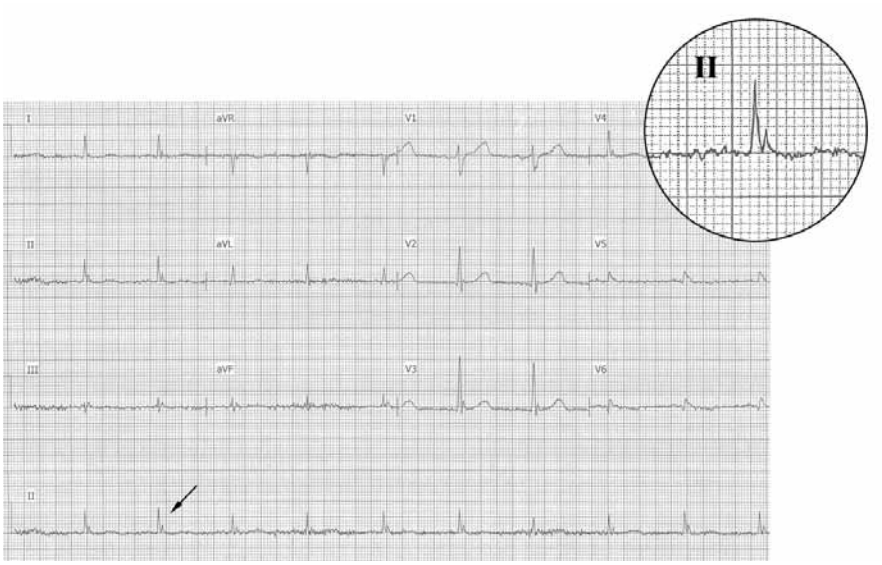
Netherlands
The Journal of Medicine
PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



The electrocardiogram of a man found in the forest: What is your diagnosis?

NOVEMBER 2010, VOL. 68. No. 11, ISSN 0300-2977

VAN ZUIDEN COMMUNICATIONS

Netherlands
The Journal of Medicine

MISSION STATEMENT

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

EDITORIAL INFORMATION

Editorial office
Academic Medical Centre,
Department of Medicine (F-4)
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Tel.: +31 (0)20-566 21 71
Fax: +31 (0)20-691 96 58
E-mail: m.m.levi@amc.uva.nl
http://mc.manuscriptcentral.com/nethjmed

CITED IN

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

Contents

ISSN: 0300-2977

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

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

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

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

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

VAN ZUIDEN communications

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

EDITORIAL

Submission, acceptance, citation and downloads of articles in the Netherlands Journal of Medicine

M. Levi

Department of Medicine, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands, m.m.levi@amc.uva.nl

Over the last ten months, the Netherlands Journal of Medicine has seen a further increase in the number of submissions from all parts of the world (*table 1*). Since the transfer of the editorial office to Amsterdam the number of submissions has almost doubled.[1] The journal impact factors for 2010 predict a further rise of the impact factor, which may be a significant factor in the increased submission rate. Unfortunately, an increasing number of submissions and a fixed space for publication will lead to lower acceptance rates. The evolvement of the acceptance rate over the last three years is shown in *table 1*. In addition, *table 2* shows the acceptance rate of the various article types. It is clear that the acceptance rate of case reports (11%) has become very low. In fact, we have adopted the policy that case reports could only be published if they substantially increase our insight into the pathogenesis or background of a disease or if they report a really original clinical finding. Some case reports merely (nicely) illustrate a classic disorder and for these reports the format of a photo quiz may be more appropriate. Indeed, our acceptance rate for photo quizzes is substantially higher (*table 2*).

The impact of articles published in scientific journals is often measured by the number of citations. In addition, we can track the number of downloads of full-text articles from our website and this may be another estimate of the 'impact' of a journal.[2] Interestingly, the number of downloads of review articles, original articles and case reports also shows a marked increase over the last few years (*figure 1*). Both downloads and citations may be considered to be an expression of acknowledgement and interest. Hence, not entirely surprisingly, there seems to be

