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Contents

ISSN: 0300-2977

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EDITORIAL

Arterial and venous thrombosis: more in common than previously thought

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Traditionally, arterial and venous thrombotic disease are regarded as separate disease entities. Indeed, the clinical manifestation of each of these types of thrombosis is quite different. Arterial thrombosis usually leads to obstruction of organ perfusion with resulting tissue ischaemia and necrosis and subsequent organ dysfunction, whereas venous thrombosis is merely associated with congestion and consequent symptoms, such as swelling or pain. There only seems to be a connection between the two diseases when venous thrombi switch from the venous to the arterial side (as occurs in case of pulmonary embolism). Interestingly, the two types of thrombosis are also usually seen by different medical professionals, whereby arterial thrombotic complications are usually taken care of by the specialist who handles the threatened end-organ (i.e. cardiologists, neurologists, etc.) in contrast to venous thromboembolism, which is most often covered by internists, sometimes specialised in haematology or vascular medicine. The separation between arterial and venous thrombosis is further accentuated by very different insights into pathogenesis, whereby arterial thrombosis is regarded as a result of vascular damage, platelet-vessel wall interaction, and high shear stress, and venous thrombosis may result from immobilisation, changes in blood composition, and (surgical) trauma.[1]

However, recent studies point to the fact that arterial and venous thrombosis have more in common than previously thought. There is increasing evidence that there is a link between arterial atherothrombotic disease and venous thrombosis, as these two conditions occur in similar patients and share common risk factors, such as age and obesity. Prandoni *et al.* were the first to demonstrate a higher prevalence of subclinical atherosclerosis in patients with previous idiopathic venous thromboembolism (VTE) in 2003 and in patients with previous VTE an increased risk of arterial cardiovascular complications has been consistently shown. [2-4] Vice-versa, large population-based studies demonstrated that the presence of thrombophilia (known to increase the risk of venous thromboembolism) also increased the risk of atherothrombosis, albeit to a modest extent.[5,6] A recent study showed that microalbuminuria, traditionally seen as a risk factor for atherothrombotic disease, was associated with a higher risk of venous thromboembolism.[7] Conversely, patients with haemophilia were shown to have less extensive atherosclerosis as compared with controls.[8] Importantly, interventions aimed at reducing atherothrombotic events in a high-risk population by means of the administration of statins were also effective in preventing venous thromboembolism. This study was the first to show this effect of statins and the results were obtained in the setting of a large prospective trial with a strong study design. It should be mentioned, however, that despite a significant 43% reduction in venous thromboembolism in patients using a statin, the absolute risk reduction was quite modest. In fact, over a median two-year follow-up the absolute incidence of thrombosis was 3.8 per 1000 in the statin group as compared with 6.7 per 1000 in the placebo group, which means that 342 patients should be treated with statins over a two-year period to prevent one venous thromboembolic event. However, it would be interesting to see whether this beneficial effect of statins would be confirmed in a population with a higher risk of venous thrombosis, such as in patients with a previous episode of venous thromboembolism. Prospective evaluation of this question and validation of the previous results are planned in a large Dutch multicentre study.

Further insight into the link between arterial and venous thrombosis is provided by the interesting study by Roshani *et al.* in this issue of the Netherlands Journal of Medicine.[9] The authors studied whether patients with prior venous thromboembolism had a higher risk of arterial cardiovascular complications. They recruited 861 subjects from families in which patients experienced venous thromboembolism or arterial thrombosis before the age of 50 years. The authors show that there is a mildly elevated risk of arterial cardiovascular complications in patients with prior venous thromboembolism; however,

this cannot be ascribed to the presence of risk factors for arterial cardiovascular disease or thrombophilic defects. Hence, based on these observations, traditional risk factors cannot be held responsible for the link between arterial and thrombotic disease.

A common pathogenesis for venous and arterial thrombosis cannot easily be found along the lines of established mechanisms of disease. New pathogenetic pathways need to be identified that may better explain this connection. One of these potential mechanisms that links arterial and venous thromboembolism may be mediated by inflammation-induced effects on the vessel wall, which appear to be important in both the pathogenesis of atherosclerosis and venous thromboembolism.[10] Inflammation is a known risk factor for venous thrombosis, either by changes in blood composition rendering it hypercoagulable, or by direct effects on the vessel wall. Simultaneously, the involvement of inflammation in the pathogenesis of atherosclerosis and arterial thrombosis becomes increasingly clear from recent studies. Interestingly, it seems that these effects are indeed modified by the use of statins, which then may explain the statin effect on venous thromboembolism.

What does this notion of a more close relationship between arterial and venous thrombosis mean for individual patients? A more integral approach in (secondary) prevention of thrombotic events may be warranted. Also, (pharmaceutical) preventive strategies could possibly be more integrated. Obviously, statins cannot be considered as adequate prophylaxis for venous thrombosis if this is indicated, although patients who have an indication for statin treatment may benefit from the additional (albeit modest) lowering of the risk of venous thromboembolism.[11] Similarly, aspirin cannot be considered adequate prophylaxis for venous thrombosis; however, large studies point to some (additional) benefit for patients with (risk of) venous thromboembolism.[12]

The days of a clear separation between arterial and venous thrombosis seem to be over. In the next years we will have to find out how this insight can be translated into better strategies to prevent or treat both arterial and venous thrombosis.

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REVIEW

Vaccination of immune-compromised patients with the focus on patients with autoimmune-inflammatory diseases

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ABSTRACT

Among immunocompromised patients morbidity and mortality due to vaccine-preventable infections is high. Although vaccination seems indicated, controversy exists about which vaccines should be offered, at what moment, and to whom. Guidelines are needed as the number of immunocompromised individuals increases due to the wider use of immunosuppressive drugs and, in particular, because since the introduction of biological agents, the spectrum of immunosuppressive drugs is rapidly expanding. In this review we will highlight controversies about vaccination in immunocompromised patients and will discuss indications for the several vaccines available to prevent infectious diseases with the focus on patients with autoimmune-inflammatory diseases.

KEYWORDS

Vaccination, immunocompromised, infection, autoimmunity

INTRODUCTION

Patients with a deficient immune response have an increased morbidity and mortality due to vaccine-preventable diseases. For example, in two large retrospective studies an increased risk for influenza-related morbidity and mortality was demonstrated in groups of elderly patients (≥65 years) with rheumatic diseases, vasculitis, chronic renal failure, dementia or stroke (all considered at intermediate risk of contracting influenza). Measured over a period spanning six seasons, the odds ratio (OR) in this group was 1.6 (95% confidence interval [CI] 1.2 to 2.0) for admission for pneumonia or influenza and 2.7 (CI 2.3 to 3.2) for death compared with low-risk elderly.[1] Another study reported that 4.5 to 7% of unvaccinated patients with rheumatic diseases, vasculitis, dementia or stroke were admitted for pneumonia/influenza or died, compared with 0.8% in unvaccinated healthy controls.[2] Based on these data vaccination in patients at risk seems indicated. However, clear vaccination guidelines are not available. This can be attributed to several factors.

First, a clear definition of an immunocompromised state is lacking. In general, it includes conditions commonly classified as primary immunodeficiency and secondary immunodeficiency. Primary immunodeficiencies are generally inherited and include conditions defined by an absence or quantitative deficiency of cellular and/or humoral components of the immune system such as X-linked agammaglobulinaemia, severe combined immunodeficiency disease, and chronic granulomatous disease. These diseases are relatively rare. The fast majority of immunocompromised patients have a secondary immunodeficiency. This is generally acquired and defined by a deficiency in humoral and/or cellular immunity that occurs as a result of a disease process or its therapy. Examples of secondary immunodeficiency include HIV infection, haematopoetic malignancies, and treatment with cytostatic/immunosuppressive drugs as given to patients with malignancies, after (solid organ) transplantation, and to patients with autoimmune inflammatory rheumatic disorders (AIIRD). The level of immunosuppression within this very heterogeneous group of patients varies largely between but also within certain patient categories. For example, in HIV-infected individuals the response on vaccination is related to the number of circulating $CD4^+$-T cells and the treatment with combination antiretroviral

therapy (cART).[3-6] cART-induced suppression of HIV replication is associated with an increase in $CD4^+$-T cell and B-cell numbers. As a result, cART improves the efficacy of the immune response to vaccination in these patients, although responses in many patients remain suboptimal.[7,8] Also, the degree to which immunosuppressive drugs cause clinically significant immunodeficiency varies by drug and is generally dose-related.

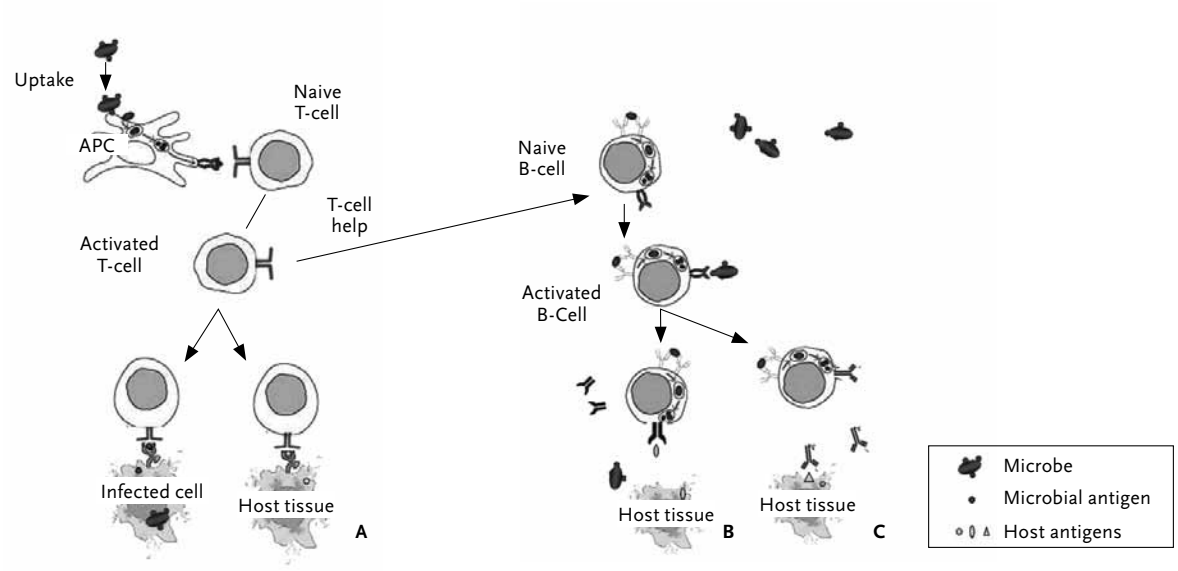
Secondly, although the risk for (severe) infection in these immunocompromised patients is increased and vaccination therefore seems indicated, due to the hampered immune response vaccination might not result in the level of protection aimed at. Moreover, administration of live-attenuated vaccines to immunocompromised individuals might result in infection because of reduced ability to mount an effective immune response. Not only infectious disease might be introduced, debate is also ongoing on whether vaccination might be responsible for the induction of certain diseases, including autoimmune disease, or might reactivate underlying disease through molecular mimicry, epitope spreading, bystander activation, or polyclonal activation (*figure 1*).[9]

Finally, apart from the retrospective studies mentioned above, only limited data on the incidence of vaccine-preventable infections (VPI) in immunocompromised patients are available and, moreover, most vaccination studies performed in these groups of patients did not have contraction and outcome of VPI as a primary study endpoint. Nearly all studies used humoral (antibody) response on vaccination as an outcome parameter. Except for a few vaccinations such as those against influenza and hepatitis B, no generally accepted definitions of protective antibody levels are available.[10] Furthermore, it is still a matter of debate whether antibody response as a surrogate endpoint is valid. For example, in the elderly it has been demonstrated that the cellular response after influenza vaccination might be a better correlate of protection than the antibody response (*table 1*).[11,12]

PRIMARY IMMUNODEFICIENCY

Most patients with primary immunodeficiency have a defect in the humoral immune response (X-linked

Figure 1. *Simplified schematic representation of mechanisms by which microorganisms might induce autoimmunity*



After invasion, microbes will be internalised by antigen-presenting cells (APCs). The microbes will be processed and microbial antigens will be presented by MHC molecules to T cells. These T cells can react with (virus) infected cells presenting the viral antigen (panel A, left). Whenever there is sufficient homology with certain self-antigens (molecular mimicry) these T cells can cross-react with self-antigens expressed by host tissue, leading to (reactivation of) autoimmune disease (panel A, right). B lymphocytes in themselves can be directly activated by microbes when polysaccharide microbial antigens are recognised by the B-cell receptor. Otherwise T-cell help is needed for B-cell activation. Activated B cells produce antibodies directed to microbial antigen, but, via a process of so-called epitope spreading also antibodies to structurally related subdominant (cryptic) epitopes may be produced which might be directed to self-antigens. Epitope spreading is a common characteristic of the adaptive immune responses mounted against different pathogens and describes the ability of the B- and T-cell immune response to diversify, at the level of specificity, from a single determinant to many different sites on a given antigen (panel B). In particular when the immune system is activated for a longer period (repetitive or constantly) the spectrum of B cells that are activated can expand and B cells with other specificity will proliferate. In some of these cases, a monospecific response can emerge, accompanied by circulating immune complexes and eventually resulting in damage to self tissues (panel C). Finally, underlying disease might reactivate through bystander activation. Bystander activation occurs whenever the initial immune response to an antigen induces tissue damage and the release of sequestered antigens which then in turn can activate autoreactive lymphocytes that were not directly involved in the initial response.

Table 1. *Controversies to overcome for the development of vaccination guidelines for immunocompromised individuals*

Problem	Example
Definition of immunocompromised state is lacking	Not clear at what time (depending on dosage and duration of use) individual immunosuppressive drugs hamper the immune response
Vaccination might induce harm	Vaccination studies are underpowered for safety Researchers are reluctant to perform studies with live-attenuated vaccines in immunocompromised patients
Data of incidence and outcome of most vaccine preventable infections is lacking	Data on contracting infectious, vaccine preventable infections after vaccination are lacking
Correlates of protection are not present or do not represent true protection	For most vaccine preventable infections no generally accepted definitions for seroprotection are available Moreover, discussion exists whether cellular instead of humoral (antibody) responses might not be a better correlate of protection

agammaglobulinaemia (XLA), autosomal recessive agammaglobulinaemia, IgA deficiency (IgAD), common variable immunodeficiency (CVID), IgG-subclass deficiency (IgGSD), and specific antibody response deficiency) and frequently experience recurrent bacterial upper and lower respiratory tract infections. Although these patients seem to be at risk for complicated influenza and infections with encapsulated micro-organisms, the prevalence, morbidity and mortality of influenza, pneumococcal, meningococcal and *Haemophilus influenza* B infections in these patients is unknown. One might question any efficacy of vaccination in patients with primary immunodeficiency. Indeed, mononuclear cells from X-linked agammaglobulinaemia patients do not mount any response to influenza vaccination. However, it has been shown that peripheral blood mononuclear cells from a subset of CVID patients are capable of producing antibodies in response to influenza antigen.[13,14] Antibody responses to polysaccharide and polypeptide vaccines have been demonstrated in 18 and 23%, respectively, of CVID patients.[15] Therefore, although data on safety of and response to other vaccines is lacking, it seems reasonable to offer patients with a primary immunodeficiency at least the seasonal flu vaccine and pneumococcal vaccine. For an overview see *table 2*.

SECONDARY IMMUNODEFICIENCY

As stated, the spectrum of patients with a secondary immunodeficiency is broad and covers many different disease entities. In order to present a condensed overview, we will focus on patients with autoimmune-inflammatory

Table 2. *Vaccination in immunocompromised patients*

Defect	Specific immunodeficiency	Recommended vaccines	Contraindicated vaccines	Comments
Innate				
Complement deficiency	Deficiency of early components (C1-C4)	Influenza Pneumococcal Meningococcal	None	All routine vaccines probably effective
	Deficiency of late components (C5-C9, properdin, factor B)	Influenza Pneumococcal Meningococcal	None	All routine vaccines probably effective
Phagocyte deficiency	Chronic granulomatous disease, leukocyte adhesion defect, myeloperoxidase deficiency	Influenza Pneumococcal	Live bacterial vaccines	All inactivated vaccines and live viral vaccines safe and probably effective
Adaptive				
Humoral (B-lymphocyte)	X-linked and autosomal recessive agammaglobulinaemia, CVID, selective IgA deficiency, IgG subclass deficiency, selective antibody response deficiency	Influenza Pneumococcal	Oral poliovirus Smallpox BCG Live oral typhoid	The effectiveness of any vaccine will be uncertain if it depends only on the humoral response
Cellular (T-lymphocyte)	SCID, DiGeorge syndrome, Wiskott-Aldrich syndrome, ataxia teleangiectasia	Influenza Pneumococcal Meningococcal *Haemophilus influenzae type b*	All live vaccines	Vaccines may be ineffective, depending on the degree of immune suppression

*** Adapted from vaccination of persons with primary immune deficiencies, in: General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP) Centers for Disease Control and Prevention (CDC). MMWR Recomm Rep 2006 Dec 1;55(RR-15):1-48. CVID = common variable immunodeficiency; SCID = severe combined immunodeficiency; BCG = Bacillus Calmette-Guérin.**

rheumatic diseases (AIIRD). Vaccination of HIV-positive individuals[16] and renal transplant candidates and recipients[17] has been recently reviewed elsewhere and general recommendations for immunocompromised adults can be found on the website of the Centres for Disease Control and Prevention.[18]

The group of AIIRD consists of a variety of disorders (*table 3*). These patients are at increased risk of contracting infectious diseases,[19-22] resulting in significant morbidity and mortality. The increased susceptibility to infection can be contributed to several factors. Risk increases due to the underlying disease itself (such as hypocomplementaemia and lymphopenia in systemic lupus erythematosus (SLE) or neutropenia in Felty's syndrome in rheumatoid arthritis (RA)), but mostly because of treatment with immunomodulatory or immunosuppressive drugs such as (high-dose) corticosteroids, disease-modifying antirheumatic drugs (DMARDs) or biological agents. Even stem cell transplantations are performed for treatment of several AIIRD.[23] Biologicals are of particular interest since more indications are being recognised for these agents, they are increasingly used earlier in the course of AIIRD, and new agents become available.[24] Importantly, the administration of these drugs aims to reduce disease activity but might also influence the response on vaccination. In the remaining part of this review we will discuss per VPI the literature available concerning the efficacy and safety of vaccination in AIIRD and the influence of immunosuppressive drugs on the vaccination response. Most studies included RA or SLE patients.

