viruses and atypical pathogens are preferred over serological tests. Although bacterial infections are generally associated with increased expression of procalcitonin (PCT) and soluble triggering-

Table 3. *Guideline for the choice of initial therapy for community-acquired pneumonia*

Severity	Antibiotic	Route	Dose	Frequency
Mild pneumonia				
1st choice	amoxicillin	Oral	500-750 mg	q6h-q8h
2nd choice	doxycycline	Oral	100 mg (first dose 200 mg)	q24h
Moderately severe pneumonia				
1st choice	penicillin	IV	1 MU	q6h
	amoxicillin	IV	1000 mg	q6h
Severe pneumonia				
Mono-therapy	moxifloxacin *or*	IV/oral	400 mg	q24h
	levofloxacin	IV/oral	500 mg	q12h
Combination therapy	penicillin *plus*	IV	1 MU	q6h
	ciprofloxacin	IV/oral	400 mg (500 mg orally)	q12h
Combination therapy	cefuroxime *or*	IV	750-1500 mg	q8h
	ceftriaxone *or*	IV	2000 mg	q24h
	cefotaxime *plus*	IV	1000 mg	q6h
	erythromycin	IV	500-1000 mg	q6h

IV = intravenous, MU = million units; Q = every (x) hour.

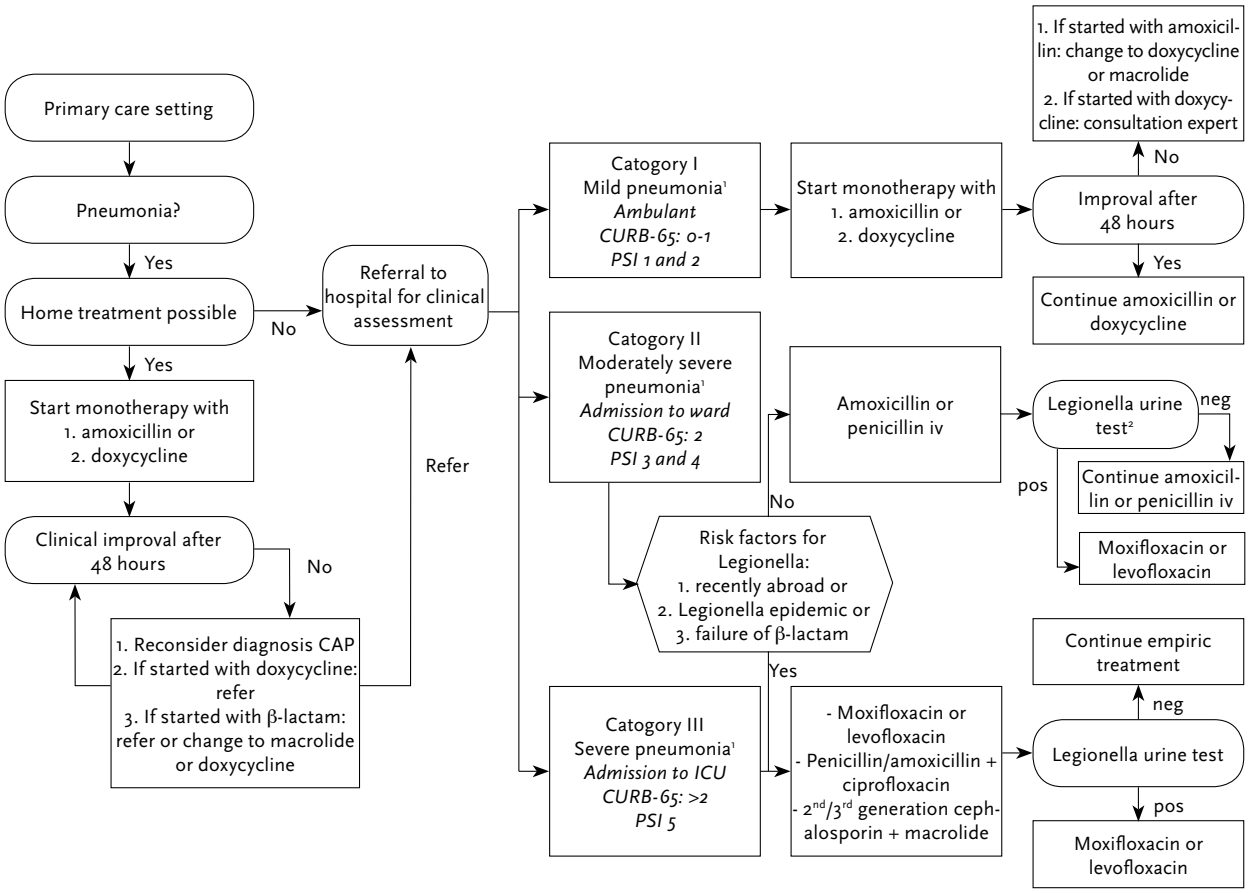
receptor-expressed-on-myeloid cells (TREM)-1, when compared with non-infectious inflammation or viral infections in the setting of CAP, their positive and negative predictive values are still ill defined and seem to be insufficient to reliably differentiate between bacterial and viral infection or to guide antibiotic therapy.[55-62]

EMPIRIC ANTIBIOTIC THERAPY FOR CAP

Risk category I (mild CAP): CURB-65: 0-1, PSI: 1-2, Pragmatic: non-hospitalised

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

Figure 1. *Flow chart of guideline recommendations on antibiotic treatment of community-acquired pneumonia*



1• Oral 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
- In the event of penicillin allergy, give a second- or third-generation cephalosporin or moxifloxacin.
- In the event of aspiration, the possibility of anaerobes or enterobacteriacae should be taken into account: penicillin is replaced by amoxicillin-clavulanate
- In the case of fulminant pneumonia after an episode of influenza, penicillin is replaced by a beta-lactam antibiotic with activity against *S. aureus*. If CAP occurs directly following an episode of influenza, the influenza should also be treated pending results from PCR testing
- Patients with documented colonisation of the respiratory tract with *Pseudomonas* spp. receive penicillin plus ceftazidime or ciprofloxacin for category II and penicillin plus ciprofloxacin for category III
- Recommended treatment options for severe CAP (monotherapy with a fourth-generation quinolone; combination therapy with penicillin (or amoxicillin) and ciprofloxacin or combination therapy with a second- or third-generation cephalosporin and a macrolide) are considered to be three equally acceptable choices
- *Legionella* pneumonia should be treated with a fluoroquinolone. Most evidence is available for levofloxacin
- For patients with CAP who recently visited a country with a high prevalence of penicillin-resistant *S. pneumoniae* (PRPS) the dose of penicillin is increased to 2 million IU 6 dd (or continuous infusion) or 2000 mg ceftriaxone once daily is given
- A urinary antigen test for *S. pneumoniae* should be performed in all patients treated as severe CAP. In patients with a positive test result and without another pathogen detected, antibiotic treatment can be streamlined to amoxicillin or penicillin once clinical stability (often within 48 hours) has been reached.