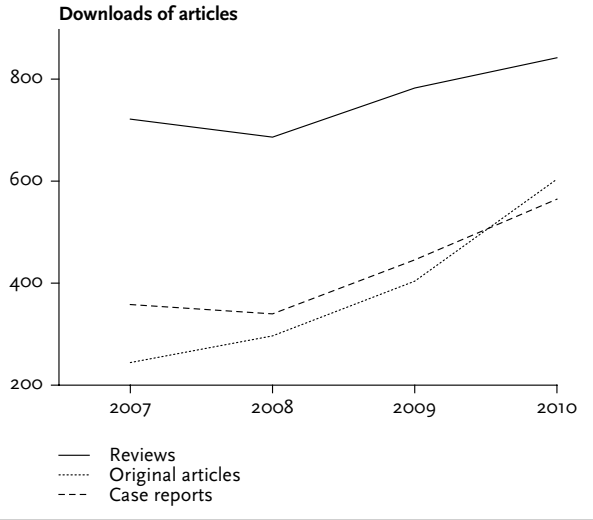
Table 1. *Number of submissions to the Netherlands Journal of Medicine over the last few years and acceptance rate (= published papers divided by submitted papers)*

	2010	2009	2008
Submissions	555	328	245
Overall acceptance rate	23%	30%	42%
Origin of submissions			
• Netherlands	47%	61%	70%
• Other European countries	21%	16%	14%
• North America	10%	7%	4%
• Rest of the world	22%	16%	12%

Table 2. *Yearly number of submissions and acceptance rate of various manuscript types in the Netherlands Journal of Medicine*

	Number of submissions	Acceptance rate
Review	50	71%
Original article	100	14%
Case report	272	11%
Photo quiz	71	56%
Other	62	16%

Figure 1. *Download of articles published in the Netherlands Journal of Medicine over the years 2007-2010*



a clear relationship between the number of downloads and the number of citations of an individual article. In *table 3* we show the papers that have generated the largest number of downloads from the Netherlands Journal of Medicine in the last ten months, which are also the papers that are most often cited.

Acceptance or rejection of a manuscript is a result of labour-intensive peer review, and we thank the many reviewers of the Netherlands Journal of Medicine who again have helped us tremendously. Also, the assistance of our highly active group of junior associate editors, composed of residents in training for Internal Medicine who have themselves been very active in research, has again shown to be invaluable for guiding the review process.

Table 3. *Most downloaded articles in the Netherlands Journal of Medicine in 2010*

Reviews

- Smeding L, *et al.* Clinical implications of heart-lung interactions.[3]
- Mebis L, *et al.* The hypothalamus-pituitary-thyroid axis in critical illness.[4]
- Beishuizen SJ, *et al.* Immune reconstitution inflammatory syndrome: immunopathogenesis, risk factors, diagnosis, treatment and prevention.[5]

Original articles

- van Tuijn CF, *et al.* Reduction of the door-to-needle time for administration of antibiotics in patients with a severe infection: a tailored intervention project.[6]
- van Hateren KJ, *et al.* Five-year incidence of type 2 diabetes mellitus in patients with familial combined hyperlipidaemia.[7]
- Kleefstra N, *et al.* Self-monitoring of blood glucose in tablet-treated type 2 diabetic patients (ZODIAC).[8]

Case reports

- van den Brand M, *et al.* Glycogenic hepatopathy: a rare cause of elevated serum transaminases in diabetes mellitus.[9]
- Haringhuizen A, *et al.* Fatal cerebral oedema in adult diabetic ketoacidosis.[10]
- Sie MP, *et al.* Human recombinant insulin and amyloidosis: an unexpected association.[11]

REFERENCES

1. Levi M. The Netherlands Journal of Medicine: the next episode. Neth J Med. 2009;67(4):115.
2. Levi M. Big hits in the Netherlands Journal of Medicine. Neth J Med. 2009;67:204-5.
3. Smeding L, Lust E, Plotz FB, Groeneveld AB. Clinical implications of heart-lung interactions. Neth J Med. 2010;68(2):56-61.
4. Mebis L, van den Berghe G. The hypothalamus-pituitary-thyroid axis in critical illness. Neth J Med. 2009;67(10):332-40.
5. Beishuizen SJ, Geerlings SE. Immune reconstitution inflammatory syndrome: immunopathogenesis, risk factors, diagnosis, treatment and prevention. Neth J Med. 2009;67(10):327-331.
6. van Tuijn CF, Luitse JS, van de Valk M, et al. Reduction of the door-to-needle time for administration of antibiotics in patients with a severe infection: a tailored intervention project. Neth J Med. 2010;68(3):123-7.
7. van Hateren KJ, Kleefstra N. Five-year incidence of type 2 diabetes mellitus in patients with familial combined hyperlipidaemia. Neth J Med. 2010;68(1):332.
8. Kleefstra N, Hortensius J, Logtenberg SJ, et al. Self-monitoring of blood glucose in tablet-treated type 2 diabetic patients (ZODIAC). Neth J Med. 2010;68(1):311-6.
9. van den Brand M, Elving LD, Drenth JP, van Krieken JH. Glycogenic hepatopathy: a rare cause of elevated serum transaminases in diabetes mellitus. Neth J Med. 2009;67(11):394-6.
10. Haringhuizen A, Tjan DH, Grool A, van Vught R, van Zante AR. Fatal cerebral oedema in adult diabetic ketoacidosis. Neth J Med. 2010;68(1):35-7.
11. Sie MP, van der Wiel HE, Smedts FM, de Boer AC. Human recombinant insulin and amyloidosis: an unexpected association. Neth J Med. 2010;68(3):138-40.