Table 3. *Autoimmune inflammatory rheumatic diseases (AIIIRD) and vaccines*

Disease	Vaccines
Adult Still's disease	Bacillus Calmette-Guérin (BCG)#
Antiphospholipid syndrome	Cholera
Anti-synthetase syndrome	Diphtheria
Behçet disease	Hepatitis A
Churg-Strauss syndrome	Hepatitis B
Clinically amyopathic dermatomyositis	*Haemophilus influenzae* b
Dermatomyositis	Human papillomavirus
Eosinophilic fasciitis	Influenza
Eosinophilic myositis	Japanese encephalitis
Giant cell arteritis	Measles*
Goodpasture's disease	Mumps*
Microscopic polyangiitis	*Neisseria meningitidis* (A/C/Y/W135, C conjugated)
Mixed connective tissue disease	Pertussis
Mixed essential cryoclobulinaemia	Poliomyelitis (parenteral and oral*)
Periodic fever syndromes	Rabies
Polyarteritis nodosa	Rubella*
Polychondritis	*Streptococcus pneumoniae* (polysaccharide and conjugated)
Polymyalgia rheumatica	Tetanus toxoid
Polymyositis	Tick-borne encephalitis
Rheumatoid arthritis	Typhoid fever (parenteral and oral#)
Scleroderma	Varicella zoster*
Sjögren's syndrome	Yellow fever*
Spondylathropathies	
Sporadic inclusion body myositis	
Systemic lupus erythematosus	
Takayasu arteritis	
Wegener granulomatosis	

 # contains live bacteria; * contains live virus

INFLUENZA VACCINE

As mentioned before, in two retrospective studies, elderly patients (≥65 years) with chronic underlying diseases (including rheumatic diseases and vasculitis) showed an increased risk for influenza resulting in admission for pneumonia or death in comparison with healthy controls.[1,2] Whether this risk in patients with AIIRD can be reduced by influenza vaccination has not been addressed. All but one of the studies performed[25] have used the humoral response, that is the development of a protective level of antibodies to influenza (≥40, as measured by the haemagglutination inhibition assay) as outcome measure but have not used the contraction and severity of influenza infection as a primary endpoint.

Rheumatoid arthritis

In RA patients the majority of studies evaluating efficacy of influenza vaccination have shown similar efficacy compared with healthy controls. Use of DMARDs did not diminish the humoral response.[26-37] In several studies anti-tumour necrosis factor (TNF) treatment did not decrease the response to influenza vaccination either.[31-33,35] Only one study reported a modestly impaired response in anti-TNF users, not resulting in a lower percentage of seroprotection.[34] The combination of anti-TNF and methotrexate, however, might act synergistically and in patients on this combination a somewhat reduced response to influenza vaccination has been reported.[38] B-cell depleting therapy using rituximab severely hampers the immune response:[37,39,40] influenza vaccination in the first eight weeks after treatment with rituximab did not result in an antibody response. Six to ten months after rituximab treatment a partial restoration of the response could be observed.[37] None of the studies showed a significant increase in disease activity in RA patients following vaccination. However, it should be noted that none of these studies has been powered for safety.

Systemic lupus erythematosus

In SLE several controlled studies on the efficacy of subunit influenza vaccine have shown a similar humoral response in patients compared with healthy controls.[27,41-44] In one RCT[45] and three controlled studies[46-48] a modestly reduced response to influenza vaccination in SLE patients was found. In two other studies SLE patients showed a similar[30] or a slightly reduced *in vitro* response to immunisation, compared with disease controls (RA patients).[28] In most of the aforementioned studies that also addressed the influence of the use of immunosuppressive drugs on efficacy, no effect on the vaccination response was found.[41,43,45,46] Others, however, did find a lower response to vaccination in SLE patients on azathioprine,[47,49] steroids,[27] and hydroxychloroquine.[50] Occasionally, disease flares in SLE patients after influenza vaccination have been reported.[42,43] However, most of these reports originate from uncontrolled studies. Although disease flares have also been reported in controlled studies, this frequency is not increased in vaccinated patients compared with unvaccinated patient controls, and therefore represents the natural course of the disease.[25,30,45,49,51]

Other AIIRD

In two studies including patients with Wegener granulomatosis[52,53] and one study in patients with systemic sclerosis (SSc)[54] humoral responses following influenza vaccination were similar between patients and healthy controls. Use of immunosuppressive drugs did not affect the humoral response and disease flares developed at the same, low, frequency compared with unvaccinated patients.

PNEUMOCOCCAL VACCINE

The efficacy of pneumococcal vaccination is even more difficult to determine as no generally accepted response criteria are available. Moreover, different vaccines (polysaccharide and conjugated pneumococcal vaccines) are available containing different numbers of antigens of pneumococcal serotypes.

Rheumatoid arthritis

In RA, similar as well as reduced responses to pneumococcal vaccination have been reported.[55,56] Although small studies in RA patients on TNF-blocking agents suggested a reduction of efficacy by the use of these drugs,[56,57] such an effect could not be demonstrated in larger studies.[32,58,59] Two studies reported an impaired response to pneumococcal vaccination only in patients on the combination of methotrexate and anti-TNF, but not in those on these individual drugs.[58,60] Probably, the combination of methotrexate and anti-TNF results in a synergistic immunosuppressive effect. Finally, rituximab reduced the response to pneumococcal polysaccharide vaccine in patients vaccinated 28 weeks after rituximab administration.[61] Safety has only been addressed in uncontrolled trials. In these studies, no increase in disease activity following pneumococcal vaccination has been reported.[32,55,56,60, 61]

Systemic lupus erythematosus

Similar responses,[62,63] as well as reduced responses,[55,64-66] were observed in several controlled studies comparing SLE patients with healthy controls. Also in uncontrolled studies results vary. In one study all 20 patients showed a significant rise in antibodies to pneumococcal antigens,[67] whereas another study reported that only half of the patients developed a fourfold antibody rise.[68] In the studies that addressed the effect of immunosuppressives drugs, the combination of steroids and azathioprine or cyclophosphamide did not hamper the responses.[55,63,64,66] With respect to safety, in all studies that compared SLE patients with to those without pneumococcal vaccination the disease activity after vaccination did not differ between groups.[62,64,67] Also in uncontrolled studies no increase in SLE activity could be demonstrated following pneumococcal vaccination.[55,63,66,68,69]

Other AIIRD

In patients with psoriatic arthritis and ankylosing spondylitis similar responses after pneumococcal vaccination were found in patients with or without use of TNF-blocking agents.[56,70] In an uncontrolled study in 18 SSc patients, pneumococcal vaccination resulted in a protective level of antibodies to at least three out of four serotypes tested in 83% of the patients.[71] An increase in disease activity after vaccination was not reported in any of these studies.

HEPATITIS B VACCINE

A few studies addressed the efficacy of hepatitis B vaccination in AIIRD patients (SLE,[72] RA,[73] ankylosing spondylitis[57] and Behçet's disease[74]). In the majority of patients, irrespective of the underlying AIIRD, an adequate response to vaccination could be demonstrated; however, a clear conclusion can not be drawn due to low numbers and absence of controls in these studies. Influence of steroids or DMARDs was absent. In contrast, use of TNF-blocking agents severely hampered the response to hepatitis B vaccine in ankylosing spondylitis patients.[57] Hepatitis B vaccine (HBV) vaccination did not lead to more RA disease flares in 22 vaccinated RA patients compared with 22 unvaccinated RA patients.[73]

One uncontrolled prospective study regarding HBV vaccination in SLE patients revealed no significant change

in disease activity, as measured by SLE disease activity index (SLEDAI) score after vaccination.[72]
In a study of 13 Behçet patients, three developed aphtae following HBV vaccination but no severe flares were present.[74]

TETANUS TOXOID VACCINE

Rheumatoid arthritis

Tetanus toxoid vaccination also seems to be as efficacious in RA patients as in healthy controls.[26,75] The use of steroids or DMARDs did not reduce efficacy. Also treatment with RTX did not diminish response to tetanus vaccine in RA patients when administered 24 weeks after RTX treatment.[61] Adverse events occurred in comparable frequency in RTX-treated patients (22%) and in eight patients on methotrexate monotherapy (24.2%). These included itching, rash, soreness at the injection site, and malaise. No data are available regarding the efficacy of tetanus toxoid vaccine within 24 weeks after treatment with RTX.

Systemic lupus erythematosus

Controlled studies in SLE patients revealed a similar response to tetanus toxoid in comparison with healthy controls.[75-77] In a larger, uncontrolled study, comprising 73 SLE patients, >90% of patients achieved protection after vaccination.[68] Side effects of immunisation (mild local tenderness or erythema, low-grade fever, or malaise) were noted in only six (8%) of patients and none of the side effects required systemic treatment or change in medical therapy for SLE. Only one small study (nine patients) showed a diminished response to tetanus vaccination in SLE patients.[78] The use of steroids or DMARDs did not reduce efficacy.

OTHER VACCINES

In an uncontrolled study on vaccination responses in SLE patients, next to tetanus toxoid, also *Haemophilus influenza* B (HIB) vaccine was administered. This resulted in protection in 88% of the 73 included patients. A trend towards a lower response was observed in patients on immunosuppressive drugs.[68]
Severe complications, such as vaccine-associated poliomyelitis, have followed vaccination with live-attenuated viral and live-attenuated bacterial vaccines among persons with altered immunocompetence.[79-84] Based on these data live vaccines are considered contraindicated in AIIRD patients under immunosuppressive therapy[85] including biological DMARDs.[24] However, these recommendations are based on case reports from decades ago, in the majority dealing with severely immunocompromised HIV-infected individuals, before the introduction of cART.[79,81,83,84] Although the risk of administrating live virus by vaccination can not be denied, it has to be acknowledged that people with a reduced immune response are at increased risk of natural severe, even life-threatening infections with for example measles and Varicella.[86] The risk-benefit ratio might be in favour of vaccination. To address this question studies with live-attenuated virus vaccines have been undertaken in immunosuppressed patients including HIV-infected patients, those with renal failure, and other medical conditions associated with reduced immunocompetence. These studies showed safety of varicella vaccine and as a consequence in the USA the use of this vaccine in HIV-infected children with CD4+-T cell counts ≥15% of normal for their age can be considered.[87] Recently in 25 children and adolescents with juvenile rheumatic diseases varicella vaccine was administered, despite the use of methotrexate, steroids and other DMARDs.[88] A small proportion (20%) of patients developed a mild varicella rash in the first two weeks after immunisation; none of the patients had worsening of their underlying disease in the three months after vaccination as compared with the three months before vaccination. Efficacy was similar compared with a group of age-matched healthy controls. In a retrospective cohort study vaccination with live measles, mumps and rubella vaccine in children with juvenile idiopathic arthritis (JIA), the majority receiving methotrexate, did not result in an increase of disease activity, nor did it lead to measles, mumps or rubella infection.[89] In a prospective nested case-control study in JIA patients treated with methotrexate alone or in combination with etanercept these results could be confirmed.[90] Although no definite conclusions can be drawn from these studies for reasons as sample size and heterogeneity of the patients included, results are encouraging, and expanding of such studies in a broader group of even more immunocompromised patients is advocated.[91]

ADVISED VACCINATIONS IN AIIRD

Based on systematic literature review and expert opinion a working party of the European League Against Rheumatism recently formulated vaccination recommendations for patients with AIIRD.[92] As can be appreciated from the studies discussed above, influenza and pneumococcal vaccination should be strongly considered in this category of patients. As data on other vaccines are limited, the working party refers to national vaccination guidelines (for adults) concerning tetanus toxoid vaccination, hepatitis A and B vaccination, and for vaccines indicated for those who plan to travel. Two exceptions were

defined. First, patients with contaminated wounds who received rituximab within the last 24 weeks should get passive immunisation with tetanus immunoglobulins. Secondly, although it is advised to avoid live-attenuated vaccines whenever possible in immunosuppressed patients with AIIRD, herpes zoster vaccination may be considered in a subgroup of immunocompromised patients based on the prevalence of herpes zoster and burden of disease (post-herpetic neuralgia). A short overview of the major recommendations is given in *table 4*.

SUMMARY

Immunocompromised patients have an increased risk for morbidity and mortality due to (vaccine preventable) infectious diseases and vaccination for these patients seems indicated. However, comprehensive, generally accepted guidelines are lacking. This is due to the absence of a clear definition of an immunocompromised state, doubts about efficacy and safety (in particular with live-attenuated virus), and the lack of clear definitions of correlate of protection after vaccination. More studies with appropriate endpoints are needed to develop evidence-based vaccination guidelines and to allow their implementation. Until then, although merely based on expert opinion, patients at risk might be vaccinated according to the proposal as presented in this overview.

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Table 4. *Vaccine choice in immunodeficient patients*

Defect	Specific immunodeficiency	Vaccines to be considered*	Vaccines to be avoided	Comments
AIIRD	No immunosuppressive drugs	Strongly considered: influenza and 23-valent pneumococcal vaccine		
	On DMARDs	Strongly considered: influenza and 23-valent pneumococcal vaccine	Live-attenuated vaccines	Vaccination can be administered during DMARD therapy
	On TNF-blocking agents	Strongly considered: influenza and 23-valent pneumococcal vaccine	Live-attenuated vaccines	Vaccination can be administered during TNF-blocking therapy
	On B-cell depleting agents	Strongly considered: influenza and 23-valent pneumococcal vaccine	Live-attenuated vaccines	Vaccination ideally should be administered before starting B-cell depleting therapy
AIIRD	Hyposplenic/asplenic patients	Recommended: influenza, pneumococcal, Hib, meningococcal	Live-attenuated vaccines	

***For other vaccines (hepatitis B, HPV, tetanus toxoid, etc) it is recommended to adhere to national vaccination guidelines.**
AIIRD = autoimmune inflammatory rheumatic disorders; DMARD = disease-modifying antirheumatic drugs.

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Netherlands
The Journal of Medicine

REVIEW

An overview of the current diagnosis and recent developments in neuroendocrine tumours of the gastroenteropancreatic tract: the diagnostic approach

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ABSTRACT

Neuroendocrine tumours of the gastroenteropancreatic tract (GEP-NETs) comprise a group of very heterogeneous neoplasms, which are considered 'rare diseases'. Epidemiological studies on the incidence of GEP-NETs worldwide have reported a remarkable increase in the detection of these tumours. In a recent study, based on pathology reports (PALGA) to investigate the incidence of pancreatic and duodenal neuroendocrine tumours in the Netherlands from 1991 until 2009, we also noticed a significant increase in the incidence of these tumours. In particular, the incidence of non-functioning neuroendocrine tumours had significantly increased over this period. Remarkably, a substantial discrepancy was observed between the numbers of neuroendocrine tumours diagnosed in the clinical as opposed to the pathological setting, emphasising that these tumours provide a real diagnostic challenge. To improve the diagnosis of GEP-NETs, we advocate that these complex neoplasms should receive more specialised attention. In this mini-review we provide an overview of the current diagnostic approach to GEP-NETs, and add the recent developments in establishing the diagnosis of these tumours, in order to increase knowledge and awareness of GEP-NETs among clinicians and pathologists. Early detection in order to prevent morbidity from GEP-NETs is advocated.

KEYWORDS

Carcinoid, diagnosis, gastrointestinal, neuroendocrine tumour, pancreas

INTRODUCTION

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) are considered to be rare, heterogeneous and complex neoplasms.[1] They include the pancreatic (PNETs) and gastrointestinal (GI) neuroendocrine tumours (GI-NETs) or carcinoids, which share their origin of cells from the diffuse neuroendocrine system, but further show many differences regarding pathogenesis, clinical behaviour and prognostic outcome.[2,3] Characteristic for GEP-NETs is their ability to produce bioactive substances (*table 1*).[4] Based on the clinical symptoms and syndrome caused by these peptides, they can be divided into functioning (F-NETs) and non-functioning tumours (NF-NETs). Due to their heterogeneity, GEP-NETs often provide a diagnostic challenge to physicians. Although GEP-NETs are generally more indolent than carcinomas, the majority are malignant, showing aggressive tumour behaviour and presenting with metastases at diagnosis.[1] GEP-NETs can occur sporadically, or as part of a hereditary syndrome such as multiple endocrine neoplasia syndrome type 1 (MEN-1), von-Hippel Lindau disease (vHLD), neurofibromatosis type 1, or tuberous sclerosis.[5]

In 2007, a summit meeting on the major clinical, pathological and scientific challenges in the field of GEP-NETs was held to debate on potential solutions.[6] There was consensus between the participants that there is worldwide a substantial lack of knowledge, experience and reliable research concerning GEP-NETs. In line with these observations, we feel that also in our country, GEP-NETs indeed present a relatively unknown and underdeveloped subject with fairly limited knowledge among most physicians. However, since several epidemiological studies

Table 1. *Symptoms and syndromes associated with neuroendocrine tumours of the gastroenteropancreatic tract*[1-4]

Gastrointestinal neuroendocrine tumours	
Functioning neuroendocrine tumours	**Non-functioning neuroendocrine tumours**
Carcinoid Flushing, diarrhoea, and wheezing	Abdominal pain, weight loss, anorexia, jaundice, nausea and vomiting, intra-abdominal haemorrhage

Pancreatic neuroendocrine tumours

Functioning neuroendocrine tumours

Insulinoma
Neuroglycopenic symptoms such as headache, blurred vision, confusion, dizziness, lethargy, and amnesia. Autonomic nervous system symptoms such as sweating, weakness, anxiety, tremor, palpitations, and nausea

Gastrinoma
Diarrhoea, abdominal pain, heartburn, weight loss, nausea and vomiting, faecal blood loss

Glucagonoma
Necrolytic migratory erythema, diabetes mellitus, cachexia

VIPoma
Watery diarrhoea, hypokalaemia, achlorhydria, hyperglycaemia, hypercalcaemia, flushing

Somatostatinoma
Diabetes mellitus, cholelithiasis, steatorrhoea, anaemia, weight loss

Other (rare) pancreatic functioning neuroendocrine tumours

ACTHoma
Cushing's syndrome

GRFoma
Acromegaly

PTH-RP tumour
Hypercalcaemia

Non-functioning neuroendocrine tumours

Abdominal pain, weight loss, anorexia, jaundice, nausea and vomiting, intra-abdominal haemorrhage

have shown an increase in the incidence of GEP-NETs worldwide, in combination with the fact that these tumours, when accurately managed, provide a relatively good prognosis for the patients, we feel that it can be worthwhile to increase the awareness of and knowledge about GEP-NETs among clinicians and pathologists, in order to further increase the early detection and prevent morbidity from GEP-NETs.[7-10]

In this mini-review, we describe the current diagnostic approach of GEP-NETs, in combination with several common pitfalls and some recent developments, to improve the diagnosis of these tumours. In addition, we provide a diagnostic algorithm to facilitate their diagnostic approach.