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

fall in this category. For this group, initial therapy with amoxicillin (first choice) or doxycycline (second choice) is recommended (*table 3, figure 1*). This is in accordance with the 2011 guideline for patients treated by GPs.[5] Doxycycline is not a first choice for this group in view of the 10% resistance of *S. pneumoniae* to doxycycline. The choice of a drug active against the most frequently occurring causative agent (*S. pneumoniae*) is essential in this case. Phenethicillin is not considered a first choice in view of the suboptimal gastrointestinal resorption. As a result of the increasing resistance of pneumococci to macrolides (2 to 3% in 1996 versus 10% in 2009), monotherapy with macrolides is discouraged unless there is a penicillin allergy or it is not possible to administer doxycycline (e.g. because of pregnancy or lactation). In that case, either clarithromycin or azithromycin are preferred over erythromycin, because of its gastrointestinal side effects. In pregnant women erythromycin is recommended. If there is a clinical suspicion of *Legionella* infection, then the *Legionella* urine antigen test must be carried out and empirical therapy must be adjusted. For patients in risk category I who receive amoxicillin or penicillin as initial therapy but do not improve within 48 hours, therapy should be switched to monotherapy with a macrolide or doxycycline. If therapy was initiated with doxycycline a switch to macrolides is not rational. In that case, referral to a hospital must be considered.

Risk category II (moderate-severe CAP): CURB-65: 2, PSI: 3-4, Pragmatic: hospitalised on non-ICU ward

For this category, initial therapy should be beta-lactam monotherapy, and the first choice is either intravenous penicillin or intravenous amoxicillin (*table 3, figure 1*). Doxycycline and macrolides cannot be recommended because of the increasing pneumococcal resistance. Broad-spectrum antibiotics such as amoxicillin-clavulanate, cefuroxime, ceftriaxone or cefotaxime are not recommended because the expected pathogens do not justify the broader spectrum. In case of a penicillin allergy, the best alternatives are a second- or third-generation cephalosporin or a fourth-generation quinolone. 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 of category II has one or more of the following risk factors, initial therapy should also cover *Legionella* spp.: 1) recent visit to a foreign country, 2) coming from an epidemic setting of *Legionella* spp. infections, 3) failure to improve despite ≥48 hours treatment with a beta-lactam antibiotic at adequate dosage without evidence of abnormal absorption or non-compliance.

Risk category III (severe CAP): CURB-65: >2, PSI: 5, Pragmatic: hospitalised in ICU ward

In this group, it is recommended to always cover *S. pneumoniae* and *Legionella* spp. For this purpose there are three equally acceptable choices, all with excellent antimicrobial activity against all expected causative agents (*table 3, figure 1*). On the one hand, the choice is dependent on the risk of development of antimicrobial resistance at the population level; on the other hand, the costs, the ease of administration and the profile of side effects play an important role:

- Monotherapy with a third- or fourth-generation quinolone (levofloxacin or moxifloxacin).
- Combination therapy with penicillin (or amoxicillin) and ciprofloxacin.
- Combination therapy with a second- or third-generation cephalosporin and a macrolide.

Moxifloxacin is preferred over levofloxacin because of its high activity against pneumococci, favourable pharmacodynamic characteristics and good tissue penetration. Potential prolongation of the QT interval should be taken into account. With regard to macrolides, the unfavourable pharmacodynamics and side effects of intravenous erythromycin (including prolongation of the QT interval) should be weighed against the potential of resistance development when using quinolones.

For all patients in category III, a *Legionella* urinary antigen test should be carried out as a routine procedure within 12 hours of admission. If the test is positive, monotherapy directed against *Legionella* spp. is recommended. If the test is negative, the patient is still treated further with combination therapy (coverage of both *S. pneumoniae* and *Legionella* spp.) because the sensitivity of the urinary antigen test is not 100%. A urinary antigen test for *S. pneumoniae* should be performed in all patients hospitalised with severe CAP. In patients with a positive test result and without another pathogen detected, antibiotic treatment can be streamlined to penicillin or amoxicillin once clinical stability (often within 48 hours) has been reached. Because of its low sensitivity, a negative test result does not justify broadening of empirical antibiotic therapy when no other pathogen is detected and the patient is clinically stable.

PATHOGEN-DIRECTED THERAPY

In the event of a culture-proven causative agent, pathogen-directed antibiotic treatment is to be preferred at all times (*table 4*). *Legionella* pneumonia should be treated with a fluoroquinolone. Although *in-vitro* activity of moxifloxacin is comparable with that of levofloxacin, levofloxacin has the most clinical evidence to support its use. In the case

Table 4. *Pathogen-directed therapy in community-acquired pneumonia*

Pathogen		Oral	Intravenous
S. pneumoniae	Penicillin susceptible	1 Amoxicillin 2 Phenethicillin 3 Macrolide or doxycycline[1]	1 Penicillin G 2 Amoxicillin 3 2nd or 3rd generation cephalosporin or 3rd or 4th generation quinolone[1]
	Penicillin resistance (MIC ≥2 µg/ml): agents chosen on basis of susceptibility, including cefotaxime, ceftriaxone, fluoroquinolone, vancomycin, linezolid, high-dose amoxicillin.		
H. influenzae	Non-β-lactamase producing	1 Amoxicillin 2 Macrolide or doxycycline[1]	1 Amoxicillin 2 2nd of 3rd generation cephalosporin [1]
	β-lactamase producing	1 Amoxicillin-clavulanate 2 Doxycycline or macrolide [1]	1 Amoxicillin-clavulanate 2 2nd of 3rd generation cephalosporin[1]
Legionella spp.		1 Fluoroquinolone 2 Azithromycin or clarithromycin 3 Doxycycline	1 Fluoroquinolone 2 Erythromycin
M. pneumoniae *C. psittaci* *C. pneumoniae*		1 Macrolide 2 Doxycycline	1 Macrolide 2 Doxycycline
C. burneti		1 Doxycycline 2 Ciprofloxacin	1 Doxycycline 2 Ciprofloxacin
S. aureus	Methicillin susceptible	1 Flucloxacillin 2 Amoxicillin-clavulanate 3 1st generation cephalosporin	1 Flucloxacillin 2 Amoxicillin-clavulanate 3 1st generation cephalosporin 4. Vancomycin[1] ± aminoglycoside or rifampicin
	Methicillin resistant (MRSA)	1 Vancomycin 2 Linezolid	1 Vancomycin 2 Linezolid 3 Teicoplanin ± rifampicin
P. aeruginosa		1 Ciprofloxacin	1 Ceftazidime ± aminoglycoside 2 Ciprofloxacin
K. pneumoniae		1 Amoxicillin-clavulanate 2 Trimethoprim/sulfamethoxazole	1 Amoxicillin-clavulanate 2 2nd or 3rd generation cephalosporin 3 Trimethoprim/sulfamethox
Anaerobic bacteria [2]		1 Amoxicillin-clavulanate 2 Clindamycin 3 Metronidazole	1 Amoxicillin-clavulanate 2 Clindamycin 3 Metronidazole