Netherlands
The Journal of Medicine

REVIEW

Chronic granulomatous disease: recent advances in pathophysiology and treatment

R.A. Seger

Division of Immunology/Haematology/BMT, University Children's Hospital of Zurich, Zurich, Switzerland, tel.: +41 44 266 73 15, fax: +41 44 266 79 14, e-mail: reinhard.seger@kispi.uzh.ch

ABSTRACT

Chronic granulomatous disease (CGD) was characterised half a century ago as a primary immunodeficiency disorder of phagocytic cells resulting in failure to kill a specific spectrum of bacteria and fungi and in concomitant hyperinflammation with widespread tissue granuloma formation. CGD now comprises five genetic defects, each impairing one of five essential subunits of the phagocyte NADPH oxidase generating reactive oxygen species. In the past few years CGD has lead to a new understanding of the importance of phagocyte oxygen metabolism for intra- and extracellular host defence and for resolution of the concomitant inflammatory process. In a not too distant future, this may help to tailor novel pharmacological and cellular interventions to the requirements of individual patients.

This review covers recent advances in the pathophysiology of CGD and outlines today's clinical presentation as well as the basic principles for treatment of this relatively rare genetic disease. 'Fatal' granulomatous disease 50 years later has become a chronic inflammatory disorder with a median survival of 30 years and is of interest to both paediatricians and internists.

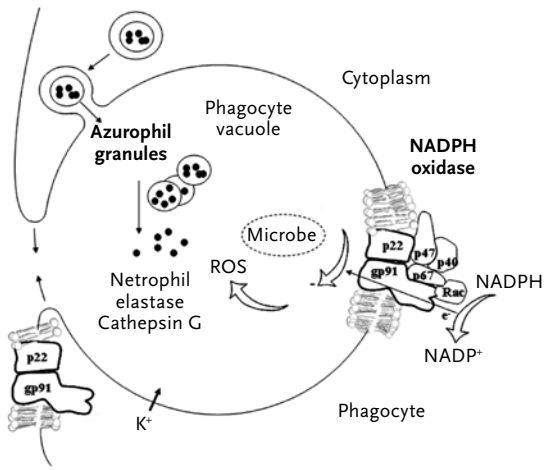
KEYWORDS

Chronic granulomatous disease, NADPH oxidase deficiency, microbial killing defect, hyperinflammation, stem cell transplantation, gene therapy

INTRODUCTION

Chronic granulomatous disease (CGD) is a group of five genetic disorders of the phagocyte (neutrophil, monocyte, macrophage, eosinophil) nicotinamide adenine dinucleotide phosphate (NADPH) *oxidase complex* generating reactive oxygen species (ROS) in response to physiological stimuli such as the phagocytosis of microbes.[1,2] The catalytic core of the phagocyte NADPH oxidase (phox) is *gp91phox*, a phagocyte-specific transmembrane glycoprotein with an apparent molecular mass of 91kDa, recently renamed NOX2.[3] Gp91phox/NOX2 transports electrons from cytosolic NADPH via flavin adenine dinucleotide (FAD), and two haemes onto molecular oxygen, which is then converted into superoxide anion and subsequently to several ROS (e.g. the highly diffusible hydrogen peroxide and hypochlorous acid) (*figure 1*). In the cell membrane NOX2 is stabilised by *p22phox*, which also serves as an anchoring site for three regulatory proteins: *p47phox, p67phox* and *p40phox*. The last three form a complex in the cytoplasm of resting phagocytes, which is translocated en bloc to the endocytosed cell membrane (the phagocytic vacuole