EPIDEMIOLOGY

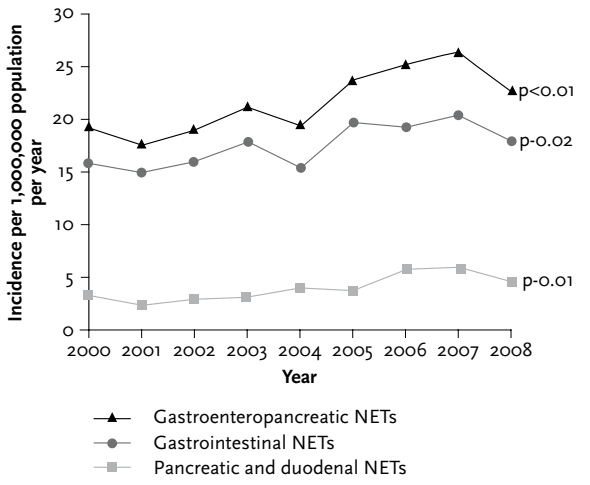
Based on pathology information from PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands, we calculated incidence of GEP-NETs from 2000 until 2008 in the Netherlands.[8,11] For both pancreatico-duodenal NETs and GI-NETs a significant increase in incidence over time was noticed (*figure 1*).

However, these calculated incidence rates are based on pathology information only and therefore might represent an underestimation. In our study, we found that this was approximately 25%, due to the fact that some patients with clinically diagnosed gastrinomas were not included in the PALGA database, because they had not undergone any surgery, biopsy and/or other pathological evaluation for their tumour.[8] This discrepancy between clinical and pathology incidence of GEP-NETs is an important issue concerning these tumours, which will be discussed later. Nonetheless, this pattern of increasing incidence rates indicates and confirms that GEP-NETs might not be as rare as previously thought. Whether this increase is due to improved detection methods rather than to a true rise in the existence of these tumours is debatable. In that context it is important to note that we observed that 4% and 14% of the GI-NETs and pancreaticoduodenal NETs respectively, were found incidentally at autopsy, which indicates that, despite improved detection methods, some GEP-NETs still remain undetected.

CURRENT DIAGNOSTIC PROCEDURE FOR GEP-NETS

Symptoms of patients with GEP-NETs are in general related to the localisation and hormonal production of the

Figure 1. *Current incidence of GEP-NETs in the Netherlands from 2000 till 2008.*



Using linear regression, trends in annual incidence rates over 2000 till 2008 were analysed. A statistically significant increase was observed in the overall annual incidence of all GEP-NETs, and GI-NETs and pancreaticoduodenal NETs separately, over the study period

tumour.[1] Frequently, symptoms are vague and aspecific, although symptoms associated with a clinical syndrome may arise suspicion for a F-PNET, *table 1*.[1]

Next to standard medical history and physical examination, laboratory analyses are crucial in the diagnosis.[12,13] To diagnose NETs, chromogranin A (CgA) levels can be determined in plasma/serum, or immunohistochemically.[14,15] Increased plasma/serum levels of CgA have been reported to correlate with a worse prognosis in these patients. Increased levels of 5-hydroxyindoleacetic acid (5-HIAA, the breakdown product of serotonin) can be determined in a 24-hour urine sample collection, and indicate the presence of a serotonin-producing tumour. Increased levels of hormones such as insulin, indicate the presence of a hormone-secreting functioning PNET.

Imaging of GEP-NETs includes endoscopy or gastroscopy, octreoscan, computerised tomography (CT) scan, or magnetic resonance imaging (MRI) scan.[16]

Pathological examination of biopsies or surgical specimens reveals the verification of the neuroendocrine nature of the tumour by immunohistochemistry, for pan-neuroendocrine markers such as keratin, CgA, neuron specific enolase (NSE), synaptophysin, grimelius, and CD56. A proliferation marker (Ki67 or MIB1) must be used to assess the degree of differentiation and proliferation, to grade the tumours according the World Health Organisation (WHO) classification.[17] Tumour characteristics as localisation, size, composition, relationship to anatomic structures, resection margins, and the presence of metastases, should be assessed in order to classify the tumour according to the tumour node metastasis (TNM) staging system.[4]

PITFALLS IN THE DIAGNOSIS OF GEP-NETS

One of the major pitfalls in the nomenclature of neuroendocrine tumours is the use of the term 'carcinoid'. In 1907, Oberndorfer introduced this term for neuroendocrine tumours with a relatively 'benign' course.[18] Increasing knowledge about these tumours, however, led to the conclusion that carcinoids also encompass low-grade and high-grade malignant tumours. Therefore, Soga *et al.* called the term 'carcinoid' a 'misnomer'.[19] In fact, this term has been used for different goals; whereas pathologists label all tumours with neuroendocrine features as a 'carcinoid', clinicians use 'carcinoid' for serotonin-producing tumours that lead to the carcinoid syndrome. Therefore, Capella *et al.* suggested replacing 'carcinoid' by 'neuroendocrine tumour' to include all tumours with neuroendocrine features, but also realised that abandoning this term completely would be too confusing, and therefore proposed to utilise it for the specification of a NET with serotonin production or producing any other substance which may lead to the carcinoid syndrome.[20] As consensus in the use of the GEP-NETs nomenclature is highly desirable, we propose that henceforth 1) the term 'carcinoid' should be used solely in the clinical setting, and only for those tumours that lead to the carcinoid syndrome as a result of the hypersecretion of serotonin, prostaglandins, or tachykinins by the tumour, characteristic of symptoms such as flushing, diarrhoea and wheezing; 2) pathologists distinguish the various types of neuroendocrine tumours; neuroendocrine tumours should be defined according to the classification of the WHO, thereby replacing 'carcinoid' by 'neuroendocrine tumour' for well-differentiated low-grade malignant carcinoids, whereas malignant carcinoids should be defined as 'neuroendocrine carcinomas'.

Another misunderstanding among pathologists and clinicians has arisen due to the lack of a standardised definition of functioning and non-functioning tumours, as pointed out by Halfdanarson *et al.*[7] Although non-functioning tumours are characterised by the lack of a clinical syndrome, they might secrete hormonal peptides as well, but only those tumours leading to clinical symptoms are referred to as functioning. For example, increased blood levels of pancreatic polypeptide or neurotensin can be found in NF-PNETs.[21] Warner *et al.* already reported that plasma hormone levels do not always correlate with the presence of a clinical syndrome.[22] For example, in case of the Zollinger-Ellison syndrome, fasting serum gastrin levels may be non-diagnostic (i.e., <1000 ng/l), or symptoms might be masked by the use of proton pump inhibitors or histamin receptor antagonists, or pernicious anaemia. Furthermore, it is reported that the hormonal secretion by the tumour is not always reflected in immunohistochemical staining for this hormone at pathology.[23] For a standardised approach, we recommend that the clinical diagnosis is superior to the pathological observations concerning the designation of the tumour as 'functioning' or 'non-functioning'. In other words, in the absence of immunohistochemical positivity for a certain hormone in combination with increased serum levels of that particular hormone and/or the presence of a clinical syndrome, the tumour should be defined as 'functioning'. In the opposite situation, i.e., a positive staining at pathology, but absence of increased serum levels and/or a clinical syndrome, the clinical presentation should be decisive, and the tumour should be defined as 'non-functioning'.

Next, the existence of 'benign' GEP-NETs is disputed. Whereas the majority of GEP-NETs are considered to be malignant, insulinomas and appendiceal carcinoids are not. However, we believe that all GEP-NETs have malignant potential, and that early diagnosis of these tumours, because of the symptoms they cause, leads to the assumption that they are benign. Namely tumour size and/or invasion, and the presence of metastases, all characteristics which can

be 'prevented' by early detection, lead a tumour to being referred to as malignant.[17,20] The fact that the majority of NF-NETs have a poor prognosis underlines that absence of clinical symptoms leads to a delay in diagnosis and a consequently more progressed tumour.

Another difficulty in diagnosing GEP-NETs arises as these tumours show a relatively high frequency of 'ectopic occurrence'. For example, gastrinomas, which are usually located in the pancreaticoduodenal region and lymph nodes, have been reported on ectopic locations such as ovaries, biliary tract, kidneys, stomach and liver.[24] Recently, we reported on a patient with recurrent hepatic gastrinomas, in whom no pancreatic, duodenal or other primary tumour could be detected in an intensive, 20-year follow-up.[25] In the literature, primary hepatic gastrinomas were described in about 20 patients, but real evidence for their primary origin (rather than being metastatic) was lacking. We believe that it is therefore uncertain whether these ectopic locations comprise primary gastrinomas rather than metastases of occult primaries. Furthermore, GEP-NETs have been reported in rare locations such as the oesophagus, gallbladder and biliary ducts, Meckel's diverticulum, ampulla of Vater, genital tract and skin.[26,27] Lack of awareness that neuroendocrine lesions can also occur on these unusual sites results in the consequence that these tumours are frequently misdiagnosed or overlooked.[27] Therefore, we recommend that when imaging is not successful in detecting a neuroendocrine tumour in the usual sites, an intensive search for occult tumours at unusual sites should be started.

Additionally, it is important to realise that GEP-NETs frequently occur as or together with a second primary malignancy.[28] The presence of a simultaneous second primary or metastatic malignancy must be thoroughly examined, as several case reports describe the existence of a second tumour synchronous with a carcinoid lesion.[28-32] For example, gastrointestinal stromal tumours (GIST) are frequently seen in combination with (gastric) carcinoids.[29,31] Furthermore, patients suffering from hereditary syndromes such as MEN-1, vHLD, neurofibromatosis type 1 or tuberous sclerosis, are at increased risk for a GEP-NET. Therefore, alertness for synchronous (neuroendocrine) tumours among clinicians is advocated. Furthermore, members from hereditary GEP-NET disorder families should be checked for such tumours, preferably by genetic counselling and, if possible, DNA profile, or by measurement of markers for these or associated tumours.

RECENT DEVELOPMENTS IN THE DIAGNOSIS OF GEP-NETS

As CgA is produced by all types of neuroendocrine cells, it serves as a highly sensitive neuroendocrine cell marker.[14,15] In 2006, Kidd *et al*. demonstrated that also CgA mRNA and protein levels were useful in the detection of gastrointestinal carcinoids and metastases.[33] Recently, Modlin *et al*. showed that measurement of circulating mRNA of CgA (and other markers such as Tph1 and NSE) provides a promising new diagnostic method for NETs.[34] Next to CgA, several studies into other markers have been reported. In particular, investigators are interested to find markers which can discriminate between the diverse GEP-NET subtypes. Long *et al*. demonstrated that PAX8 might be a useful immunohistochemical marker in the discrimination of pancreatic and ileal NETs, as the latter lack expression of this transcription factor.[35] However, Hosoda *et al*. found that immunohistochemistry on endoscopic ultrasound (US) biopsy specimens using a selected panel of markers, including CK-7, CDX-2, synaptophysin, CgA, and the KRAS mutational status, could be used to discriminate endocrine tumours from two other major types of pancreatic cancers (i.e., invasive ductal carcinoma and acinar cell carcinoma).[36] A comparable study was performed by Burford *et al*., who found that strong immunohistochemical expression for E-cadherin and B-catenin were characteristic for PNETs, and could be used to discriminate from solid pseudopapillary neoplasm, in which staining is absent.[37] Another selected panel, including CDX-2, NESP-55, TTF-1 and PDX-1, was described to be useful to discriminate between metastatic NETs of pancreatic, gastrointestinal and pulmonary origin, in a study by Srivastava *et al*.[38] In contrast, Fendrich *et al*. found that PDX-1 expression was present in pancreatic but not duodenal gastrinomas, and PDX-1 expression in combination with Shh and PP expression in resected metastases might aid to locate undetected or occult primary gastrinomas.[39] However, all the above-mentioned studies are non-conclusive, and further research and validation studies are needed before these diagnostic tools can be used in practice. Based on a literature review and analysis of the utility of plasma/serum CgA measurements in NETs, Modlin *et al*. concluded that CgA still serves as the most specific (86%) and sensitive (68%) biomarker in plasma/serum to diagnose NETs that is currently available.[40]

The improvement of imaging techniques is one of the most probable explanations for the incidence increase of GEP-NETs. For example, in a study by Ishikawa *et al*., endoscopic US combined with contrast enhancement showed the best results in the preoperative localisation of PNETs in comparison with other imaging techniques, such as CT and US.[41] Prasad *et al*. reported that occult primary NETs could be detected by PET/CT using 68Ga-DOTA-NOC receptor in 59% of patients with confirmed NETs on biopsies from metastatic lesions, which was approximately three times higher than with CT alone.[42] Also, research is ongoing in the field of genetic and molecular pathology. Previously, three detailed review articles

Figure 2. *Diagnostic algorithm for neuroendocrine tumours of the gastroenteropancreatic tract (GEP-NETs)*

I. CLINICAL DIAGNOSIS

1. Detailed personal history and physical examination

See *table 1* for an overview of symptoms related to the various types of GEP-NETs

2. Determine localisation if possible, using:

- EUS or endoscopy in combination with CT scan or MRI scan
- Somatostatin receptor scintigraphy or Octreoscan

→ Positive imaging: Continue with 3

→ Negative imaging: thorough search for occult tumours at unusual locations, continue with 3

3. Measure plasma or serum CgA levels

To verify the neuroendocrine nature of tumour

4. Measure hormone levels in serum

To detect possible peptide production by the tumour in order to define the tumour as 'functioning' or 'non-functioning'.
Note: Only define a tumour as a 'carcinoid' in case of increased serotonin serum levels and/or urinary 5-HIAA elevations, and/or the presence of the classical 'carcinoid syndrome' (see table 1).

5. Confirm diagnosis with a specific diagnostic test

Positive test: Diagnosis confirmed, continue with II

Negative test: consider non-functioning tumour and/or differential diagnosis, continue with II

6. Investigate the presence of a hereditary syndrome

- Detailed family history
- Investigation for associated tumours and/or lesions
- Gene testing

Note: Consider the presence of synchronous tumours in case of gastric carcinoids (GISTs) or the presence of a hereditary syndrome.

II. PATHOLOGICAL DIAGNOSIS

1. Immunostaining

- Staining for general neuroendocrine markers including chromogranin A, synaptophysin, NSE, keratin and grimelius, to determine the neuroendocrine nature of the tumour.
  Note: For the definition of a neuroendocrine tumour, at least one of above-mentioned general neuroendocrine markers should show a positive staining
- In case of a clinical (diagnosis or suspicion for) functioning tumour; Stain for specific hormones including serotonin, gastrin, insulin, glucagon, somatostatin, and/or VIP

  Note: Be aware that, also in case of a clinical functioning tumour, immunohistochemical staining for the particular hormone can be absent. Immunohistochemical staining should aid in determining the diagnosis, and determine the actual diagnosis

2. Determine WHO classification

- Determination of proliferation index by Ki67 or MIBG1
- Determination of mitotic count
- Investigate tumour characteristics:
  - size
  - histological pattern
  - relation to other structures/invasion
  - angioinvasion
  - metastases

Note: Define the tumour as NET or NEC, not carcinoid. The term carcinoid should only be designated (by clinicians) to tumours with serotonin production and/or in the presence of the classical carcinoid syndrome (table 1).

3. Determine TNM stage

- Determine tumour localisation
- Determine tumour size
- Determine invasion of the tumour into surrounding organs/structures
- Determine the presence of lymph node metastases
- Determine the presence of distant metastases

EUS = endoscopic ultrasound; CT = computerised tomography; MRI = magnetic resonance imaging; 5-HIAA = hydroxyindoleacetic acid; NSE = neuron specific enolase; VIP = vasoactive intestinal peptide; WHO = World Health Organisation; NET = neuroendocrine tumour; NEC = neuroendocrine carcinoma; TNM = tumour node metastasis staging system.

that describe recent advances in the molecular genetics of sporadic and familial GEP-NETs, were reported.[5,43,44] Therefore, this review will not discuss this subject in detail.

DIAGNOSTIC ALGORITHM

The algorithm comprises a clinical and a pathological part. Although the pathological evaluation is important in the diagnosis, the clinical presentation largely determines the definition of a NET. However, we advocate an interdisciplinary cooperation between clinicians and pathologists in the diagnostic approach of GEP-NETs.

Although research into specific biomarkers to detect GEP-NETs is ongoing, studies are still inconclusive. Therefore, we recommend CgA as a highly specific and sensitive neuroendocrine marker in the diagnosis of NETs. CgA measurement in plasma/serum, and immunostaining for this marker on biopsy or surgical specimens, should be performed routinely by clinicians and pathologists, respectively, in order to adequately diagnose (or exclude) a NET.

Imaging techniques to detect NETs are improving. The use of various imaging tools combined is advocated. In specialised centres, relatively new imaging modalities including PET scan can be used in the localisation of a NET. Repeatedly negative imaging results in detecting a primary NET should raise a physician's suspicion for an ectopically localised NET. Furthermore, the presence of a secondary tumour should be investigated, in particular when a hereditary syndrome is present.

For standardised documentation and in order to determine the therapeutic approach, tumours should be categorised according the WHO and TNM classification (*figure 2*).

CONCLUSION

GEP-NETs compose a complex and heterogeneous tumour entity, which forms a diagnostic challenge to physicians. In this review, we aimed to provide a clear overview of current diagnostic procedures and common pitfalls for GEP-NETs. Taking some recent diagnostic developments into account, we propose a diagnostic algorithm for GEP-NETs, to generate a more standardised diagnostic approach, facilitate the diagnosis, and eventually improve the early detection of these tumours.

AUTHORS NOTE

These data were presented at the Meeting of the 'Nederlandse Vereniging van Gastro-enterologie (NvGE)' 19 March 2010, Veldhoven, the Netherlands.

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Netherlands
The Journal of Medicine

REVIEW

Therapy in pneumonia: What is beyond antibiotics?

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ABSTRACT

Community-acquired pneumonia (CAP) is a common and serious disease with significant mortality, morbidity and associated healthcare costs. Severity of pneumonia is related to the extent of the inflammatory response. Primary goal in the treatment of pneumonia is starting adequate antibiotic therapy as soon as possible. However, antimicrobial resistance among the most common bacteria causing pneumonia is increasing. For those two reasons, extended inflammatory response and increasing antibiotic resistance, it is interesting to look at adjunctive non-antibiotic therapeutic strategies aimed at modulation of the inflammatory response or at the micro-organism itself. In this review, we discuss the current knowledge regarding these therapies and their possible role in the future.