These recommendations are based on NethMap 2010, the Infectious Diseases Society of America, British Thoracic Society and Dutch Association of Chest Physicians (NVALT) guidelines.[2,4,14] [1]In the event of penicillin allergy; [2]Usually polymicrobial.

of *Legionella* pneumonia, there is no convincing clinical evidence for added value of adding rifampin to treatment with quinolones.[63,64]

TIMING OF FIRST DOSE OF ANTIBIOTICS, TREATMENT DURATION AND SWITCH FROM INTRAVENOUS TO ORAL ROUTE

Available literature is not convincing that prompt administration of antibiotics as soon as the diagnosis of CAP is confirmed is associated with improved clinical outcome.[65-70] For patients with severe CAP admitted through the emergency department (ED), the first antibiotic dose should be administered within four hours of presentation and preferably while still in the ED. In patients with severe sepsis and septic shock, the recommendation of the SWAB Sepsis guideline applies.[71] Although the guidelines emphasise the importance of initiating antibiotic treatment rapidly, maximal efforts should be made to avoid inaccurate diagnosis of CAP and/or inappropriate utilisation of antibiotics.

If adult patients with mild to moderate-severe CAP are treated with a beta-lactam antibiotic or fluoroquinolones, the length of antibiotic treatment can be shortened to five days in those patients who have substantially improved after three days of treatment.[72-74] As there have been no studies on the optimal duration of treatment for CAP with doxycycline, we recommend continuing seven days of treatment in these cases. Pneumonia caused by *S. aureus* should be treated for at least 14 days.[2] Pneumonia caused by *M. pneumoniae* or *Chlamydophila* spp. is generally advised to be treated for 14 days.[2] For *Legionella* pneumonia a treatment duration of seven to ten days is sufficient in

patients with a good clinical response. Of interest, two recent studies have shown that PCT measurements can be used to shorten the duration of antibiotic therapy in patients with CAP.[75,76] However, in both studies the mean duration of antibiotic therapy in the control arm was much longer (10.7 to 12 days) when compared with the standard duration of therapy as advised by this guideline (five days), therefore measurement of PCT levels to guide duration of antibiotic therapy is not recommended when standard treatment duration is limited to five to seven days.

Patients should be switched from intravenous to oral therapy when they have substantially improved clinically, have adequate oral intake and gastrointestinal absorption and are haemodynamically stable.[77-79] For patients who fulfil these criteria, inpatient observation is no longer necessary.[2,80]

THE ROLE OF ADJUNCTIVE IMMUNOTHERAPY FOR PATIENTS WITH CAP

Over the last decade a whole range of potential immunomodulating therapies as adjunctive to antibiotics have been investigated in patients with CAP. Dexamethasone as an adjunctive treatment was reported to reduce length of stay in patients with CAP, but reports are not consistent that corticosteroid therapy improved outcome in patients hospitalised with CAP.[18,81] As corticosteroid therapy is associated with – among other things – increased risk of hyperglycaemia, corticosteroids are not recommended as adjunctive therapy for the treatment of CAP. Targeting the coagulation system by administration of recombinant human tissue factor pathway inhibitor or adding granulocyte-colony-stimulating factor does not reduce mortality in patients with CAP.[82,83]

RECOMMENDED POLICY IN PATIENTS WITH PARAPNEUMONIC EFFUSION

Parapneumonic effusion (PPE) is defined as any pleural effusion associated with pneumonia. Parapneumonic effusion associated with loculations with or without pus and thickening of the pleura is called loculated parapneumonic effusion (complicated parapneumonic effusion). Empyema is defined as any pleural effusion with pus or micro-organisms in Gram stain or culture. In about 50% of cases empyema is caused by bacterial pneumonia. About half of the strains cultured from empyema are streptococci of the *S. intermedius ('milleri')* group and *S. pneumonia,* 20% are anaerobic pathogens and in 8% *S. aureus* is cultured.[84] Mortality of CAP increases if pleural effusion is present.[85] In patients with PPE with a significant quantity of pleural fluid, thoracocentesis should be performed to determine the pH and to send a sample for Gram stain and culture. Drainage of the pleural space is indicated in the presence of pus or PPE with a pH 7.2.[86] For patients in whom a loculated PPE is suspected, ultrasonography or chest CT should be performed.[87,88] In general intravenously administered antibiotics penetrate well in the pleural cavity[89,90] and installation of antibiotics into the pleural cavity is not recommended. Fibrinolytic therapy can be beneficial in selected cases of patients with loculated PPE and empyema, especially if the pleural fluid is not viscous, and fibrinolytic therapy is administered within 24 hours after admission.[91-94] Intrapleural fibrinolytic therapy does not reduce mortality in PPE and empyema, and does not improve the long-term functional or radiographical outcome.[92,95-97] When given, intrapleural fibrinolytic therapy should preferably be administered within 24 hours of admission. The most frequently used dosage regimen for intrapleural fibrinolytic therapy is streptokinase 250,000 IU or urokinase 100,000 IU once daily for three days. The chest tube should be clamped for two to four hours after administering the fibrinolytic agent. Surgical intervention should be considered as soon as it is clear that conservative treatment has failed, preferably within three days.

QUALITY INDICATORS FOR ANTIBIOTIC THERAPY IN CAP

Quality indicators must comply with high quality standards. Optimally, they should measure the quality in a valid and reliable manner with little inter- and intra-observer variability so that they are suitable for comparison between professionals, practices, and institutions. However, it should be emphasised that many current quality indicators are constructed based on relatively weak evidence and rather represent present best practices for CAP.[98] Reasonable process quality indicators for empirical antibiotic therapy in patients with CAP include the following: 1) rapid initiation of antibiotic therapy, 2) choosing an empirical antibiotic regimen according to national guidelines, 3) adapting dose and dose interval of antibiotics to renal function, 4) switching from iv to oral therapy, according to existing criteria and when clinically stable, 5) changing broad spectrum empirical into pathogen-directed therapy (streamlining therapy), 6) taking two sets of blood samples for culture, 7) using a validated scoring system (e.g. PSI score or CURB-65 score) to assess severity of illness, 8) urine antigen testing against *Legionella* spp. upon clinical suspicion and/or in severely ill patients. It should be emphasised here that these process quality indicators can be used as internal indicators in local quality improvement projects. It is not recommended

What's new: Top 5 changes in recommendations since the 2005 guidelines were published

- Concerns regarding increased antimicrobial resistance have grown in recent years. Notably, the resistance of *S. pneumoniae* to macrolides (10%) and doxycycline (7 to 11.5%) has increased, which limits the use of these agents for empirical treatment of CAP
- A urinary antigen test for *S. pneumoniae* should be performed in all patients hospitalised with severe CAP. In patients with a positive test result and without another pathogen detected, antibiotic treatment can be streamlined to penicillin or amoxicillin once clinical stability (often within 48 hours) has been reached. Because of its low sensitivity, a negative test result does not justify broadening of empirical antibiotic therapy when no other pathogen is detected and the patient is clinically stable
- If adult patients with mild to moderate-severe CAP are treated with a β-lactam antibiotic or fluoroquinolone, the length of antibiotic treatment can be shortened to five days in those patients who improve substantially after three days of treatment. Procalcitonin (PCT) measurements are useful for shortening the duration of antibiotic therapy in patients with CAP who are treated for ten days or more. It is not recommended to use the PCT test to tailor the duration of antibiotic therapy in patients with CAP when standard treatment duration is limited to five to seven days
- During annual epidemics of influenza, which usually occur from late fall to early spring in the Netherlands, infection with this virus should be considered in patients presenting with CAP. PCR results from nasopharyngeal swabs are considered the most reliable indicator for influenza virus replication in the human body. Antiviral treatment is recommended for patients with confirmed or suspected influenza who have complicated illness, for instance pneumonia. Oseltamivir is the recommended antiviral medication of choice as recent viral surveillance and resistance data indicate >99% susceptibility among currently circulating strains. If CAP occurs directly following an episode of influenza, the influenza should also be treated pending results from PCR testing. In cases of fulminant pneumonia after an episode of influenza, penicillin should be replaced by a beta-lactam antibiotic with activity against *S. aureus*
- Concerns have arisen about potential unintended consequences of implementation of the rule that in patients with suspected CAP antibiotics be started within four hours of admission. Although these guidelines emphasise the importance of rapid administration of the first dose of antibiotics, maximal effort should be made that this recommendation does not cause the inaccurate diagnosis of CAP and/or inappropriate utilisation of antibiotics

that these indicators be used as external (performance) indicators to compare hospitals, as long as they have not been validated for this purpose.

ACKNOWLEDGMENT AND DECLARATION OF INTEREST

The Guidelines Committee would like to thank all individuals and societies who contributed to the development of this guideline. In particular, we thank H.C. Dyserinck, Academic Medical Center, Amsterdam, for excellent library support, Prof. Dr. M. D. de Jong, Department of Microbiology, AMC, Amsterdam, the Netherlands, for help with the paragraphs on influenza, G. C. Koh, the Wellcome Trust Sanger Institute, Cambridge, UK for assistance in preparation of this manuscript and H.K. de Jong, Center for Experimental Molecular Medicine, AMC, Amsterdam, the Netherlands, for assistance in the preparation of *figure 1*. Members of the preparatory committee reported the following potential conflicts of interest: WJW: none; MJB: Novartis Europe advisory board Daptomycine, Pfizer Netherlands advisory board vaccines, grant from Pfizer Netherlands for investigating aetiology of CAP; WGB: received a grant from GSK and Astra Zeneca for research and a fee from Pfizer for medical advice; REJ: none; RMA: none; BJK: none; JAS: none; JED: none; RJ: none; TJV: received two grants for research and a fee for consultation from Pfizer; APES: received support for conference attendance from Pfizer and AstraZeneca; JMP: none.

REFERENCES

1. Bjerre LM, Verheij TJM, Kochen MM. Antibiotics for community acquired pneumonia in adilt outpatients. Cochrane Database Syst Rev. 2009;(4):1-43.
2. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44 Suppl 2:S27-S72.

3. Schouten JA, Prins JM, Bonten MJ, et al. Revised SWAB guidelines for antimicrobial therapy of community-acquired pneumonia. Neth J Med. 2005;63(8):323-35.

4. NVALT (National Society for Respiratory Physicians). Guideline for Diagnosis and Treatment of Community-acquired Pneumonia (CAP). Alphen aan den Rijn: Van Zuiden Communications; 2003.

5. Verheij T, Hopstaken RM, Prins JM, et al. NHG-standaard Acuut hoesten. Eerste herziening. H&W 2011;54:68-92.

6. 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.

7. 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(17):952-6.

8. 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(7):1730-54.

9. 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(11):1405-33.

10. 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(2):347-82.

11. ERS Task Force Report. Guidelines for management of adult community-acquired lower respiratory tract infections. European Respiratory Society. Eur Respir J. 1998;11(4):986-91.

12. BTS Guidelines for the Management of Community Acquired Pneumonia in Adults. Thorax. 2001;56 Suppl 4:IV1-64.:IV1-64.

13. SWAB. NethMap 2010 – Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands. Amsterdam: 2010

14. Lim WS, Baudouin SV, George RC, et al. BTS guidelines for the management of community acquired pneumonia in adults: update 2009. Thorax. 2009;64 Suppl 3:iii1-55.

15. 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(3):110-7.

16. 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(5):543-7.

17. Oosterheert. Diagnosis and treatment of community-acquired lower respiratory tract infections. 2005.

18. Snijders D, Daniels JM, de Graaff CS, van der Werf TS, Boersma WG. Efficacy of corticosteroids in community-acquired pneumonia: a randomized double-blinded clinical trial. Am J Respir Crit Care Med. 2010;181(9):975-82.

19. van der Eerden MM, Vlaspolder F, de Graaff CS, et al. Comparison between pathogen directed antibiotic treatment and empirical broad spectrum antibiotic treatment in patients with community acquired pneumonia: a prospective randomised study. Thorax 2005;60(8):672-8.

20. 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(17):836-40.

21. Boersma WG, Daniels JM, Lowenberg A, Boeve WJ, van de Jagt EJ. Reliability of radiographic findings and the relation to etiologic agents in community-acquired pneumonia. Respir Med. 2006;100(5):926-32.