KEYWORDS

Pneumonia, therapy, corticosteroids, immunomodulation

INTRODUCTION

Community-acquired pneumonia (CAP) is the most common infectious disease that necessitates hospitalisation.[1] Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) are serious complications of hospital stay and mechanical ventilation, respectively.[2,3] Despite advances in prevention by vaccination, microbiological diagnostics and antibiotic therapy, pneumonia is still characterised by a high mortality and morbidity and is associated with significant healthcare costs.[4,5] The mainstay of CAP therapy is early diagnosis and initiation of appropriate antibiotic therapy within four hours to minimise the door-to-needle time.[6] Antibiotic therapy for HAP and VAP is even more challenging due to the increase in antibiotic resistance of Gram-negative bacteria.[7] Unfortunately, there is also a trend of increasing antibiotic resistance in the most common bacteria that cause CAP, *Streptococcus pneumoniae* and *Haemophilus influenzae*.[8,9] Despite adequate antibiotic therapy, a substantial number of patients at high risk of deterioration require additional therapies for pneumonia. These therapies are aimed either at the micro-organism or at the host. Therapeutic targets are improvement of recognition of microbial antigens (with complement or toll-like receptors), improvement of effector mechanisms of the immune response (with immunoglobulins) and limiting immunopathology caused by the cytokine storm (with corticosteroids, statins or activated protein C (APC)). Certain antibiotics, such as macrolides, can also limit the damage caused by an overactive immune system. We will limit this review to treatment options for an immunocompetent hospitalised patient receiving appropriate antibiotics.

THERAPY TARGETED AT IMPROVEMENT OF BACTERIAL OPSONISATION

Complement cascade

The complement system can be activated in three ways: classical pathway activation after antibodies have been bound to the micro-organisms, alternative pathway activation, and activation by mannose residues (mannose-binding lectin (MBL) route). Complement activation via either route ultimately results via a cascade of steps in the formation of a membrane-attack complex, which results

in lysis of the pathogen. Other complement split products are deposited on the surface of the micro-organism which promote its phagocytosis. MBL binds to several respiratory pathogens including *Haemophilus influenza,*[10,11] *Mycoplasma pneumonia*[12] and *Legionella pneumophila,*[13] and to a lesser degree *Streptococcus pneumoniae.*[14]

Polymorphisms in the structural and promoter sequences of the *MBL2* gene lead to a deficiency in MBL production, with a frequency of approximately 10 to 15% in the normal healthy population.[15]

Susceptibility to lower respiratory tract infections does not seem to be affected by MBL deficiency.[16-18] However, MBL deficiency is associated with a more severe clinical course of pneumonia and the development of more severe forms of sepsis, ICU admission and fatal outcome in lower respiratory tract infections.[18,19] Also in invasive pneumococcal disease, several studies found an increased frequency of MBL deficiency.[20-22]

Thus far, MBL substitution therapy has only been tested in phase I and II trials, and to date no negative clinical effects are reported.[23,24] However, over-substitution should be avoided because high MBL genotypes are associated with nephropathy in patients with diabetes mellitus type 1 and vascular tissue damage in myocardial ischaemia-reperfusion injury.[25,26] To date, MBL replacement has not been used in pneumonia patients. MBL substitution might be of value as adjunctive therapy for MBL-deficient patients.

Toll-like receptors

Toll-like receptors (TLRs) are a family of receptors that activate the inflammatory response after recognition of molecular patterns that are present on different pneumonia-associated micro-organisms.[27,28] The role of TLRs in sepsis has been recently reviewed.[29,30] Polymorphisms in the genes coding for TLRs are associated with increased susceptibility to (severe) sepsis, including pneumonia or sepsis caused by *S. pneumoniae.*[31,32] Because they are a major trigger for the inflammatory response, TLRs are regarded as a promising target for anti-inflammatory therapy.

In an animal model for pneumococcal pneumonia, triggering of TLR5 with its ligand, flagellin, leads to substantially better survival.[33] This shows the importance of the immediate activation of the innate response in clearance of a pulmonary infection.

As indicated above, over-activation of the inflammatory response can cause substantial damage and should therefore be avoided. TAK-242 is an agonist of another TLR, TLR4, and inhibits intracellular signalling, thereby preventing up-regulation of the inflammatory response. TLR4 is a lipopolysaccharide (LPS) binding receptor, and Gram-negative LPS containing bacteria are a major cause of severe sepsis in critically ill patients. TLR4 is the only receptor in which blocking seems an interesting additive therapy.[34,35] The first recently published double-blind randomised trial comparing TAK-242 to placebo in patients with severe sepsis and septic shock did not show a difference in mortality. Furthermore, treatment with TAK-242 did not succeed in decreasing cytokine levels, which suggests that other inflammatory pathways are involved.[36] These studies have been performed in critically ill patients, and subgroup analyses of patients with pneumonia are lacking. Furthermore, TLR4 is mainly involved in the inflammatory response to LPS-containing bacteria, and these bacteria are uncommon in community-acquired pneumonia.

Currently, there is no role for TLR antagonists in the treatment of pneumonia, or severe sepsis or septic shock.

THERAPY TARGETED AT IMPROVEMENT OF EFFECTOR MECHANISMS OF THE IMMUNE RESPONSE

Immunoglobulins

In the period before antibiotics were available (up to 1940) treatment of pneumococcal pneumonia consisted of the passive administration of serotype specific antibodies.[37] Nowadays, substitution therapy with immunoglobulins is used for long-term treatment of patients with humoral immunodeficiency diseases.[38,39] By replacing or increasing the levels of immunoglobulins, especially Immunoglobulin G (IgG), the inflammatory response to the bacteria could be improved by trapping endotoxins and facilitating phagocytosis. Clinical studies on the use of intravenous immunoglobulins IgG (IVIG) in infectious disease are limited and mainly focused on patients with streptococcal toxic shock syndrome and severe sepsis and septic shock.[40] Although consecutive reviews showed improved outcome of patients treated with IVIG, the use of immunoglobulins in critically ill patients is still controversial.[41-44] It is unclear whether the benefit of IVIG therapy was due to the antibody concentration or to volume resuscitation with proteins, or to an anti-inflammatory effect.[45] All studies contained numerous patients with severe sepsis or septic shock due to pneumonia, but there were no subgroup analyses investigating the effect of IVIG in patients with pneumonia. Therefore, the use of immunoglobulins for pneumonia in general remains unclear and remains restricted to patients with severe sepsis or septic shock.

THERAPY TARGETED AT LIMITING IMMUNOPATHOLOGY

Corticosteroids

The inflammatory cytokine response in the lung is characterised by a short intense elevation in TNF-α

followed by increases in IL-1β and IL-6. A subsequent increase in IL-10, which is an anti-inflammatory cytokine that inhibits macrophage and neutrophil production, is the beginning of the anti-inflammatory response that prevents an uncontrolled inflammatory response.[46-48] In pneumonia patients, this hierarchy of cytokine response is not observed, because the inflammatory response is already ongoing upon admission to the hospital.[49] In most patients these cytokines control and eliminate the primary infection; however, in some patients, the cytokine activation becomes widespread. This indicates the need for a delicate balance between a sufficient and excessive cytokine response. The extended systemic inflammatory response is presumed to play a role in the organ dysfunction that is characteristic of severe sepsis and septic shock.[50] Among patients with pneumonia, non-survivors of CAP exhibit persistent elevation in inflammatory cytokine levels over time, compared with survivors.[49,51] Modulation of this inflammatory response during infection is an attractive concept.

Corticosteroids are the most important physiological inhibitors of inflammation. They can switch off genes that encode pro-inflammatory cytokines and switch on those that encode anti-inflammatory cytokines.[53] Prolonged (>5 days) treatment with low-dose corticosteroids can down-regulate inflammatory cytokine transcription and accelerate the resolution of critical illness.[53] In addition to this direct effect on gene transcription, recent observations have shown that corticosteroids might be effective in patients with severe sepsis due to relative adrenal insufficiency associated with critical illness and systemic inflammation-induced glucocorticoid receptor resistance. Not only in severe sepsis and septic shock, but also in pneumonia there are different reasons in support of a beneficial effect of corticosteroids.[54,55] Corticosteroids might be effective in reducing excessive pulmonary inflammation and thereby reducing lung injury.[56] Furthermore, in some cases of pneumonia, bronchospasm can play a significant role (e.g., in patients with COPD/asthma or viral-induced pneumonia), which can be counteracted by corticosteroids.[57,58]

Over the last several decades, corticosteroids have been used as adjunctive therapy in patients with sepsis and septic shock. Initial trials investigating short courses of high doses found no beneficial effect on mortality, whereas more recent trials showed that a low dose (< 300 mg/d) of hydrocortisone for a longer duration (>5 days) may improve survival.[59-62]

In contrast to this large number of studies on sepsis and septic shock, randomised controlled trials (RCT) using corticosteroids as an adjunctive treatment to antibiotics in pneumonia are limited and have variable results. The use of corticosteroid treatment in CAP dates back to 1956, when favourable effects of hydrocortisone in patients with pneumococcal pneumonia were reported.[63] Two more recent studies found a significant reduction in hospital mortality and length of hospital stay in patients with severe CAP who were treated with adjunctive corticosteroids. Confalonieri *et al*. found a marked improvement in the ratio of the partial pressure of oxygen in arterial blood (Pa_{O_2}) to the fraction of inspired oxygen (Fi_{O_2}) as well as a survival advantage in patients with severe CAP treated with hydrocortisone for seven days. A retrospective study showed that patients with severe CAP who were treated with systemic corticosteroids had a reduced risk of mortality compared with patients without adjunctive corticosteroids.[64] A smaller randomised controlled trial (RCT) compared prednisolone for three days with a placebo in patients with CAP of any severity and found no effect on hospital stay; however, in patients with moderate or severe CAP, corticosteroids promoted resolution of clinical symptoms and reduced the duration of intravenous antibiotic treatment.[65] To date, a recent study by Snijders *et al*. is the largest to evaluate the role of prednisolone in patients with CAP of any severity.[66] In that RCT no beneficial effects of adjunctive corticosteroids on CAP were found.

There may be some potential adverse effects of the use of corticosteroids for CAP. From a theoretical point of view, the risk of gastrointestinal bleeding, muscle weakness and metabolic disorders is increased. In addition, down-regulation of the host response to infection might increase the risk of nosocomial infections and reactivation of viral infections. In a systematic review of 20 RCTs that involved adjunctive corticosteroid therapy in sepsis, these serious adverse events did not occur more often than in placebo-treated patients. However, hyperglycaemia and hypernatraemia were observed more frequently in the corticosteroid-treated patients.[67]

Statins

In addition to modulation of the inflammatory response by corticosteroids, in experimental studies statins have shown to have significant anti-inflammatory properties.[68] These benefits are not ascribed to their cholesterol-lowering activity but rather to a pleiotropic effect on isoprenoid synthesis that results in the down-regulation of intracellular inflammatory signalling; this leads to modulation of the immune response, which results in a reduction in cytokine levels.[68] Moreover, statins improve endothelial function and may modify the balance of coagulation towards a less prothrombotic state, as seen in sepsis. Large retrospective observational studies have shown a potential positive effect on mortality in patients with severe infections or sepsis.[69,70] However, in a prospective cohort study, statins were not associated with

reduced mortality or less ICU admissions.[71] Large RCTs are needed to evaluate the effect of an intervention with statin therapy during CAP.

Activated protein C

An exaggerated inflammatory response can result in a decline in protein C, which is a soluble anticoagulant and prefibrinolytic enzyme. Reduced levels of activated protein C (APC), which leads to a procoagulant state, are associated with an increased risk of death in septic patients.[72] In patients with severe sepsis, APC has been shown to reduce mortality (PROWESS trial).[73] This may be due to its anticoagulant activity, but there is also evidence that APC is an inhibitor of the systemic inflammatory response.[74] In a subanalysis of the PROWESS trial, a survival benefit was seen in patients with CAP-associated sepsis and a high mortality risk (APACHE >25) who were treated with APC compared with placebo.[75] However, administration of APC increases the risk of serious bleeding, with reported rates of up to 10%.[76] Therefore, recent guidelines recommend that APC should only be considered in patients with severe sepsis and a high risk of death but not in the overall CAP population.[77]

Macrolide antibiotics

Several antibiotics that are used in the therapy of CAP appear to have actions beyond direct antibacterial activities. Macrolides are known to also possess immunomodulatory effects.[78] Macrolides accumulate in inflammatory cells and modulate their actions, which results in modification of leukocyte function, cytokine expression and mucus production. Macrolides infer a biphasic effect on the host. First, they have direct antimicrobial activity by stimulating the host defence against bacteria via stimulation of leukocyte degranulation, phagocytosis and oxidative burst. Second, after the acute infection, neutrophils that are primed by cytokines or lipopolysaccharide (LPS, is an endotoxin) are inhibited by macrolides, which leads to resolution of the inflammatory response. Moreover, macrolides may also improve macrophage function, which results in the increased removal of apoptotic debris.[79] Another potential explanation for the beneficial effects of macrolides is reduction in bacterial load with less cell wall lysis than beta-lactam antibiotics; this results in a more gradual reduction in bacterial load and, therefore, a more gradual release of immunologically reactive components, which may prevent an extended systemic inflammatory response.

The beneficial effect of macrolides has been recognised in chronic pulmonary diseases, probably through inhibition of quorum-sensing in bio films, but some studies found improved outcomes in CAP patients who were treated with macrolide-containing antibiotic regimes.[80,81] The outcome in pneumococcal pneumonia was improved when a macrolide was added to standard treatment, even when the bacteria was sensitive to standard treatment.[82,83] This effect appears to be most prominent in severe bacteraemic pneumococcal pneumonia.[84] However, other studies were unable to show a beneficial effect of macrolides in CAP.[85,86]

CONCLUSION

At this moment, timely administration of appropriate antibiotics is still the most important therapy in pneumonia.[87] Beyond antibiotics, there are other targets for adjunctive therapy. For immunoglobulins, APC and TLR4 antagonists, the majority of evidence is extrapolated from studies on severe sepsis and septic shock. Many patients in these studies suffered from pneumonia, but reliable subgroup analysis was only performed in some of these studies. Furthermore, results from these studies are conflicting and most meta-analyses do not provide firm conclusions. The only conclusion that can be drawn is that immunoglobulins are a promising therapy in patients with pneumonia and severe sepsis or septic shock. APC might be used in patients with pneumonia and severe sepsis or septic shock with an APACHE score >25. To date, for the patient with CAP there is no role for therapy with TLR4 antagonists or MBL. Adding macrolides to the antibiotic regimen is an interesting and promising strategy, but prospective RCTs are necessary. Currently, there is consensus on the use of corticosteroids in septic shock. Nevertheless, the use of corticosteroids in patients with pneumonia without severe sepsis or septic shock is still unclear, but the results of new studies will be reported in the near future.

In conclusion, in this review we have discussed the various options for supportive therapy of patients who are treated with otherwise effective antibiotics. In view of increasing resistance, these supportive therapies might become the only option left. However, probably neither corticosteroids, nor APC, immunoglobulins or any of the others can be used as monotherapy. As adjunctive therapy so far, corticosteroids, APC, and immunoglobulins are available and can be used in patients with CAP complicated by severe sepsis or septic shock. Complement, including MBL and TLR agonists and antagonists, are attractive options but warrant additional studies because insufficient evidence is available to date.

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Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Risk factors of arterial cardiovascular complications in patients with prior venous thromboembolism

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ABSTRACT

Background. The effect of cardiovascular risk factors (CVRs) and thrombophilic defects on the risk of arterial cardiovascular complications in patients with prior venous thromboembolism (VTE) is unclear.

Objective. We investigated whether the risk of arterial cardiovascular complications is increased after VTE and whether CVRs and thrombophilic defects influence this risk.

Methods. Subjects were selected from three family cohorts of probands with VTE or an arterial cardiovascular complication before the age of 50 and thrombophilic defects (i.e. hyperhomocysteinaemia, prothrombin G20210A or elevated FVIII). For this analysis, probands with arterial cardiovascular complications before inclusion and their relatives as well as relatives without the studied thrombophilic defects were excluded. We calculated the incidence of arterial cardiovascular complications (e.g. myocardial infarction, ischaemic stroke, transient ischaemic attack or peripheral arterial disease) in subjects with and without VTE and adjusted the relative risk for at least one CVR, two or more thrombophilic defects and quintiles of a propensity score (considering risk factors conditional to VTE history).

Results. 861 subjects were included, of whom 399 had experienced VTE before inclusion. Twelve arterial cardiovascular complications occurred in subjects with and nine in subjects without VTE history. Hence the annual incidence was 1.0 (95% CI 0.5 to 1.7) and 0.7 (0.3 to 1.2) in subjects with and without VTE (RR 1.5, 0.6 to 3.6). Adjusting for possible confounders did not change this relative risk.

Conclusion. The mildly elevated risk of arterial cardiovascular complications in patients with prior VTE appears to be independent of cardiovascular risk factors and thrombophilic defects.

KEYWORDS

Cardiovascular diseases, risk factors, thrombophilia, venous thromboembolism

INTRODUCTION

Following the observation of higher prevalence of subclinical atherosclerosis in patients with previous idiopathic venous thromboembolism (VTE) in 2003,[1] several studies have investigated the association between venous and arterial thrombosis. A mildly increased risk of arterial cardiovascular complications in patients with previous VTE has consistently been demonstrated.[2-6] A plausible explanation for such an association might be the presence of shared risk factors between VTE and arterial cardiovascular complications.[7-9] However, two large cohort studies were unable to establish a link between atherosclerosis at baseline and venous thrombosis during follow-up.[10,11] As the study populations in various published cohorts differ, we intended to confirm the increased risk of arterial cardiovascular complications after an episode of VTE in three prospective cohorts of thrombophilic

families. More important, we aimed to investigate whether the presence of multiple conventional cardiovascular risk factors and thrombophilic defects is able to explain the risk increase.

MATERIALS AND METHODS

Study population

The study subjects were selected from three cohorts of thrombophilic families which were identified by probands with documented VTE or premature arterial cardiovascular complications (any event before 50 years of age) and either hyperhomocysteinaemia, prothrombin G20210A or persistently elevated levels of factor VIII. Subjects were recruited between August 1997 and May 2004 from three academic hospitals: Academic Medical Center, Amsterdam, University Medical Center, Groningen and Academic Hospital Maastricht. Details of these studies have been published previously.[12-14] The study was approved by the institutional review boards of the participating hospitals. Additional thrombophilia tests for factor V Leiden and deficiencies of antithrombin, protein S and protein C were performed in all participants. Detailed information about previous episodes of VTE and arterial cardiovascular complications, exposure to exogenous risk factors for thrombosis and anticoagulant treatment was collected by validated questionnaire and by reviewing medical records at baseline. Also, cardiovascular risk factors namely smoking, diabetes mellitus, hyperlipidaemia and hypertension were recorded at inclusion.

Outcome

The outcome of this analysis was the first arterial cardiovascular complication, such as myocardial infarction (MI), ischaemic stroke, transient ischaemic attack (TIA) or peripheral arterial disease. Coronary and peripheral arterial disease had to be symptomatically and angiographically proven while MI was diagnosed according to clinical, enzymatic and electrocardiographic criteria. Ischaemic stroke was defined as the onset of rapidly developing symptoms and signs of cerebral function loss which lasted at least 24 hours and had an apparent vascular cause, as demonstrated by computed tomography scan or magnetic resonance imaging. If a cerebral event completely resolved within 24 hours without cerebral lesions at scanning, it was classified as TIA.[12] We contacted subjects every six months until April 2006, with a detailed questionnaire to identify new episodes of VTE and arterial cardiovascular complications, exposure to risk factors and medication use.

Statistical analysis

To evaluate whether the risk of an arterial cardiovascular complication is higher in subjects with a history of VTE than those without, we excluded probands who had had an arterial cardiovascular complication prior to enrolment, as well as their relatives, because of higher risk of a recurrent event or the possible hereditary inclination to develop an event. Similarly, relatives with an arterial cardiovascular complication before baseline were excluded. Furthermore, in order to compare subjects with comparable genetic backgrounds regarding thrombophilic defects, we excluded relatives with none of the three thrombophilic defects originally qualifying for inclusion.

The annual incidence (95% confidence interval [95% CI]) of the outcome was computed for two groups of subjects, with and without a history of VTE. The follow-up period was defined as years between the inclusion date and the date of death, last contact visit or when an arterial cardiovascular complication occurred. The relative risk of an arterial cardiovascular complication was computed by dividing the incidences of two groups. Potential confounders for the observed relative risk were considered as the presence of conventional cardiovascular risk factors (i.e. smoking, diabetes mellitus, hyperlipidaemia and hypertension and obesity (BMI ≥25 kg/m^2)) and thrombophilic defects. We computed the Mantel-Haenszel adjusted relative risk for the presence of at least one cardiovascular risk factor and two or more thrombophilic defects. We also developed a propensity score which is the probability of experiencing VTE, based on individual characteristics (i.e. age, cardiovascular risk factors and thrombophilic defects) using binary logistic regression model and subsequently computed the Mantel-Haenszel adjusted relative risk for the quintals of the propensity score.[15] Finally, to exclude the protective effect of anticoagulation on the development of arterial thrombosis, we subtracted periods of anticoagulation treatment from the follow-up period.

RESULTS

A total of 861 subjects met the inclusion criteria for this analysis (*figure 1*): 399 subjects (317 probands and 82 relatives) had experienced VTE prior to enrolment and 462 subjects (all relatives) had not. During follow-up, 21 subjects experienced an arterial cardiovascular event, of whom 12 had a history of VTE and nine did not. The median follow-up duration was three years (range: 0.1 to 7) and did not differ between subjects with and without VTE. The annual incidence rate of arterial cardiovascular events was 1.0 (95% CI 0.5 to 1.7) in subjects with previous VTE, and 0.7 (0.3 to 1.2) in those without past VTE (RR 1.5; 95% CI 0.6 to 3.6). *Table 1* shows the baseline characteristics of the two groups. Sex and age were balanced between the two groups as were classical cardiovascular risk factors except for obesity, which was more prevalent in subjects

Figure 1. *Applied selection for current analysis*

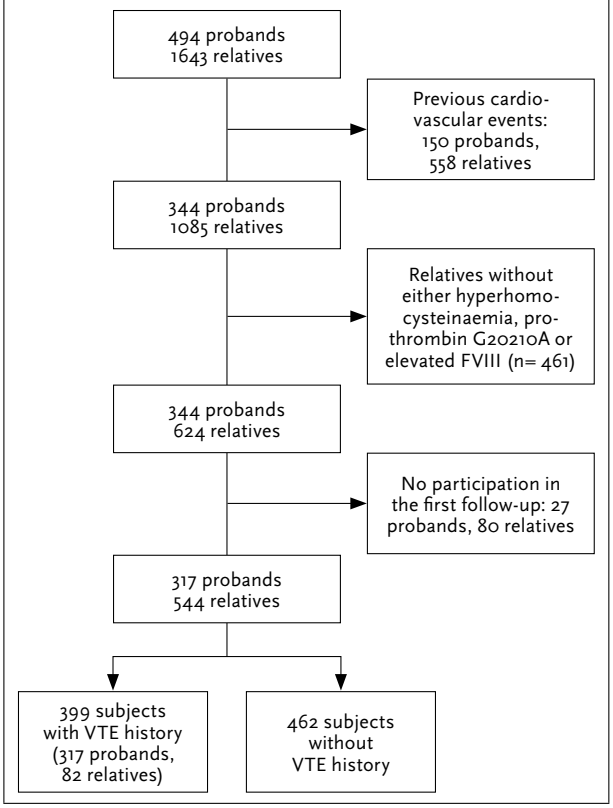

Table 1. *Baseline characteristics and number of arterial cardiovascular outcomes stratified for history of venous thromboembolism (VTE)*

	Subjects without VTE history (N=462)	Subjects with VTE history (N=399)
Sex, M/F (%)	40/60	36/64
Age, year (mean±SD)	47±17	49±16
Hypertension (%)	82 (18)	82 (21)
Hyperlipidaemia (%)	47 (10)	45 (11)
Diabetes mellitus (%)	16 (4)	15 (4)
Obesity, BMI≥30 (%)	72 (16)	86 (22)
Smoking (%)	177 (38)	132 (33)
Hyperhomocysteinaemia (%)	178 (39)	120 (30)
Prothrombin G20210A (%)	160 (35)	131 (33)
Factor VIII elevation (%)	225 (49)	232 (58)
Factor V Leiden (%)	68 (15)	97 (24)
Protein S deficiency (%)	6 (1)	13 (3)
Protein C deficiency (%)	4 (1)	6 (2)
Antithrombin deficiency (%)	1 (0.2)	8 (2)
Number of arterial cardiovascular events (N)	9	12
Number of person-years of follow-up (years)	1367	1199
Annual incidence of arterial cardiovascular events (%) (95% CI)	0.7 (0.3-1.2)	1.0 (0.5-1.7)
Relative risk of arterial thrombotic events in subjects with VTE *vs* those without (95% CI)		1.5 (0.6-3.6)

with a history of VTE (22 *vs* 16%). The thrombophilic defects that were qualified for inclusion, i.e. hyperhomocysteinaemia, prothrombin G20210A mutation and elevated levels of FVIII, were present in 30%, 33% and 58% of subjects with a history of VTE, and in 39%, 35% and 49% in the subjects without previous VTE. Overall, the prevalences of co-inherited thrombophilic defects were somewhat higher in the group with a history of VTE, i.e. 24 *vs* 15% for the FV Leiden mutation, 3 *vs* 1% for protein S deficiency, 2 *vs* 1% for protein C deficiency and 2 *vs* 0.2% for antithrombin deficiency.

Table 2 shows the distribution of cardiovascular risk factors and thrombophilic defects of the subjects who experienced arterial cardiovascular events during follow-up, stratified for a history of VTE. Adjusting the observed relative risk for arterial cardiovascular events in subjects with a history of VTE versus those without previous VTE for the presence of at least one cardiovascular risk factor and for two or more thrombophilic defects did not change the relative risk estimate (1.5; 95% CI 0.7 to 3.3 and 1.5; 0.7 to 3.3 respectively). Likewise, the adjusted relative risk for quintiles of the propensity score was 1.4 (95% CI 0.5 to 3.5). The relative risk of arterial cardiovascular events adjusted for quintiles of propensity score after subtracting periods of anticoagulation use from the follow-up was 1.7 (0.7 to 4.1).

Table 2. *Cardiovascular risk factor and thrombophilic defect distributions in subjects who developed arterial cardiovascular events stratified for venous thromboembolism (VTE) history*

	Subjects without VTE history (N=9)	Subjects with VTE history (N=12)
Sex M/ F (%)	67/33	67/33
Age, year (Mean±SD)	53±18	68±11
Hypertension (%)	1 (11)	4 (33)
Hyperlipidemia (%)	1 (11)	0 (0)
Diabetes mellitus (%)	0 (0)	1 (8)
Obesity BMI≥30 (%)	1 (11)	0 (0)
Smoking (%)	4 (44)	3 (25)
Hyperhomocysteinaemia (%)	3 (33)	3 (25)
Prothrombin G20210A (%)	4 (44)	5 (42)
Factor VIII elevation (%)	3 (33)	8 (67)
Factor V Leiden (%)	2 (22)	4 (33)
Protein S deficiency (%)	0 (0)	0 (0)
Protein C deficiency (%)	0 (0)	0 (0)
Antithrombin deficiency (%)	0 (0)	0 (0)

DISCUSSION

In this prospective analysis of subjects from three prospective family cohort studies we observed that patients with previous VTE have a 1.5 times higher risk of developing arterial cardiovascular complications than their

first-degree relatives who do not have a history of VTE. The estimated relative risk did not alter by adjusting for cardiovascular risk factors or the presence of thrombophilic defects. To our knowledge, this analysis is the first that evaluated simultaneously the effect of cardiovascular risk factors and thrombophilic defects on the risk of arterial cardiovascular complications in patients with previous VTE.

Three other cohort studies of patients with either unprovoked and provoked venous thrombosis or pulmonary embolism have also confirmed the increased risk of arterial cardiovascular complications after VTE.[6,16,17] Among which, one study adjusted the risk for age and cardiovascular risk factors where they did not notice a difference by adjustment.[16]

Our study is different from the previous ones because we included first-degree relatives of the patients with a history of VTE as the control cohort, implicitly expressing the highest possible similarity between the exposed (probands and relatives with VTE) and the control cohort for the environmental variables such as lifestyle and known and unknown genetic variables that are burdensome to adjust for and can produce residual confounding in any association under study. On the other hand, having strict inclusion criteria resulted in a small number of arterial cardiovascular complications. Hence, we could not investigate whether type of VTE (unprovoked *vs* provoked) modulates the risk of arterial cardiovascular complications. This may have led to underestimation of the observed increased risk as some but not all studies have shown that only subjects with unprovoked VTE had an increased risk of subsequent arterial cardiovascular complications.[2,3,6,17] Furthermore our results are only applicable in a highly selected cohort of thrombophilic families.

In conclusion, conventional cardiovascular risk factors and multiple thrombophilic defects do not seem to explain the mildly increased risk for arterial cardiovascular complication in subjects with a history of VTE.

ACKNOWLEDGMENTS

We would like to thank Professor Jan Vandenbroucke for his intellectual support with the statistical analysis.

This study was supported by a grant of the Netherlands Heart Foundation (1999.187).

Dr S. Middeldorp is Clinical Established Investigator of the Netherlands Heart Foundation (2008T056).

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CASE REPORT

Pneumococcal aortitis: an insidious diagnosis

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ABSTRACT

A patient with *Streptococcus pneumoniae* aortitis is presented. Because of nonspecific symptoms (fever and back pain) there was a long diagnostic delay. In addition, the aortitis was located near the renal arteries which severely hampered early surgical treatment. Although emergency surgery was performed when aortic rupture occurred, the patient did not survive. Infectious arteritis of large vessels is a diagnosis often made late and associated with high mortality.

KEYWORDS

Streptococcus pneumoniae, aortitis, fatal outcome, antibiotics, surgery

What was known on this topic?
It is long known that infections of the large vessels can be life-threatening. However, aortitis is a very rare manifestation of bacteraemia and is usually a post-mortem diagnosis. Most commonly, bacterial aortitis is caused by S*almonella* and S*taphylococcus* species. The experience with aortitis caused by *Streptococcus pneumoniae* is limited.

What does this case add?
There are two principal points that this case report adds. 1) Aortitis is a difficult diagnosis because of the nonspecific presenting symptoms and, subsequently, can have a long diagnostic delay which will inevitably complicate treatment. Hence, awareness of such a possibly fatal diagnosis is required for doctors confronted with a patient with long-lasting fever and back or abdominal pain. 2) This case report adds to our knowledge that pneumococcal aortitis can not be treated by antibiotics alone (possibly also due to point 1). Instead, surgical treatment is the ultimate treatment modality and delaying surgical intervention may result in loss of the patient. This notwithstanding, the reasons to delay surgical intervention may be obvious as is also shown in this case report. We illustrate the difficult medical decision-making when confronted with pneumococcal aortitis.

INTRODUCTION

Bacterial infections of the cardiovascular system have long been known[1,2] and are associated with high mortality as they may present insidiously.[3] Infectious aortitis is usually a post-mortem diagnosis.[4] Most commonly, bacterial aortitis is caused by S*almonella* and S*taphylococcus* species.[3,5,6] We discuss a patient with *Streptococcus pneumoniae* aortitis with a long diagnostic delay. Moreover, it was located near the renal arteries which severely hampered early surgical treatment. Despite emergency surgery when the aortitis resulted in (contained) aortic rupture, the patient did not survive. Infectious arteritis of large vessels is a diagnosis often made late and associated with high mortality.

CASE REPORT

A 63-year-old relatively healthy white male experienced fever without any other complaints two months before admission. Medical history revealed diabetes mellitus type 2 (for which he received metformin) and smoking. A week passed and fever regressed to a subfebrile temperature. He was still able to play tennis, but gradually back pain emerged for which he used acetaminophen. His general practitioner prescribed a five-day course of azithromycin but this was not effective. The pain intensified and he started non-steroidal anti-inflammatory drugs. As he now could no longer play tennis, he was

referred to the regional hospital. A five-day course of moxifloxacin temporarily lowered his temperature but did not relieve the pain.

At the outpatient clinic the patient's physical examination was unremarkable except for a temperature of 37.8 °C. The erythrocyte-sedimentation rate was raised (100 mm/h, ULN 20 mm/h), as were leukocyte count (12 x 10 E09/l, ULN 10,5) and C-reactive protein (107 mg/l, ULN 5 mg/l). Creatinine (71 µmol/l, ULN 110 µmol/l), urine analysis and chest-radiography were normal.

When opioids became necessary to relieve the pain, positron emission tomography-computed tomography (PET-CT) was planned for suspected spondylodiscitis. Because fever did not recur, blood cultures were not performed. PET-CT (*figure 1*) showed a fluorodeoxyglucose hotspot at the ventral side of the abdominal (non-aneurysmatic) aorta near the renal arteries, best compatible with aortitis. Subsequently, he was referred to our tertiary academic referral hospital.

The patient was admitted, physical examination was still unremarkable (temperature 37.6 °C), erythrocyte-sedimentation rate (117 mm/h), leukocyte count (17x10 E09/l), C-reactive protein (309 mg/l), creatinine (122 µmol/l), glucose (21.8 mmol/l, ULN 5,6 mmol/l) and HbA1c (8.5%, ULN 6%) were all increased. Blood cultures were taken and the next day penicillin sensitive Gram-positive bacteria were cultured appearing to be *Streptococcus pneumoniae*. High-dose intravenous penicillin (12 x 10 E6 international units/day) was started but lowered to 6 x 10 E6 international units/day when kidney function deteriorated (creatinine 246 µmol/l). Because of the positive cultures, impaired kidney function, the absence of a (false) aneurysm and the complex location of the aortitis, it was decided to optimise the patient by intravenous antibiotics before proceeding to surgery. CT angiography was scheduled to be performed in seven to ten days to assess aneurysm formation. Subsequently, the creatinine declined again but the patient's blood pressure started to rise to 160/90 mmHg for which nifedipine was started. Transthoracic and transoesophageal echocardiography did not reveal signs of endocarditis.

Although the patient's back pain now demanded opioids six to eight times daily he was in a relatively good condition and walking around. Hydration with saline resulted in further creatinine lowering while a MAG3 scan revealed delayed perfusion of the left kidney with a relative contribution of 41%. His temperature and infection parameters also declined (C-reactive protein 88 mg/l, leukocytes 12 x 10 E9/l). Haemoglobin concomitantly decreased slightly from 6.4 to 5.6 mmol/l, then considered to be the result of hydration and continuing infection. Despite the impaired kidney function (creatinine 139 µmol/l) CT angiography was performed after ten days. It showed a contained aortic rupture with a haematoma encompassing both renal arteries (*figure 2*). The left kidney appeared to be hardly perfused and the right kidney was supplied by an accessory artery located just below the aneurysm.