22. Ruiz M, Ewig S, Marcos MA, et al. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am J Respir Crit Care Med. 1999;160(2):397-405.

23. Logroscino CD, Penza O, Locicero S, et al. Community-acquired pneumonia in adults: a multicentric observational AIPO study. Monaldi Arch Chest Dis. 1999;54(1):11-7.

24. Ostergaard L, Andersen PL. Etiology of community-acquired pneumonia. Evaluation by transtracheal aspiration, blood culture, or serology. Chest. 1993;104(5):1400-7.

25. Arancibia F, Bauer TT, Ewig S, et al. Community-acquired pneumonia due to gram-negative bacteria and pseudomonas aeruginosa: incidence, risk, and prognosis. Arch Intern Med. 2002;162(16):1849-58.

26. Leroy O, Vandenbussche C, Coffinier C, et al. Community-acquired aspiration pneumonia in intensive care units. Epidemiological and prognosis data. Am J Respir Crit Care Med. 1997;156(6):1922-9.

27. Den Boer JW, Nijhof J, Friesema I. Risk factors for sporadic community-acquired Legionnaires' disease. A 3-year national case-control study. Public Health. 2006;120(6):566-71.

28. Delsing CE, Kullberg BJ, Bleeker-Rovers CP. Q Fever in the Netherlands from 2007 to 2010. Neth J Med. 2010;68(12):382-7.

29. Schimmer B, Morroy G, Dijkstra F, et al. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill. 2008;13(31).

30. Fine MJ, Auble TE, Yealy DM, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med. 1997;336(4):243-50.

31. Lim WS, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax. 2003;58(5):377-82.

32. Bont J, Hak E, Hoes AW, Macfarlane JT, Verheij TJ. Predicting death in elderly patients with community-acquired pneumonia: a prospective validation study reevaluating the CRB-65 severity assessment tool. Arch Intern Med. 2008;168(13):1465-8.

33. Chalmers JD, Singanayagam A, Akram AR, et al. Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. Systematic review and meta-analysis. Thorax. 2010;65(10):878-83.

34. Aujesky D, Auble TE, Yealy DM, et al. Prospective comparison of three validated prediction rules for prognosis in community-acquired pneumonia. Am J Med. 2005;118(4):384-92.

35. Buising KL, Thursky KA, Black JF, et al. A prospective comparison of severity scores for identifying patients with severe community acquired pneumonia: reconsidering what is meant by severe pneumonia. Thorax. 2006;61(5):419-24.

36. Kauppinen MT, Lahde S, Syrjala H. Roentgenographic findings of pneumonia caused by Chlamydia pneumoniae. A comparison with streptococcus pneumonia. Arch Intern Med. 1996;156(16):1851-6.

37. Macfarlane JT, Miller AC, Roderick Smith WH, Morris AH, Rose DH. Comparative radiographic features of community acquired Legionnaires' disease, pneumococcal pneumonia, mycoplasma pneumonia, and psittacosis. Thorax. 1984;39(1):28-33.

38. Lahde S, Jartti A, Broas M, Koivisto M, Syrjala H. HRCT findings in the lungs of primary care patients with lower respiratory tract infection. Acta Radiol. 2002;43(2):159-63.

39. Hayden GE, Wrenn KW. Chest radiograph vs. computed tomography scan in the evaluation for pneumonia. J Emerg Med. 2009;36(3):266-70.

40. Syrjala H, Broas M, Suramo I, Ojala A, Lahde S. High-resolution computed tomography for the diagnosis of community-acquired pneumonia. Clin Infect Dis. 1998;27(2):358-63.

41. Hagaman JT, Rouan GW, Shipley RT, Panos RJ. Admission chest radiograph lacks sensitivity in the diagnosis of community-acquired pneumonia. Am J Med Sci. 2009;337(4):236-40.

42. 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(2):165-9.

43. Lettinga KD, Verbon A, Weverling GJ, et al. Legionnaires' disease at a Dutch flower show: prognostic factors and impact of therapy. Emerg Infect Dis. 2002;8(12):1448-54.

44. Yzerman EP, Den Boer JW, Lettinga KD, Schellekens J, Dankert J, Peeters M. Sensitivity of three urinary antigen tests associated with clinical severity in a large outbreak of Legionnaires' disease in The Netherlands. J Clin Microbiol. 2002;40(9):3232-6.

45. Murdoch DR, Laing RT, Mills GD, 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(10):3495-8.

46. Gutierrez F, Masia M, Rodriguez JC, et al. Evaluation of the immunochromatographic Binax NOW assay for detection of Streptococcus pneumoniae urinary antigen in a prospective study of community-acquired pneumonia in Spain. Clin Infect Dis. 2003;36(3):286-92.

47. Sorde R, Falco V, Lowak M, et al. Current and Potential Usefulness of Pneumococcal Urinary Antigen Detection in Hospitalized Patients With Community-Acquired Pneumonia to Guide Antimicrobial Therapy. 2011;171(2):166-72.

48. Roson B, Fernandez-Sabe N, Carratala J, et al. Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis. 2004;38(2):222-6.

49. Stralin K, Kaltoft MS, Konradsen HB, Olcen P, Holmberg H. Comparison of two urinary antigen tests for establishment of pneumococcal etiology of adult community-acquired pneumonia. J Clin Microbiol. 2004;42(8):3620-5.

50. Andreo F, Ruiz-Manzano J, Prat C, et al. Utility of pneumococcal urinary antigen detection in diagnosing exacerbations in COPD patients. Respir Med. 2010;104(3):397-403.

51. Wegdam-Blans MC, Nabuurs-Franssen MN, Horrevorts AM, Peeters MF, Schneeberger PM, Bijlmer HA. [Laboratory diagnosis of acute Q fever]. Ned Tijdschr Geneeskd. 2010;154(37):A2388.

52. Bautista E, Chotpitayasunondh T, Gao Z, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362(18):1708-19.

53. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children--diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis. 2009;48(8):1003-32.

54. Fiore AE, Uyeki TM, Broder K, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. MMWR Recomm Rep. 2010;59(RR-8):1-62.

55. Don M, Valent F, Korppi M, et al. Efficacy of serum procalcitonin in evaluating severity of community-acquired pneumonia in childhood. Scand J Infect Dis. 2007;39(2):129-37.

56. Thayyil S, Shenoy M, Hamaluba M, Gupta A, Frater J, Verber IG. Is procalcitonin useful in early diagnosis of serious bacterial infections in children? Acta Paediatr. 2005;94(2):155-8.

57. Korppi M, Remes S, Heiskanen-Kosma T. Serum procalcitonin concentrations in bacterial pneumonia in children: a negative result in primary healthcare settings. Pediatr Pulmonol. 2003;35(1):56-61.

58. Daubin C, Parienti JJ, Fradin S, et al. Procalcitonin levels and bacterial aetiology among COPD patients admitted to the ICU with severe pneumonia: a prospective cohort study. BMC Infect Dis. 2009;9:157.

59. Christ-Crain M, Opal SM. Clinical review: the role of biomarkers in the diagnosis and management of community-acquired pneumonia. Crit Care. 2010;14(1):203.

60. Gibot S, Cravoisy A, Levy B, Bene MC, Faure G, Bollaert PE. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. N Engl J Med. 2004;350(5):451-8.

61. Latour-Perez J, Alcala-Lopez A, Garcia-Garcia MA, et al. Diagnostic accuracy of sTREM-1 to identify infection in critically ill patients with systemic inflammatory response syndrome. Clin Biochem. 2010;43(9):720-4.

62. Bopp C, Hofer S, Bouchon A, Zimmermann JB, Martin E, Weigand MA. Soluble TREM-1 is not suitable for distinguishing between systemic inflammatory response syndrome and sepsis survivors and nonsurvivors in the early stage of acute inflammation. Eur J Anaesthesiol. 2009;26(6):504-7.

63. Blazquez Garrido RM, Espinosa Parra FJ, Alemany FL, et al. Antimicrobial chemotherapy for Legionnaires disease: levofloxacin versus macrolides. Clin Infect Dis. 2005;40(6):800-6.

64. Grau S, Antonio JM, Ribes E, Salvado M, Garces JM, Garau J. Impact of rifampicin addition to clarithromycin in Legionella pneumophila pneumonia. Int J Antimicrob Agents. 2006;28(3):249-52.

65. Meehan TP, Fine MJ, Krumholz HM, et al. Quality of care, process, and outcomes in elderly patients with pneumonia. JAMA 1997;278(23):2080-4.

66. Battleman DS, Callahan M, Thaler HT. Rapid antibiotic delivery and appropriate antibiotic selection reduce length of hospital stay of patients with community-acquired pneumonia: link between quality of care and resource utilization. Arch Intern Med. 2002;162(6):682-8.

67. Houck PM, Bratzler DW, Nsa W, Ma A, Bartlett JG. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. Arch Intern Med. 2004;164(6):637-44.

68. Benenson R, Magalski A, Cavanaugh S, Williams E. Effects of a pneumonia clinical pathway on time to antibiotic treatment, length of stay, and mortality. Acad Emerg Med. 1999;6(12):1243-8.

69. Marrie TJ, Wu L. Factors influencing in-hospital mortality in community-acquired pneumonia: a prospective study of patients not initially admitted to the ICU. Chest. 2005;127(4):1260-70.

70. Bruns AH, Oosterheert JJ, Hustinx WN, Gaillard CA, Hak E, Hoepelman AI. Time for first antibiotic dose is not predictive for the early clinical failure of moderate-severe community-acquired pneumonia. Eur J Clin Microbiol Infect Dis. 2009;28(8):913-9.

71. Bax HI. Dutch Working Party on Antibiotic Policy (SWAB) guidelines for Antibacterial therapy of adult patients with Sepsis. 2010.

72. File TM, Jr., Mandell LA, Tillotson G, Kostov K, Georgiev O. Gemifloxacin once daily for 5 days versus 7 days for the treatment of community-acquired pneumonia: a randomized, multicentre, double-blind study. J Antimicrob Chemother. 2007;60(1):112-20.

73. Tellier G, Niederman MS, Nusrat R, Patel M, Lavin B. Clinical and bacteriological efficacy and safety of 5 and 7 day regimens of telithromycin once daily compared with a 10 day regimen of clarithromycin twice daily in patients with mild to moderate community-acquired pneumonia. J Antimicrob Chemother. 2004;54(2):515-23.

74. el Moussaoui R., de Borgie CA, van den Broek P, et al. Effectiveness of discontinuing antibiotic treatment after three days versus eight days in mild to moderate-severe community acquired pneumonia: randomised, double blind study. BMJ. 2006;332(7554):1355.

75. Christ-Crain M, Stolz D, Bingisser R, et al. Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia: a randomized trial. Am J Respir Crit Care Med. 2006;174(1):84-93.

76. Schuetz P, Christ-Crain M, Thomann R, et al. Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. JAMA. 2009;302(10):1059-66.

77. 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(5):722-7.

78. Oosterheert JJ, Bonten MJ, Schneider MM, et al. Effectiveness of early switch from intravenous to oral antibiotics in severe community acquired pneumonia: multicentre randomised trial. BMJ 2006;333(7580):1193.

79. Ramirez JA, Vargas S, Ritter GW, 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(20):2449-54.

80. Nathan RV, Rhew DC, Murray C, Bratzler DW, Houck PM, Weingarten SR. In-hospital observation after antibiotic switch in pneumonia: a national evaluation. Am J Med. 2006;119(6):512-7.

81. Meijvis SC, Hardeman H, Remmelts HH, et al. Dexamethasone and length of hospital stay in patients with community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial. Lancet. 2011;377(9782):2023-30.

82. Wunderink RG, Laterre PF, Francois B, et al. Recombinant Tissue Factor Pathway Inhibitor in Severe Community-Acquired Pneumonia: A Randomized Trial. Am J Respir Crit Care Med. 2011;183(11):1561-8.

83. Root RK, Lodato RF, Patrick W, et al. Multicenter, double-blind, placebo-controlled study of the use of filgrastim in patients hospitalized with pneumonia and severe sepsis. Crit Care Med. 2003;31(2):367-73.

84. Maskell NA, Batt S, Hedley EL, Davies CW, Gillespie SH, Davies RJ. The bacteriology of pleural infection by genetic and standard methods and its mortality significance. Am J Respir Crit Care Med. 2006;174(7):817-23.

85. Varkey B, Rose HD, Kutty CP, Politis J. Empyema thoracis during a ten-year period. Analysis of 72 cases and comparison to a previous study (1952 to 1967). Arch Intern Med. 1981;141(13):1771-6.

86. Heffner JE, Brown LK, Barbieri C, DeLeo JM. Pleural fluid chemical analysis in parapneumonic effusions. A meta- analysis. Am J Respir Crit Care Med. 1995;151(6):1700-8.

87. Laing FC, Filly RA. Problems in the application of ultrasonography for the evaluation of pleural opacities. Radiology. 1978;126(1):211-4.