Emergency surgery was performed the same day by a left-sided retroperitoneal approach and distal thoracotomy. A severely infected aorta and surrounding tissue were encountered. The aorta was replaced by a Dacron graft from the superior mesenteric artery to the aorta bifurcation. The renal arteries could not be reconstructed

Figure 1. *Positron emission tomography – computed tomography (PET-CT) showing a fluorodeoxyglucose hotspot at the ventral side of the abdominal aorta at the level of the renal arteries best compatible with aortitis. The aorta did not show any aneurysms or other irregularities*

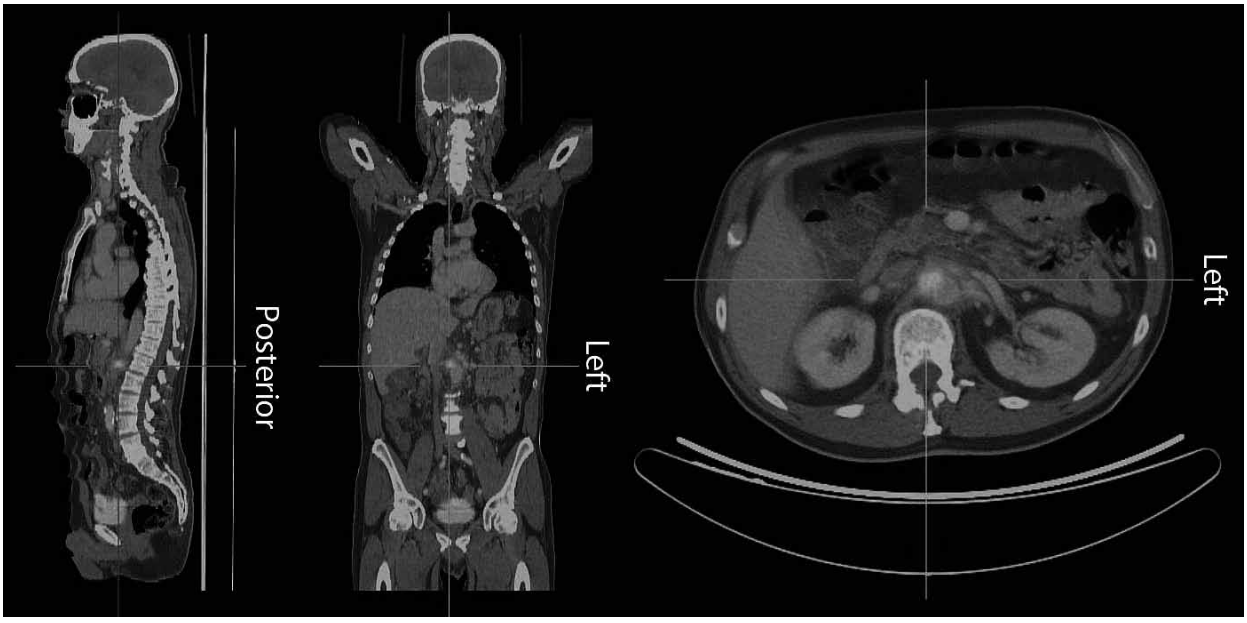
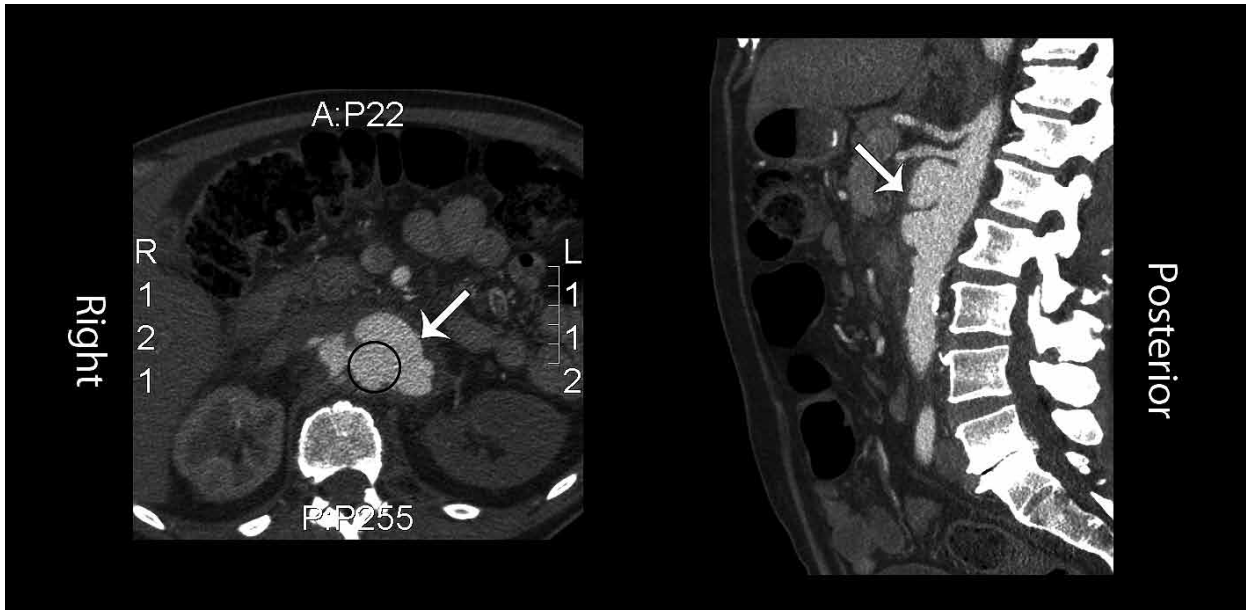


Figure 2. *CT scan showing a contained rupture of the aorta (circle) with a haematoma (arrows) encompassing both renal arteries. Additionally there is hypoperfusion of the left kidney. Notably, no unusual atherosclerotic plaques were documented*



due to severe destruction of the arterial wall and had to be sutured, resulting in the need for permanent dialysis. The remaining arterial wall was considered of acceptable quality to perform secure anastomoses. Gentamycin sponges were wrapped around the prosthesis and native aorta. After an operation of 5.5 hours the patient was stable and transferred to the intensive care unit. There he remained stable for 4.5 hours after which a sudden sharp fall in blood pressure and haemoglobin (5.2 to 2.5 mmol/l) quickly resulted in cardiac arrest. Although resuscitation was started within seconds, the patient died while being transferred back to the operating theatre. The relatives did not authorise autopsy.

DISCUSSION

We present an insidious case of pneumococcal aortitis with fatal outcome despite treatment with high-dose intravenous penicillin and emergency surgery. As autopsy was not performed we can only speculate on the sudden death due to severe bleeding. Rupture of an anastomosis due to poor suture holding or any other technical shortcoming is most likely.

Infectious aortitis is rare but potentially life-threatening. Aortitis with *Streptococcus pneumoniae* has been reported,[7-10] although pneumococcal bacteraemia with the clinical presentation of aortitis is very rare.[11] While there are reports of aortitis in children, the usual presentation is an older male febrile patient with underlying atherosclerotic disease and back or abdominal pain.[7,8] As far as we are aware, there are no reports of successful outcome with antibiotic treatment alone, also surgical intervention yields only around 50% survival.[6,7]

The present case was in a sense typical for pneumococcal aortitis with long diagnostic delay.[7] Blood cultures might have been positive earlier, although bacteraemia may not always be present.[6] Whether earlier intravenous penicillin would have resulted in a different outcome is uncertain but likely. This might have prevented emergency surgery in a severely infected aorta as opposed to elective surgery. Although the patient's atherosclerotic burden on CT was not unusual for his age, his diabetes mellitus and smoking did pose him to higher cardiovascular risk, in this case the ominous nesting of *Streptococcus pneumoniae* near the renal arteries.

In conclusion, infectious aortitis is an insidious diagnosis associated with high mortality. It often presents in a nonspecific manner but long-lasting elevated temperature and abdominal or back pain should raise suspicion and warrant further investigations. When suspected, direct initiation of broad-spectrum intravenous antibiotics while awaiting blood cultures is mandatory. Surgical intervention with debridement of the focus of infection is the ultimate treatment modality, although timing is difficult. Imaging techniques (e.g. CT, PET-CT, MRI)[7,9,10] may help to confirm the diagnosis and guide treatment.

ACKNOWLEDGEMENTS

We would like to express our regards to the patient and his family. We would like to thank Drs. A.J. van Wieringen (general practitioner) and J.A.C. Brakenhoff (Waterland Hospital, Purmerend) for patient referral and the Nuclear Medicine Department at the Medical Center Alkmaar and Prof. B.L.F. Van Eck-Smit (Nuclear Medicine Department, AMC Amsterdam), for the nuclear investigations.

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CASE REPORT

Life-threatening hypokalaemic paralysis associated with distal renal tubular acidosis

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ABSTRACT

A 56-year-old woman developed acute respiratory failure requiring mechanical ventilation due to acute hypokalaemic paralysis. There was no gastrointestinal potassium loss ~~or she was not taking diuretics~~. Additional analyses revealed a normal anion gap metabolic acidosis with a positive urine anion gap. An acid-load test revealed a renal urine acidification defect, leading to the diagnosis of distal renal tubular acidosis. Normalisation of the serum potassium was established with oral bicarbonate suppletion and temporary potassium suppletion.

KEYWORDS

Acute paralysis, distal renal tubular acidosis, hypokalaemia, metabolic acidosis

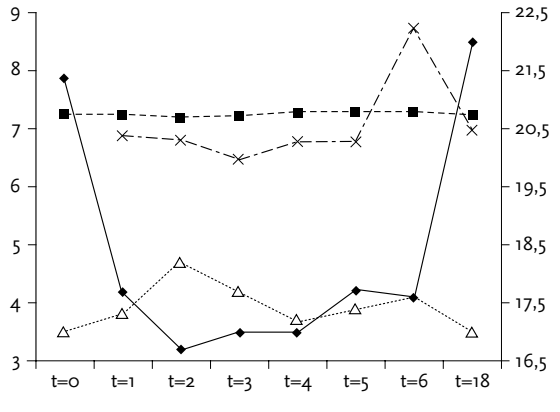
INTRODUCTION

Renal tubular acidosis (RTA) type I or distal RTA is a rare disorder consisting of a defect in renal regulation of acid-base balance and, consequently, in electrolyte excretion. The disease is generally asymptomatic and discovered upon the presence of a chronic, normal anion gap metabolic acidosis in combination with a persistently high urine pH. Although uncommon, patients with RTA type I may present with symptomatic hypokalaemia. We describe a rare case of a patient with this disorder, who presented with acute hypokalaemic paralysis complicated by acute respiratory failure requiring mechanical ventilation.

CASE REPORT

While on holiday, a 56-year-old woman was admitted to the intensive care unit of an Indonesian hospital for acute paralysis complicated by respiratory failure necessitating mechanical ventilation. Severe hypokalaemia (K 1.8 mmol/l) was noted, without symptoms indicating gastrointestinal potassium loss or use of diuretics. Chloroquine was used as malaria prophylaxis. She was referred to our Outpatient Department of Nephrology for investigation of persistent hypokalaemia despite potassium substitution (potassium chloride 600 mg orally four times daily). Besides a transient episode of loss of strength and sensibility in her fingers and complaints of a dry sensation of the mouth but not eyes, her medical history was unremarkable. Physical examination revealed no abnormalities; blood pressure was 100/70 mmHg. Relevant laboratory investigation after cessation of potassium supplementation revealed persistent, mild metabolic acidosis (pH 7.30; base excess -5.4) with a normal serum anion gap (AG 6 mmol/l; N <11 mmol/l) and normal renal function (creatinine 92 µmol/l). Urine pH was high (pH 7.0), urine sodium amounted to 91 mmol/l and urinary calcium excretion to 1.6 mmol/l. A positive urine AG was calculated (38 mmol/l). Additional laboratory investigation revealed a raised erythrocyte sedimentation rate (61 mm/h; N 0 to 20 mm/h), transient hypergammaglobulinaemia (IgG 17.30 g/l; N 7.00 to 16.00 g/l; negative for monoclonal gammopathy) and positive antinuclear antibodies with specificity for anti-SSa and anti-SSb. Plasma aldosterone level (0.45 nmol/l; N 0.04 to 0.35 nmol/l) and plasma renin activity (5.0 ng/ml/h; N 0.3 to 3.5 ng/ml/h) were elevated. Because of the persistent hypokalaemic state with normal AG metabolic acidosis and a positive urine AG, we suspected a renal tubular defect as the primary cause. An acid-load test with NH_4Cl demonstrated a renal acidification defect, indicated by a urine pH persistently above 5.3 despite low plasma bicarbonate levels (*figure 1*). Findings were compatible with a diagnosis of distal RTA. The patient was treated with sodium bicarbonate 1.5 g orally three times daily and temporary potassium suppletion, resulting in normalisation of serum potassium level and of metabolic acidosis.

Figure 1. *Results of acid load test (0.1 g/kg NH_4Cl, orally)*



x-axis presents time in hours after acid load; ■ = serum pH (left y-axis); ◆ = serum bicarbonate levels (mmol/l , right y-axis); = △ = potassium (mmol/l, left y-axis); × = urine pH (left y-axis).

DISCUSSION

Here we present a patient who suffered from life-threatening hypokalaemic paralysis due to RTA type I. The diagnosis was suspected upon the presence of a normal AG metabolic acidosis with a high urine pH and high urine AG, in the absence of gastrointestinal bicarbonate loss or urine tract infection with an urea-splitting organism.[1] No definitive differentiation between the various types of RTA could be made based solely upon these findings, since clinical data describing the values of the urine AG accompanying proximal RTA during acidosis are few and contradictory.[1,2] Therefore an acid-load test was performed, demonstrating a renal defect in urine acidification due to diminished proton excretion, which only occurs in case of distal RTA.[1]

The incomplete type of distal RTA is a subtype usually presenting with nephrolithiasis or nephrocalcinosis with (near) normal serum pH and bicarbonate levels. Contrary to RTA 1, which originates from a defect in proton excretion, proximal RTA (RTA type II) is caused by a proximal renal bicarbonate reabsorption defect. In the presence of a high plasma bicarbonate level, the distal tubular reabsorption capacity of bicarbonate is exceeded and a higher amount of bicarbonate is excreted. Thus, RTA type II can be verified by determining the presence of a high bicarbonate excretion fraction (>15%) after bicarbonate infusion in a patient with low plasma bicarbonate levels, in contrast to a maximum of 3% in normal subjects and patients with another type of RTA.[1] Features of the different types of RTA are summarised in *table 1*.

Distal RTA can arise as a primary condition, but is usually secondary to other diseases (*table 2*). Hypergammaglobulinaemia was detected initially in our patient, which in itself is a possible cause of distal RTA. The combination of dry mouth and anti-SSa and anti-SSb suggests Sjögren's syndrome. However, signs and/or symptoms necessitating immunosuppressive treatment were absent. Hypokalaemic paralysis may be the solely presenting symptom of Sjögren's syndrome[4-8] or another autoimmune disease (such as systemic lupus erythematosus),[9] sometimes preceding other symptoms by five years.[1]

Several mechanisms in the pathogenesis of the hypokalaemic state in RTA type I are proposed and summarised in *figure 2*. Some small studies and case reports have suggested both the absence of H-ATPase in the apical and cytoplasmic anion-exchanger 1 (AE 1, a bicarbonate/Cl-ATPase) in the basal membrane after immunohistochemical staining of tubular cells underlying RTA I in Sjögren's syndrome.[10,11] Furthermore, higher antibody titres against carbon anhydrase II

Table 1. *Features of the different types of renal tubular acidosis (RTA)*

	RTA I	Incomplete RTA I	RTA II	RTA IV /aldosterone resistance
Defect	Reduced distal H secretion	Reduced distal H secretion	Reduced proximal HCO_3 resorption	Hypoaldosteronism
Plasma pH	(Very) Low	Normal	Low	Low
Plasma HCO_3	Very low – low	Normal	Low	Low - normal
Urine pH	>5.3	>5.3	Variable	<5
Plasma potassium	(Very) Low*	Normal	Low	High
Confirmation test	Acid-load test	Acid-load test	Bicarbonate loading	Plasma
Symptoms & signs	Hypokalaemia (/hyperkalaemia) Osteopenia/osteomalacia Nephrolithiasis/ nephrocalcinosis	Nephrolithiasis/ nephrocalcinosis	Hypokalaemia Osteomalacia	Hyperkalaemia

Omitted is RTA III: a rare, genetic disorder due to carbon anhydrase II deficiency with combined features of type I and II; * = in some cases hyperkalaemia can be present; ** = during metabolic acidosis; * = not obligatory, diagnosis can be made upon laboratory findings alone. Adapted from Rose *et al.* and Soriano *et al.*[1,2]**

Table 2. *Aetiology of distal renal tubular acidosis*

Primary

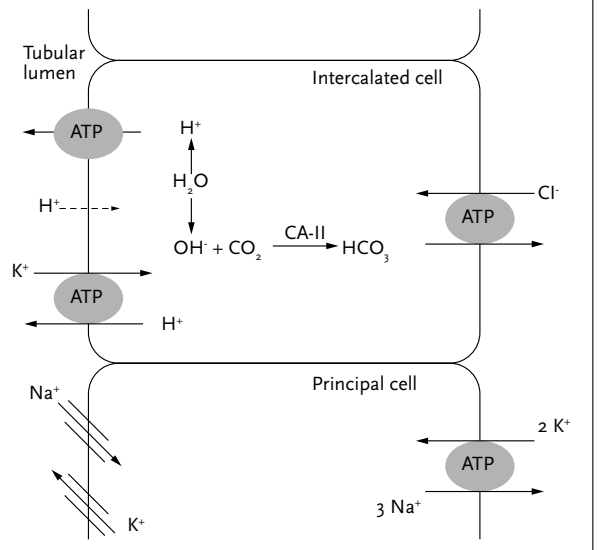
- Idiopathic
- Familial/genetic disorders (autosomal dominant or recessive, Marfan's syndrome, Ehler-Danlos syndrome, Wilson's syndrome)

Secondary

- Autoimmune disorders: Sjögren's syndrome, rheumatoid arthritis, fibrosing alveolitis, systemic lupus erythematosus*
- Hypercalciuria: idiopathic, hypervitaminosis D, familial hypercalciuria, primary hyperparathyroidism, medullary sponge kidney
- Dysproteinaemia: hypergammaglobulinaemia, cryoglobulinamia, amyloidosis
- Medication / toxins: e.g. iphosphamide, analgesics, toluene amphotericin B, lithium carbonate, amiloride, trimethoprim
- Liver disease: (primary biliary) cirrhosis, chronic active hepatitis
- Hyperthyroidism: hypercalcaemic
- Renal disease: transplant rejection*, medullary sponge kidney, obstructive nephropathy*
- In context of genetic diseases: e.g. Sickle cell anaemia*, hereditary ovalocytisus
- Marked volume depletion

***may be accompanied by hyperkalaemia.**[1,2]

Figure 2. *Possible pathophysiological mechanisms underlying RTA type I (schematic representation of cortical part of distal tubules)*



1. Absence or nonfunctional H-ATPase decreasing H excretion of the intercalated cells; 2. Anion Exchanger 1 (HCO3/Cl-ATPase) deficiency/ malfunctioning, preventing HCO3 resorption, and as a result less H formation and excretion; 3. Increased permeability allowing H back diffusion from the lumen (amphotericin B); 4. Inhibition/dysfunction of CA II diminishing H production for secretion; 5. Diminished Na resorption (reduced Na delivery [volume depletion] or Na/K-ATPase dysfunction).
Mechanisms 1 to 4 lead to hypokalaemia through enhanced K excretion in the principal cells in order to balance electronegativity; in case of mechanism 5 decreased amounts of both H and K are secreted, creating a hyperkalaemic, acidotic state; adapted from Rose *et al.*, Nicoletta *et al.*, and Koul *et al.*[1,3,4]

been noted in Sjögren's patients with RTA, suggesting a role in the pathophysiology of RTA I.[12] In absence of (properly functioning) H-ATPase, AE 1 or CA II, less protons are secreted in distal tubular cells, leading to metabolic acidosis. The electronegative gradient created by distal sodium resorption is therefore compensated by an increased potassium secretion, leading to renal (potassium) salt wasting. Notably, during metabolic acidosis, less sodium absorption takes place in the proximal tubuli as a result of a lower amount of glomerular filtrated bicarbonate. The enhanced delivery of sodium distally creates a hyperreninaemic hyperaldosteronism state, stimulating distal sodium reabsorption and thereby additional potassium wasting. Our patient also had an elevated plasma aldosterone level and plasma renin activity. Whether the use of chloroquine, known to be able to induce hypokalaemia possibly by stimulating potassium transport to the intracellular compartment,[13] aggravated the hypokalaemic state is unclear. Our patient, however, only used prophylactic doses of this medicine which makes chloroquine intoxication less likely.

Clinical signs and symptoms accompanying distal RTA, other than hypokalaemia and symptoms related to the primary disorder causing distal RTA, are the effects of metabolic acidosis. Acidosis promotes the release of calcium from bone tissue, causing osteoporosis and (rarely) osteomalacia.[1,14,15] The enhanced calcium release and, consequently, increased excretion, combined with a high urine pH and hypocitraturia (due to acidosis) promotes urinary precipitation of calcium phosphate, resulting in nephrolithiasis and nephrocalcinosis.[16,17]

Increased calcium excretion could not be detected in our patient. Nevertheless, the presence of high urine pH (and possible concomitant hypocitraturia) predisposes for nephrocalcinosis or nephrolithiasis development.