88. Eibenberger KL, Dock WI, Ammann ME, Dorffner R, Hormann MF, Grabenwoger F. Quantification of pleural effusions: sonography versus radiography. Radiology. 1994;191(3):681-4.

89. Taryle DA, Good JT, Jr., Morgan EJ, III, Reller LB, Sahn SA. Antibiotic concentrations in human parapneumonic effusions. J Antimicrob Chemother. 1981;7(2):171-7.

90. Joseph J, Vaughan LM, Basran GS. Penetration of intravenous and oral ciprofloxacin into sterile and empyemic human pleural fluid. Ann Pharmacother. 1994;28(3):313-5.

91. Diacon AH, Theron J, Schuurmans MM, Van de Wal BW, Bolliger CT. Intrapleural streptokinase for empyema and complicated parapneumonic effusions. Am J Respir Crit Care Med. 2004;170(1):49-53.

92. Rahman NM, Maskell NA, West A, et al. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med. 2011;365(6):518-26.

93. Bouros D, Schiza S, Tzanakis N, Chalkiadakis G, Drositis J, Siafakas N. Intrapleural urokinase versus normal saline in the treatment of complicated parapneumonic effusions and empyema. A randomized, double-blind study. Am J Respir Crit Care Med. 1999;159(1):37-42.

94. Davies RJ, Traill ZC, Gleeson FV. Randomised controlled trial of intrapleural streptokinase in community acquired pleural infection. Thorax. 1997;52(5):416-21.

95. Cameron R, Davies HR. Intra-pleural fibrinolytic therapy versus conservative management in the treatment of adult parapneumonic effusions and empyema. Cochrane Database Syst Rev. 2008;(2):CD002312.

96. Diacon AH, Theron J, Schuurmans MM, Van de Wal BW, Bolliger CT. Intrapleural streptokinase for empyema and complicated parapneumonic effusions. Am J Respir Crit Care Med. 2004;170(1):49-53.

97. Maskell NA, Davies CW, Nunn AJ, et al. U.K. Controlled trial of intrapleural streptokinase for pleural infection. N Engl J Med. 2005;352(9):865-74.

98. Seymann GB. Community-acquired pneumonia: defining quality care. J Hosp Med. 2006;1(6):344-53.

99. Graffelman AW, Knuistingh NA, le Cessie S, Kroes AC, Springer MP, van den Broek PJ. Pathogens involved in lower respiratory tract infections in general practice. Br J Gen Pract. 2004;54(498):15-9.

100. 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(12):902-6.

LETTER TO THE EDITOR

Transplacental passage of nevirapine, nelfinavir and lopinavir

S. van Hoog[1], K. Boer[1], J. Nellen[2], H. Scherpbier[3], M.H. Godfried[2]

[1]Department of Obstetrics, [2]Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, [3]Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, tel.: +31 (0)20-5664154

Dear Editor,

Since 1998, the cornerstone of preventing mother-to-child transmission of human immunodeficiency virus (HIV) is the use of highly active antiretroviral therapy (c-ART) in pregnancy and as post-exposure prophylaxis to the neonate. Individual agents differ with respect to their transplacental passage.[1] Our purpose was to examine transplacental passage of nevirapine, lopinavir and nelfinavir by studying the C/M ratio (cord /maternal venous blood concentration), determined by high performance liquid chromatography and the possible influence of the birth weight ratio indicating placental insufficiency rate.

Seventy-nine HIV-infected women out of 263 who delivered between 2003 and 2010 in the Academic Medical Centre, Amsterdam, were included since paired venous mother-cord blood samples were available for them. Data were retrospectively collected from the electronic medical records. Maternal and cord venous samples were drawn simultaneously at delivery and the interval from last c-ART intake registered. All women received intravenous zidovudine during labour. Nine patients were excluded (seven because the post-dose interval was longer than in the average population or unknown and two because swapping of maternal and cord samples was evident).

Nevirapine showed a relatively high median C/M ratio of 0.67, while nelfinavir and lopinavir had lower ratios of 0.14 and 0.24, respectively (*table 1*). *Figure 1* illustrates differences in transplacental passage between the three agents. No association existed between the C/M ratio and the newborn birth weight in any of the antiretroviral drugs.

The C/M ratios in this study were similar to previous reports. The low cord-to-mother ratio for lopinavir and nelfinavir suggests a low transplacental transfer. A reason for this could be that protease inhibitors (PIs) are large molecules with a high protein binding (98%). Moreover, PIs are a substrate for the efflux transport molecule P-glycoprotein, which forms a functional barrier in the human placenta that limits the exposure of the foetus to xenobiotics, to protect it from their potential teratogenic effects.

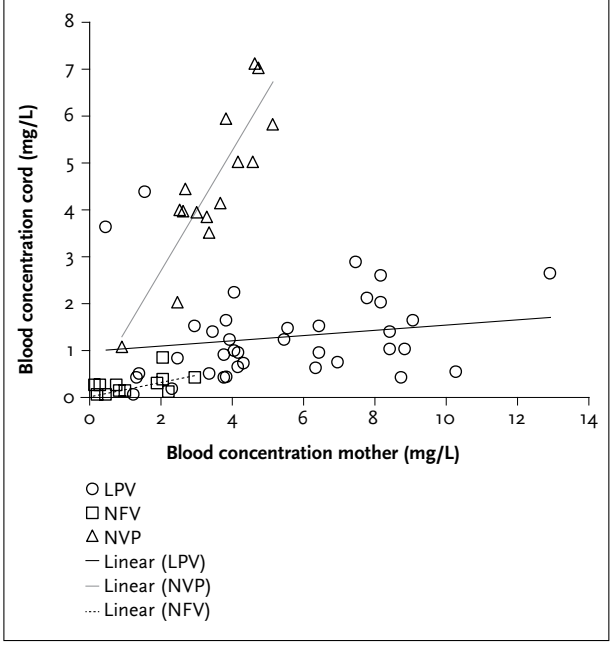
Nevirapine, in contrast, shows a high transplacental passage, which is almost similar to passive diffusion. This could be explained by the lower protein binding (60%), the lower molecular weight of nevirapine and the fact that nevirapine is not a substrate for P-glycoprotein.