Treatment of distal RTA is based upon alkali supplementation correcting the metabolic acidosis, and not upon potassium suppletion. Preferably, potassium citrate (1-2 Eq/kg daily, orally) should be given which is better tolerated and, in contrast to sodium bicarbonate, produces no natriuresis.[1] Unfortunately, potassium citrate is not readily available in our region. Generally, no additional potassium supplementation is required, since correction of the serum pH and bicarbonate levels reduces renal potassium wasting by diminishing distal sodium delivery. However, a mild hypokalaemic state can persist since potassium wasting is not completely abolished. This effect can be corrected by simultaneous potassium supplementation using potassium citrate. Treatment of the underlying disease may partially reverse the acidification defect in distal RTA, as in cases of Sjögren's syndrome.[1]

In conclusion, distal RTA should be considered in case of unexplained hypokalaemia, particularly with concomitant

(normal AG) metabolic acidosis. The disorder can be primary or hereditary, but usually occurs secondarily to other conditions. Several pathophysiological mechanisms have been proposed to originate the defect in renal acidification and potassium balance. Therapy primarily consists of bicarbonate suppletion in order to prevent a hypokalaemic state and negative effects of chronic metabolic acidosis. In some cases, however, additional potassium supplementation may be required to correct mild, residual hypokalaemia.

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PHOTO QUIZ

Hoarseness due to a thyroid mass

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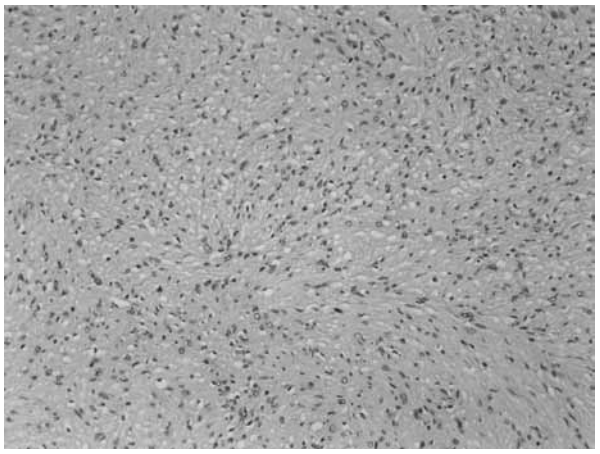
A 70-year-old African American man with a long-standing history of tobacco use presented to our clinic with hoarseness of voice, weight loss of 40 pounds in the year prior to presentation, and progressive dysphagia over a three-year period. He could only tolerate a liquid diet. CT scan revealed a left thyroid mass measuring 3.6 x 5.4 cm that replaced the entire left thyroid lobe and extended posteriorly causing oesophageal compression. Fine needle aspiration biopsy of the mass revealed absence of follicular cells in the aspirate and therefore it was non-diagnostic.

Histological examination showed typical Antoni A cells with an encapsulated mass composed of more cellular proliferation with elongated cells having elongated wavy nuclei forming fascicles; and Antoni B cells with spindle cell proliferation that is loosely textured, containing round-to-oval nuclei and some pseudoinclusions (*figure 1*).

The patient underwent standard left thyroid lobectomy and his symptoms resolved postoperatively. On frozen section, the thyroid mass was noted to be a spindle cell neoplasm.

Immunohistochemistry showed that the tumour cells expressed S-100 and Vimentin but did not express desmin, muscle-specific antigen, thyroid transcription factor-1 (TTF-1), cytokeratin (AE1/AE3), thyroglobulin, chromogranin, synaptophysin, or smooth muscle actin. Additional histological examination is shown in *figure 1*.

Figure 1. *Schwannoma Antoni A pattern: Spindle cell proliferation that is loosely textured containing round to oval nuclei some pseudoinclusions representing Antoni A pattern*



WHAT IS YOUR DIAGNOSIS?

See page 40 for the answer to this photo quiz

ANSWER TO PHOTO QUIZ (PAGE 39)

HOARSENESS DUE TO A THYROID MASS

Peripheral nerve sheath tumours (PNSTs) of the thyroid gland are subdivided into malignant and benign. Benign PNSTs include neurofibromas and schwannomas.[1] PNSTs of the thyroid gland are quite rare and usually asymptomatic. Most tumours are benign. Isolated neurofibromas are exceedingly rare and usually asymptomatic.[2]

Schwannomas are the most common type of PNSTs. And they originate from neuronal sheath cells (Schwann cells). They are slowly growing tumours producing symptoms from compression of vital structures. Their highest incidence has been reported at between 40 and 60 years of age. Schwannomas are classified microscopically into two types, Antoni type A, which has pallisading, compact, and spindle-shaped nerve-sheath cells, and Antoni type B, which has a sparsely cellular pattern with either cystic degeneration or xanthomatous change.[3] In our patient, a combination was seen. The list of differential diagnoses is short, as benign nonepithelial tumours of the thyroid glands are rare and include vascular, smooth muscle, and nerve tumours.

Fine needle aspiration is generally unsuccessful for the diagnosis of schwannomas of the thyroid because follicular cells are absent and the remaining cells are difficult to identify. The immunohistochemical staining for schwannomas is usually positive for S-100 protein and neuron-specific enolase as well as actin, vimentin, cytokeratin, and smooth muscle actin, differentiating this type of tumour from other spindle cell sarcomas. The MIB1 proliferation marker is also present, which is also used for the grading and prognosis of the tumour.[4]

The only treatment for symptomatic thyroid schwannoma is surgical removal due to the difficulty of making the diagnosis before surgery. Surgical resection is mandatory for symptomatic cases.

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Netherlands
The Journal of Medicine

Skin lesions depicting a systemic disease

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CASE REPORT

A 38-year-old woman with a previous medical history of diabetes mellitus type 2, and a hysterectomy, presented to our hospital because of acute renal failure for which temporary dialysis was necessary. She did not have any physical complaints. The patient was haemodynamically stable, and had no fever.

Physical examination revealed a large ulcerative process in the abdominal scar (*figure 1*) and multiple painful red nodules on the lower extremities (*figure 2*, this is also the biopsy site). Laboratory investigations showed the following results: haemoglobin of 9.8 mmol/l, white blood cell count 24.3 /nl, C-reactive protein 180 mg/l, creatinine 1100 µmol/l, urea 27 mmol/l, modification of diet in renal disease (MDRD) 3.0 ml/min, potassium 2.8 mmol/l, sodium 133 mmol/l, amylase of 59 U/l, and no antinuclear antibodies. Routine urine analysis showed no protein, erythrocytes or cylinders. Fractional sodium excretion was 0.2%. The chest X-ray was normal. A CT scan of the abdomen showed pseudocysts indicating chronic pancreatitis (*figure 3*). On repeated imaging, there were no signs of a pancreatic carcinoma. Biopsy of one of the painful nodules on her legs revealed a septal and lobular panniculitis (*figure 4*).

WHAT IS YOUR DIAGNOSIS?

See page 42 for the answer to this quiz

Figure 1. *Large ulcerative process in the abdominal scar*

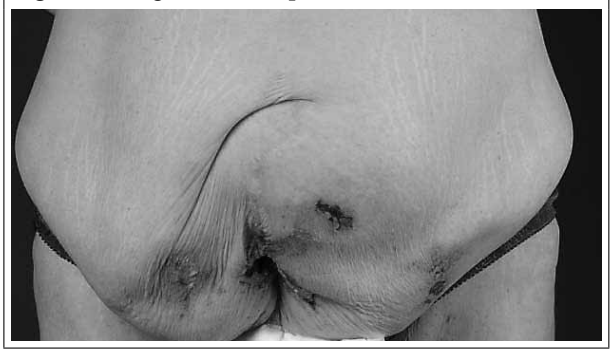


Figure 2. *Multiple painful red nodules on the lower extremities (biopsy site)*

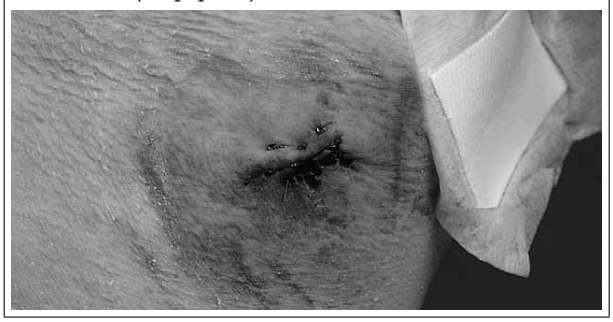


Figure 3. *CT scan showing signs of chronic pancreatitis*

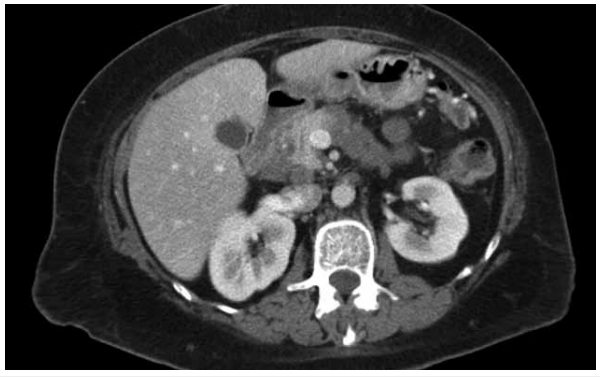
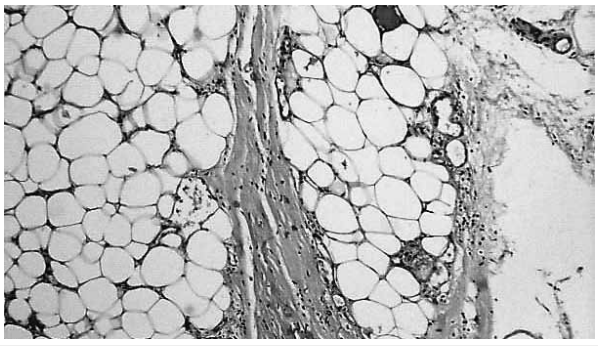


Figure 4. *Biopsy from one of the nodules revealing septal and lobular panniculitis*



ANSWER TO PHOTO QUIZ (PAGE 41)

SKIN LESIONS DEPICTING A SYSTEMIC DISEASE

The combination of the above-mentioned findings led us to the diagnosis of systemic panniculitis or Weber-Christian disease. Weber-Christian disease was initially described by Pfeiffer in 1892, and was established by Weber and Christian in the 1920s. It is an infiltrative inflammatory disease of fat tissue that usually occurs in young white females. It is characterised by tender skin nodules that are often but not always associated with constitutional symptoms, such as fever, arthralgias and myalgias.[1] Patients with Weber-Christian disease typically present with subcutaneous nodules on the extremities, but skin lesions can also occur over the posterior thorax, abdominal area, breasts, face or buttocks, and the panniculitis can also affect other organs. The disease can then present as a severe systemic illness leading to death from panniculitis involving the heart, lungs, liver, pancreas or kidneys.[1,2] The diagnosis is confirmed by excisional biopsy of a nodule. The panniculitis is typically lobular, although it may be both lobular and septal. This patient responded well to corticosteroid therapy.

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SPECIAL ARTICLE

Treatment of acute hepatitis C virus infection in HIV+ patients: Dutch recommendations for management

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ABSTRACT

With a rising incidence of acute hepatitis C virus (HCV) infection in patients coinfected with the human immunodeficiency virus (HIV), there is a need for evidence-based treatment recommendations. There are no randomised trials available and published studies differ with respect to design, patient characteristics and number of patients included, making a comparison between studies difficult. However, it is critical to standardise treatment for this group of patients in order to optimise the outcome of therapy. The Dutch Society for HIV Physicians proposed to write recommendations for the treatment of acute HCV in HIV-coinfected patients. Combination therapy with pegylated interferon-alpha and ribavirin is the preferred regimen initiated preferably within 12 weeks after the diagnosis of acute HCV. A treatment duration of 24 weeks is recommended in case of a favourable virological response (either achievement of a rapid virological response or a >2log10 decrease plus undetectable HCV-RNA at week 12). In all other patients prolonging the duration of therapy to 48 weeks should be considered.

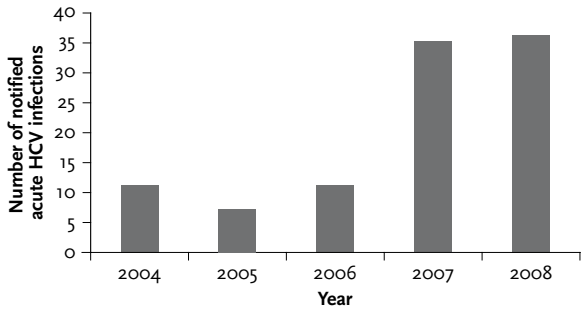
KEYWORDS

Acute hepatitis C, HIV, peginterferon-alfa, ribavirin, therapy

INTRODUCTION

In recent years, the incidence of acute hepatitis C virus (HCV) infections among men having sex with men coinfected with the human immunodeficiency virus (HIV) has markedly increased (*figure 1*).[1-4] HIV-infected patients with an acute HCV infection hardly ever present with overt clinical symptoms, thereby hampering early detection of acute HCV infection. Frequent routine laboratory assessment of transaminases is the most commonly used method of detection.[5,6] In contrast to

Figure 1. *Number of notified acute HCV infections per year, among HIV-infected MSM in the Netherlands*



Source: RIVM-report 210261005 'Sexually Transmitted Infections, including HIV, in the Netherlands in 2008'. RIVM-OSIRIS notification data.

treatment of acute HCV mono-infected patients, where sustained virological response (SVR) rates between 72 to 94% are reached with pegylated interferon-alpha (peg-IFN-α) monotherapy,[7-11] the optimal treatment strategy for acute HCV in HIV-coinfected patients is less clear.[12,13] Therefore, there is a need for evidence-based treatment recommendations, guiding clinicians in the field to treat acute HCV in this population. Here, we summarise the available literature, after which recommendations are made regarding the case definition for acute HCV (*textbox 1*), the preferred treatment regimen, the time to start therapy and the duration of therapy (*textbox 2*).

Textbox 1. *Case definition of acute hepatitis C virus*

Preferred criteria (Level II)

1) Positive anti-HCV IgG in the presence of a documented negative anti-HCV IgG in the previous 12 months

or

2) Detectable HCV-RNA in the presence of either a documented negative HCV-RNA or a documented anti-HCV IgG seroconversion within the previous 12 months

Alternative criteria (If historical data/ stored samples are lacking) (Level III)

1) Detectable HCV-RNA or positive anti-HCV IgG in association with:
   a) an acute rise in ALAT with a documented normal ALAT and no change in antiretroviral regimen within the past six months

   and

   b) anti-HAV IgM negative and anti-HBV core IgM negative, and exclusion of other causes of an acute hepatitis

Textbox 2. *Recommendations regarding acute HCV treatment*

Treatment regimen:

- Combination therapy pegylated interferon alpha-2a/2b and ribavirin (weight based 800-1400 mg)
  → level III

When to start

- Preferably within 12 weeks after the diagnosis of acute HCV
  → level III

Treatment duration

- 24 weeks in case of either
  1) a rapid virological response (RVR, HCV-RNA <50 IU/ml) or
  2) >2log10 drop of HCV-RNA at week 4 and undetectable HCV-RNA at week 12
  → level III
- in all other patients prolonging the duration of treatment to 48 weeks should be considered
  → level IV
- treatment should be stopped when <2log10 drop in HCV-RNA at week 12 of therapy
  → level IV

LITERATURE SEARCH

An English-language literature search was conducted using the PubMed database through 1 April 2010. As search terms "hepatitis C or acute hepatitis C or HCV or acute HCV" AND "peginterferon-alfa or interferon-alfa or ribavirin or combination therapy or mono-therapy or RBV or HCV treatment or HCV therapy" AND "HIV or human immunodeficiency virus or Acquired Immunodeficiency Syndrome" were used. Titles and/or abstracts were screened to determine the relevance of the studies. Of all relevant studies, full-text publications were reviewed. Furthermore, references of full-text studies were reviewed for missing publications. Lastly, conference abstracts of the annual meetings of the American Association for the Study of Liver Diseases (AASLD), the Conference on Retroviruses and Opportunistic Infections (CROI) and the European Association for the Study of the Liver (EASL) were reviewed for treatment of acute HCV.

DEFINITION OF THE DIAGNOSIS ACUTE HCV IN HIV-COINFECTED PATIENTS

To date, there is no uniform definition for the diagnosis of acute HCV. Most studies use a raise in transaminases from a previous measurement, during routine lab monitoring of HIV patients, as criterion for acute HCV.[14-21] However, sometimes a cut-off value is defined above which acute HCV is suspected (for example, >10x above the upper limit of normal or a pre-defined value of >300 U/l). Other studies combine a raise in transaminases with clinical symptoms and/or exposition to HCV as criterion for acute HCV.[22-25] Furthermore, all studies use a documented seroconversion from negative to positive anti-HCV IgG with subsequently detectable HCV-RNA as criterion for acute HCV. The time frame of seroconversion differs per study though; within six months,[25-30] 12 to 24 months[18,31-33] or unknown.[34-36]

HCV SEROCONVERSION

In the past, the sensitivity of HCV serology in HIV-infected patients has been questioned. In a large cohort study conducted in the USA, 3.2% of HIV-infected patients coinfected with *chronic* HCV were anti-HCV negative but plasma HCV-RNA positive.[37] Thomson *et al.* retrospectively tested stored serum samples of HIV patients on the presence of HCV antibodies. At the time point that HCV-RNA was first detectable, only 25% of the samples were anti-HCV IgG positive. This percentage subsequently increased to 63%, 87% and 90% at month 3, 6 and 9 respectively after the first positive HCV-RNA,[38] suggesting

that relying solely on HCV antibody testing induces the risk of missing patients with acute HCV infections. Furthermore, the study showed that at the time of HCV diagnosis, 76% of patients had an elevated alanine aminotransferase (ALAT) value (40 U/l being the upper limit of normal) making this a valuable method for detecting acute HCV. In the Utrecht cohort study, 18 of the 23 HIV-infected patients with acute HCV experienced an HCV seroconversion within the previous six months whereas extending this interval to 12 months resulted in a 100% seroconversion rate.[39]

Based on the available literature, we recommend the following case definition for acute HCV (modified from future European AIDS Treatment Network (NEAT) recommendations; *textbox 1*)

TREATMENT OF ACUTE HCV IN HIV-COINFECTED PATIENTS

What to treat with

In recent years, several retrospective studies have been published on the efficacy of peg-IFN-α monotherapy for the treatment of acute HCV in HIV-infected patients. SVR rates (i.e. HCV-RNA negative 24 weeks after discontinuation of therapy) varied from 0%,[40] 8%,[36] 67%[41] to 100% (*table 1*).[42,43] In two prospective non-randomised studies, patients were treated with either peg-IFN-α monotherapy or peg-IFN-α/ribavirin combination therapy.[25,44] Vogel *et al.* treated 21 patients with combination therapy and 15 patients with monotherapy, resulting in higher SVR rates of 73% (24 weeks) and 100% (48 weeks) for peg-IFN-α monotherapy compared with 38% (24 weeks) and 80% (48 weeks) for the patients treated with combination therapy. A protocol violation (treatment duration was extended from 24 to 48 weeks in nine patients) and a small number of patients per treatment arm (ribavirin, length of treatment) make the results from this study difficult to interpret. From 1999 through 2007, Morin *et al.* registered all patients with acute HCV, of whom 15 were also coinfected with HIV. The choice of therapy was left to the treating physician, resulting in five patients being treated with peg-IFN-α monotherapy and seven patients with peg-IFN-α/ribavirin combination therapy. In this study combination therapy was more successful than monotherapy (57 *vs* 40%). Due to the long period of inclusion, some patients were treated with conventional interferon-α again leading to very small numbers of patients in both treatment arms. In a recent study by Arends *et al.*,[45] treatment with peg-IFN-α monotherapy for acute HCV in 19 HIV-infected patients resulted in an SVR rate of only 37%. Remarkably, a large percentage of 47% of patients were null-responders to peg-IFN-α monotherapy (defined as <2log10 decline in HCV-RNA at week 12 of treatment).

All these studies differ with respect to design, patient characteristics and numbers of included patients making comparison between studies difficult. However, studies including mostly or exclusively HCV genotype 1 and 4 infected patients reported lower SVR rates after peg-IFN-α monotherapy,[36,46-48] compared with studies that also included HCV genotype 2 and 3 infected patients.[25,49-51]

Does addition of ribavirin to peg-IFN-α lead to a higher SVR rate? In most studies performed with peg-IFN-α/ribavirin combination therapy, SVR rates varied between 50 and 80% (*table 1*). For example, in a recent prospective study by Matthews *et al.* (n=27), an SVR rate of 80% was reported after 24 weeks of therapy.[52] Likewise, combination-therapy studies differ with respect to patient characteristics (percentage genotype 1 infected patients, time between seroconversion and treatment, CD4 cell count and amount of patients treated with antiretroviral therapy) and the definition of acute HCV. For example, in the prospective study by Dominquez *et al.* (n=14),[53] with an SVR of 71%, a low dose of ribavirin (800 mg) was used, the median CD4 count was low and the median time between diagnosis and treatment was 14 weeks. In contrast, in the study by Gilleece *et al.* (n=27),[54] reporting an SVR of 59%, combination therapy was already initiated after a median of four weeks after the diagnosis, the median CD4 count was not mentioned and ribavirin was dosed according to body weight. Despite these differences, the reported SVR rates were very similar.

Besides a probably higher SVR rate, there might be another argument favouring the addition of ribavirin to peg-IFN-α. A recent conference abstract by Matthews *et al.* demonstrated that the viral kinetics of HCV were better (i.e. steeper decline in HCV viral load during therapy) in patients treated with peg-IFN-α/ribavirin combination therapy compared with peg-IFN-α monotherapy.[55]

In conclusion, based on the available literature, treatment with peg-IFN-α/ribavirin combination therapy is the preferred treatment regimen with achievable success rates above 60%. Since this is considerably higher than SVR rates reached in HIV-infected patients with chronic HCV, treatment of HCV in the acute phase of the infection should be pursued.

When to start

Deciding at which time point one should start treatment in patients with acute HCV is difficult. On the one hand a chance of spontaneous viral clearance should be awaited while on the other hand deferring treatment to the chronic phase of HCV diminishes the chances of achieving a high SVR.

Studies in patients with an acute HCV mono-infection have shown that the rate of spontaneous viral clearance can be as high as 40% occurring mostly within 12 weeks after the diagnosis.[56] In HIV-infected patients with acute HCV

Table 1. *Overview of studies evaluating the efficacy of peg-IFN-alpha with or without ribavirin in HIV-infected patients with acute hepatitis C virus infection*

	Number of treated patients (total number of patients)	Patient characteristics	Genotype	Median time between diagnosis and start of therapy	CD4/HAART	Treatment regimen	SVR rate
Vogel *et al.*[66] 2005 (retrospective)	11 (16)	Male: 91% MSM: 91% Symptomatic: 82%	1 & 4: 91% 2: 9%	2.6 weeks	507 / 73%	Peg-IFN-α + RBV (n=5), peg-IFN-α (n=4) and IFN-α (n=2) Duration varied between 24 and 48 weeks	80% (peg-IFN-α + RBV) and 100% (peg-IFN-α)
Danta *et al.*[36] 2005 (retrospective)	23 (39)	Male: NM% MSM: NNM% Symptomatic: NM%	1 & 4: 85%	0 weeks for peg-IFN-α monotherapy; 12 weeks for peg-IFN-α/ RBV	549 / NM	Peg-IFN-α monotherapy (centre 1); Peg-IFN-α / RBV (centre 2)	60% (peg-IFN-α / RBV) 8% (peg-IFN-α monotherapy)
Serpaggi *et al.*[67] 2006 (retrospective)	10 (12)	Male: 100% MSM: 100% Symptomatic: 20%	1 & 4: 92% 3: 8%	49 days	625 / 90%	IFN (n=7), IFN + RBV (n=2) and peg-IFN (n=1)	0%
Luetkemyer *et al.*[68] 2006 (retrospective)	4 (9)	Male: 100% MSM: 67% Symptomatic: 44%	1: 75% 2: 25%	8 weeks	329 / 89%	Peg-IFN-α + RBV (WB) 1 patient 24 weeks and 3 patients 48 weeks	50% (1 patient only end-of-treatment available)
De Rosa *et al.*[69] 2009 (retrospective)	6 (7)	Male: 57% MSM: NB% Symptomatic: 14%	1 & 4: 71% 2: 29%	31 days	539 / 43%	Peg-IFN-α monotherapy 12 weeks	67%
Lambers *et al.*[18] 2010 (retrospective)	50 (52)	Male: 100% MSM: 100% Symptomatic: NM%	1: 65% 2 and 3: 4% 4: 19% Unknown: 12%	27 weeks	450 / NM	Peg-IFN-α + RBV Duration: 24 weeks (n=21) and 48 weeks (n=29)	75% (24 weeks) 86% (48 weeks)
Schulze zur Wiesch *et al.*[70] 2009 (case-series)	3 (3)	Male: 67% MSM: 33% Symptomatic: 0%	1: 33% 3: 67%	10 weeks	323 / 0%	Peg-IFN-α (n=2) and peg-IFN-α + RBV (n=1) Duration: mono (22 and 28 weeks) and combination 48 weeks	100% for mono and combination therapy
Fierer *et al.*[60] 2008 (prospective)	10 (31)	Male: 100% MSM: 100% Symptomatic: NM%	NM	'Acute phase'	527 / NM	Peg-IFN-α + RBV for 24 weeks	80%
Gilleece *et al.*[71] 2005 (prospective)	27 (50)	Male: NM% MSM: 100% Symptomatic: NM%	1: 74% 2, 3 and 4: NM	4 week	NB / 56%	Peg-IFN-α + RBV (WB) 24 weeks	59%
Dominquez *et al.*[72] 2006 (prospective)	14 (25)	Male: 100% MSM: 96% Symptomatic: 36%	1 & 4: 50% 3: 50%	14 weeks	345 / 86%	Peg-IFN-α + RBV (WB) 24 weeks	71%
Vogel *et al.*[25] 2006 (prospective)	36 (47)	Male: 100% MSM: 81% Symptomatic: 47%	1 & 4: 75% 2 & 3: 20%	7 weeks	416 / 61%	peg-IFN-α (n=15) and peg-IFN-α + RBV (n=21) Duration: 24 weeks (11 peg-IFN and 16 RBV) and 48 weeks (4 peg-IFN and 5 RBV)	Peg-IFN 24 weeks:73% Peg-IFN 48 weeks: 100% Peg-IFN+RBV 24 weeks: 38% Peg-IFN+RBV 48 weeks: 80%
Matthews *et al.*[73] 2009 (prospective)	22 (27)	Male: 100% MSM: 49% Symptomatic: 46%	1: 60% 2 & 3: 33 %	> 30 weeks	614 / 59%	Peg-IFN-α (n=2) Peg-IFN-α + RBV (n=20) 24 weeks	0% for peg-IFN-α; 80% for peg-IFN-α/RBV
Schnuriger *et al.*[74] 2009 (prospective)	20 (38)	Male: 100% MSM: NM% Symptomatic: 30%	1 & 4: 95% 3: 5%	4 weeks	509 / 75%	Peg-IFN-α + RBV (800 mg) 24 weeks	75% (2 patients with SVR became re-infected)
Morrin *et al.*[75] 2010 (prospective)	12 (15)	Male: 93% MSM: 67% Symptomatic: 27%	1 & 4: 73% 3: 13%	Less than 12 weeks (n=10) and more than 12 weeks (n=2)	NM	Peg-IFN-α: 5 (42%) Peg-IFN-α + RBV : 7 (58%) 7 patients 24 weeks and 5 patients 48 weeks	Mono: 2 (40%) Combi: 4 (57%)
Arends *et al.*[76] 2010 (prospective)	19 (23)	Male: 100% MSM: 100% Symptomatic: 0%	1: 68% 4: 32%	12 weeks	500 / 42%	Peg-IFN-α monotherapy 24 weeks (n=9) and 48 weeks (n=3)	37% (2 patients with SVR became re-infected)

MSM = men who have sex with men; NM = not mentioned; peg-IFN-α = pegylated interferon-alpha; SVR = sustained virological response; RBV = ribavirin.

chances of spontaneous viral clearance were lower (around 10 to 15%), but also highest within the first 12 weeks after the diagnosis.[57-60] However, in a recent study by Vogel *et al.*, evaluating spontaneous viral clearance rates in 92 HIV-infected patients with acute HCV, an unusually high clearance rate of 40% was reported.[61] An intent-to-treat (ITT) analysis on week 12 after the initial diagnosis showed a positive predictive value of 89% for development of chronic HCV in case of HCV-RNA positivity. Furthermore, patients not experiencing a 2log10 drop in HCV-RNA at week 4 after the diagnosis had an 85% chance of becoming chronically infected with HCV. The PCR used for HCV-RNA detection had a relatively high lower limit of detection of 600 IU/ml in contrast to the currently used detection limits of 10 to 50 IU/ml. This is important since it has been observed in acute HCV-infected patients that HCV-RNA fluctuates, sometimes even briefly becoming undetectable.[62]

Although the absence of spontaneous viral clearance might be predicted four weeks after the infection, this does not imply that treatment should be started thereafter. To date, no randomised study has compared the outcome of therapy in HIV-infected patients with an acute HCV infection starting treatment very early after the diagnosis compared with those who initiated treatment 12 weeks after the diagnosis. In the study by Matthews *et al.*[63] a high SVR rate of 80% was reported with treatment being initiated more than 30 weeks after the diagnosis of acute HCV. Furthermore, a recent retrospective cohort study in 50 patients from Amsterdam, evaluated the effect on SVR in patients with initiation of therapy within six months (24 weeks) after the diagnosis versus deferral of treatment thereafter.[18] In this study, the time of infection was defined as the midpoint between the last negative and first positive HCV test (serology or PCR). Although statistically not significant, in both the 24- and 48-week treatment arms a higher SVR rate was reported in patients starting treatment within six months after the diagnosis. Furthermore, in most intervention studies, treatment of acute HCV in HIV-infected patients was initiated after a median of 12 weeks after the diagnosis. In a cohort of acute HCV mono-infected Japanese patients a significantly lower SVR rate was noted when the start of therapy was postponed for one year compared with initiating therapy within eight weeks after the diagnosis (40% versus 81%).[64] Taken together, although spontaneous viral clearance generally occurs within 12 weeks after an acute HCV infection and the chances of persistence of acute HCV might be predicted already four weeks after the infection, evidence for very early initiation of therapy is lacking.

In conclusion, after establishing the diagnosis of acute HCV in HIV-coinfected patients, it is recommended to await spontaneous viral clearance for a period of no more than 12 weeks. If no spontaneous clearance occurs, anti-HCV therapy should be started.

Treatment duration

Most studies in HIV-infected patients with acute HCV achieved SVR rates above 60% after 24 weeks of treatment (*table 1*). This is supported by the previously mentioned Amsterdam cohort study evaluating the outcome of therapy in 50 HIV-infected patients with acute HCV.[18] No significant difference in SVR rates was demonstrated between 24 and 48 weeks of therapy (75 *vs* 86% in all patients starting therapy within six months after the diagnosis). In contrast, in a German study higher SVR rates were seen in the 48-week arm compared with the 24-week arm (89 *vs* 52%; p=0.062).[25] It must be noted that these patients were erroneously treated for 48 weeks due to a protocol violation and that both patients treated with peg-IFN-α monotherapy and combination therapy with ribavirin were pooled together for this analysis.

A recent re-analysis by Vogel *et al.*[65] of previously published studies, evaluated the treatment outcome and the role of HCV viral kinetics during therapy in a group of 111 HIV-infected patients treated for acute HCV. Longer treatment duration did not significantly improve SVR rates. Both achievement of a rapid virological response (in this study defined as HCV-RNA <600 IU/ml) and a complete early virological response (cEVR, undetectable HCV-RNA (<600 IU/ml) at week 12) were strong predictors of achieving an SVR. In other words, in case of favourable viral kinetics (fast decline of HCV-RNA until undetectable at week 4 (RVR) or week 12 (cEVR)), 24 weeks of treatment seems sufficient. On the other hand, only 9% of patients without a cEVR reached an SVR.

Quality of studies on which a recommendation is based:

Grade	Definition
A1	Meta-analysis of at least two independent studies of A2 level
A2	Randomised double-blind, placebo-controlled study of adequate quality and size
B	Comparative study not fulfilling the characteristics of an A2 level study (including case-control studies and cohort studies)
C	Non-comparative studies
D	Expert opinion

Level of evidence based on the quality of the study on which a recommendation is based

Level	Definition
I	Study of level A1 or at least two independent studies of level A2
II	Single level A2 study or at least two independent level B studies
III	Single level B or C study
IV	Expert opinion

CONCLUSION

In conclusion, 24 weeks of therapy is the preferred duration of treatment in HIV-infected patients with acute HCV achieving either an RVR or a >2log10 drop in HCV-RNA with an undetectable HCV viral load at week 12 of therapy (cEVR). In all other patients extending treatment duration to 48 weeks should be considered. In patients without a >2log10 drop in HCV-RNA at week 12 of therapy, treatment can be stopped.

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Netherlands
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Red-cell casts despite a negative urine dipstick analysis in a patient with Crohn's disease

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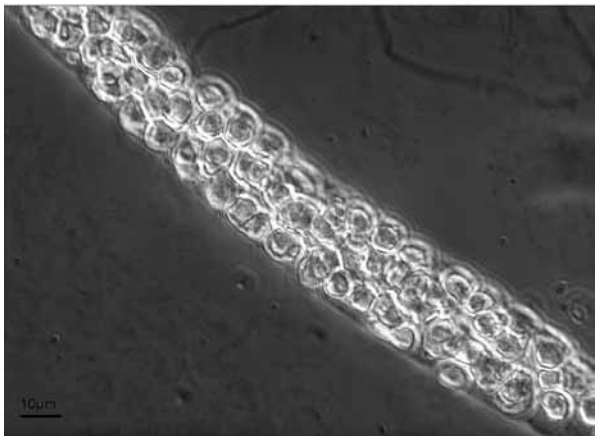
Dear Editor,

A 17-year-old cachectic man with Crohn's disease was admitted to the Department of Ophthalmology in our hospital with panuveitis. Laboratory screening revealed a high serum creatinine of 90 μmol/l (normal range18 to 62 μmol/l). Urine dipstick analysis was repeatedly negative for erythrocytes, leukocytes and protein. Although a negative dipstick is known to reliably exclude haematuria we also examined the urinary sediment for dysmorphic erythrocytes, assuming a renal disease as part of the systemic inflammatory disease comprising the panuveitis and Crohn's disease. Microscopic examination of the urine showed no dysmorphic erythrocytes. However, red-cell casts indicative of glomerular disease were seen (*figure 1*). The panuveitis, the glomerulonephritis, and the creatinine level improved after treatment with prednisone. The red-cell casts had disappeared.

This case shows that even with a repeatedly negative urine dipstick analysis, glomerular disease can be missed if microscopic examination of the urine is not performed.

Figure 1. *Blood cell cast in urine of the patient*



LETTER TO THE EDITOR

Vitamin D might reduce some vascular risk factors and, consequently, risk of dementia

W.B. Grant

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Dear Editor,

A recent paper noted that vascular risk factors are associated with an increased risk of dementia and that studies investigating the effect of a multi-component intervention aimed at vascular risk factors to prevent or slow down cognitive decline and dementia are desirable.[1] Overlooked in the discussion was any mention of the possible role of vitamin D in reducing the risk of vascular risk factors and, hence, risk of dementia. Of the vascular risk factors mentioned, serum 25-hydroxyvitamin D [25(OH)D] has been found to be inversely correlated in observational studies with risk of hypertension[2] and diabetes mellitus.[3,4] Lower serum 25(OH)D levels have also been found inversely correlated with the incidence of cardiovascular disease[4,5] and cognitive impairment.[6] While the mechanisms whereby vitamin D reduces the risk of cardiovascular disease have not been precisely delineated, there is mounting evidence that 'vitamin D may influence blood pressure through the renin-angiotensin system, parathyroid hormone levels, myocardial function, inflammation, and vascular calcification.'[7] Vascular calcification is a marker of atherosclerotic burden and a risk factor for dementia.[8]

Since there is good evidence that higher serum 25(OH) D levels reduce the risk of vascular diseases, diabetes, and cognitive impairment, and since these diseases often precede dementia, vitamin D would very likely reduce the risk of dementia.[9] Additional studies to examine this hypothesis would be very useful.

There are many other health benefits of vitamin D including reduced risk of many types of cancer and infectious diseases. It was estimated that if the population mean value of serum 25(OH)D level in the Netherlands were increased from 50-55 nmol/l to 105 nmol/l this would reduce all-cause mortality rates by 18%.[10]

DISCLOSURE

I receive or have received funding from the UV Foundation (McLean, VA), the Sunlight Research Forum (Veldhoven), Bio-Tech-Pharmacal (Fayetteville, AR), the Vitamin D Council (San Luis Obispo, CA), and the Danish Sunbed Federation (Middelfart).

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