Table 1. *Results*

Type of ART (+combivir)	Median post-dose interval (h)	Median maternal concentration (mg/l)	Median population ratio	Median cord concentration (mg/l)	c/m ratio
NVP (n=17)	3.60 (±4.60) (n=14)	4.00 (±1.95)	1.10 (±0.50) (n=9)	3.20 (±1.60)	0.67 (±0.15)
NFV (n=20)	8.40 (±8.00) (n=18)	0.75 (±1.85)	0.80 (±0.4) (n=11)	0.15 (±0.25)	0.14(±0.36)
LPV (n=42)	6.10 (±8.34) (n=36)	4.10 (±5.45)	0.80 (±0.83) (n=26)	0.96 (±1.16)	0.24 (±0.21)

Post-dose intervals, blood concentrations and ratios of nevirapine (NVP), nelfinavir (NFV) and lopinavir (LPV); c/m ratio = the ratio of the venous cord blood/maternal blood concentration.

Figure 1. *Cord and mother blood concentrations of LPV, NFV and NVP*



In a situation where maximum efficacy and minimum toxicity is required, these may be important data for treatment decisions.

Preliminary data were presented at the winter meeting of the Dutch HIV/AIDS Society held in the Netherlands on 23 January 2009. A poster presentation was given at the the 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention (IAS 2011) in Rome, Italy from 17-20 July 2011.

REFERENCE

1. Gingelmaier A, Kurowski M, Kastner R, Eberle J, Mylonas I, Belohradsky BH, et al. Placental transfer and pharmacokinetics of lopinavir and other protease inhibitors in combination with nevirapine at delivery. AIDS. 2006; 20(13):1737-43.

LETTER TO THE EDITOR

Severe hypertriglyceridaemia associated with the use of capecitabine

H.A. Polinder-Bos[1], E.E. Kok[1], A. van de Wiel[1], W. Spiering[3], J.P.M. Wielders[2], H.J. Bloemendal[1*]

Departments of [1]Internal Medicine, [2]Clinical Chemistry, Meander Medical Center, de Lichtenberg, Amersfoort, the Netherlands, [3]Department of Vascular Medicine, University Medical Center Utrecht, Utrecht, the Netherlands, *corresponding author: tel.: +31 (0)33-8505050, fax: +31 (0)33-8502695, e-mail: hj.bloemendal@meandermc.nl

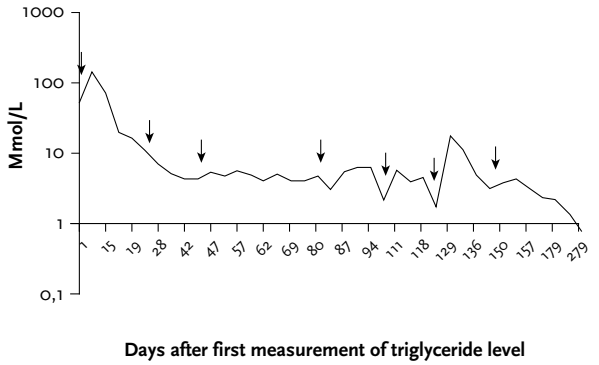
Dear Editor,

Capecitabine is frequently used as adjuvant chemotherapy in colorectal cancer and in the treatment of advanced or metastatic breast, colorectal or gastric cancer.[1,2] The main adverse effects of capecitabine are palmar-plantar erythrodysthesia, diarrhoea and stomatitis.[3] Only a few cases of capecitabine-associated hypertriglyceridaemia have been documented.[2-4]

A 52-year-old man was treated with adjuvant capecitabine/oxaliplatin (CAPOX) therapy after a laparoscopic rectum resection because of rectal carcinoma. Because of weight loss his dietician had advised a protein- and fat-enhanced diet a couple of months before. At the end of the third cycle of CAPOX severe dyslipidaemia with extremely high triglycerides levels was observed (138 mmol/l). The CAPOX cycle was discontinued, a low-fat diet was advised and gemfibrozil medication was started. After normalisation of the lipid spectrum, CAPOX was restarted in increasing dosages of capecitabine. After another cycle with a full dose of CAPOX, the patient developed hypertriglyceridaemia again (*figure 1*). Primary causes of hypertriglyceridaemia were excluded. Apolipoprotein B and HbA1c were in the normal range. The patient was not known to have a genetic disorder of lipid metabolism and lipoprotein lipase activity proved to be normal. A post-heparin lipolytic activity test was normal. Consequently an apolipoprotein C-II deficiency was not likely and we did not perform a immunoturbidimetric test. The apolipoprotein E genotype was E3/E3 excluding familial dysbetalipoproteinaemia. All known causes of secondary hypertriglyceridaemia were excluded as well, except the high-fat diet which the patient had used. But a diet leading to these high levels of triglyceride is unlikely because increases after a high-fat meal seldom exceed 5 mmol/l in the absence of other factors.

Evaluating our case and previous case reports,[2-4] the most likely cause of the severe hypertriglyceridaemia is a side effect of capecitabine treatment. According to the Modified Naranjo Scale, a method for estimating the probability of adverse drug reactions, this side effect should be scored as a definite adverse drug reaction of capecitabine.[5]

Figure 1. *Triglycerides related to capecitabine/oxaliplatin*



Lipid monitoring is not routinely performed in cancer patients receiving capecitabine. Since hypertriglyceridaemia is a serious side effect it should be considered to routinely perform a lipid spectrum in the treatment of patients with capecitabine-containing regimens.

REFERENCES

1. Stathopoulos GP, Koutantos J, Lazaki H, et al. Capecitabine (Xeloda) as monotherapy in advanced breast and colorectal cancer. Anticancer Res. 2007; 27:1653-6.
2. Bar-Sela G, Haim N. Uncontrolled hypertriglyceridemia induced by Capecitabine. Cancer Chemother Pharmacol. 2009; 63:779-82.
3. Koutras AK, Habeos IG, Vagenakis AG, et al. Capecitabine-induced hypertriglyceridemia. Anticancer Res. 2006;26:2249-514.
4. Kurt M, Babaoglu MO, Yasar U et al. Capecitabine-induced severe hypertriglyceridemia. Ann Pharmacother. 2006;40:328-31.
5. Naranjo CA, Busto U, Sellers EM, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30:239-45.

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.

Submission
All submissions to the *Netherlands Journal of Medicine* should be submitted online through Manuscript Central at http://mc.manuscriptcentral.com/nethjmed. Authors should create an account and follow the instructions. If you are unable to submit through Manuscript Central contact the editorial office at m.m.levi@amc.uva.nl, tel.: +31 (0)20-566 21 71, fax: +31 (0)20-691 96 58.

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 corresponding person (with the address, telephone number, fax number and e-mail address). 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. The letter should make it clear that the final manuscript has been seen and approved by all authors. All authors should sign the letter. The letter should either be submitted through http://mc.manuscriptcentral.com/nethjmed or faxed to the editorial office (+31 (0)20-691 96 58).

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

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

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

Keywords: Include three to five keywords in alphabetical order.

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

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

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

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

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