Figure 1. *Phagosome formation and oxidative killing of microbes by phagocytic cells*



ROS = reactive oxygen species

or phagosome) during phagocytosis. A small cytosolic GTPase, *Rac*, is also activated, translocates and induces a crucial conformational change within p67, needed to activate NOX2. p47 and p40 serve as adaptor molecules.
Mutations in all of the five structural genes of the NADPH oxidase complex have been found to cause CGD with an overall prevalence of 1:250,000. X-linked defects in NOX2 account for about 70% of cases, autosomal-recessive defects in p47 for about 20% and the remainder for the very rare p22 and p67 defects.[4,5] Last year a single p40phox deficient CGD patient was identified.[6] Clinically the NOX2 deficient form of CGD runs a more severe course than the p47 deficient form, with earlier presentation and earlier death.[4,5]
A provisional diagnosis of CGD is made by a DHR assay using flow cytometry or by nitroblue tetrazolium (NBT) using light microscopy. DHR (dihydrorhodamine-1, 2, 3) freely enters the phagocytes and is oxidised intracellularly to rhodamine-1, 2, 3 by diffusible H_2O_2 after phagocyte stimulation.[7] Since the assay relies on endogenous myeloperoxidase (MPO), it will give a false-negative DHR result in complete MPO deficiency which can be misinterpreted as variant NADPH oxidase deficiency (CGD).[8] NBT is a yellow dye that is co-phagocytosed with microbial particles and reduced by superoxide to blue, insoluble formazan, which cannot leave the phagosomes.[7] In contrast to the objective DHR assay, the NBT test is subjective being based on microscopic inspection of a limited number of cells, classical CGD patients showing no formazan formation. Variant CGD with residual ROS production may be missed in the NBT test as it manifests as faint blue staining.[9] X-linked carriers of NOX2 deficiency have a mosaic pattern of normal and defective neutrophils on oxidative testing by either DHR or NBT, ranging in most cases from 20 to 80% oxidase positive cells. It must be kept in mind, however, that up to one-third of x-linked defects arise from new mutations in germ-line cells of the mother and will not be present in her somatic cells.
The provisional diagnosis of CGD should always be checked by a specialist centre, where a definitive diagnosis can be given by immunoblotting for the components of NADPH-oxidase and by DNA-based molecular techniques. Molecular determination of the disease-causing mutation(s) is required before genetic counselling, before prenatal or preimplantation diagnosis and before gene therapy.

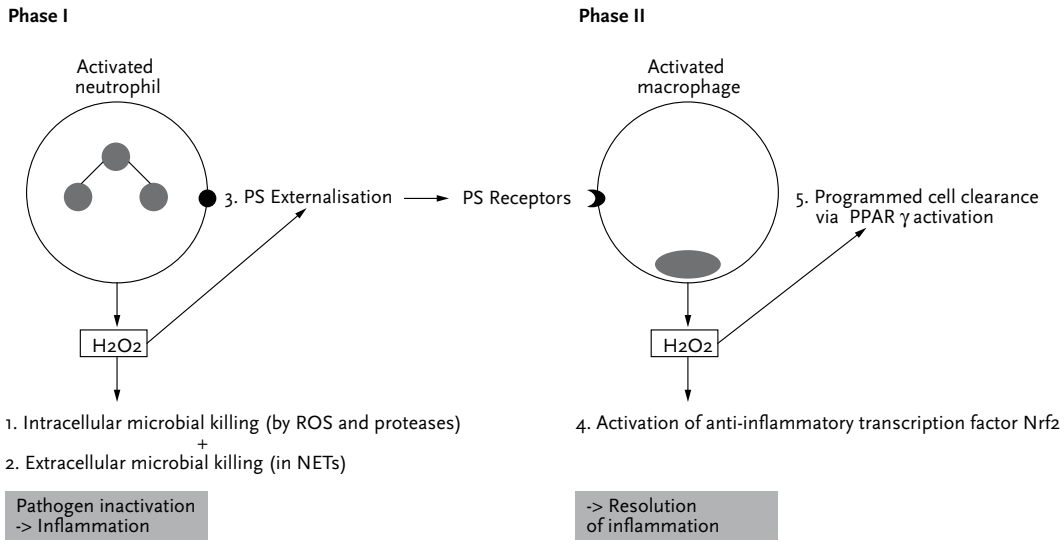
ADVANCES IN PATHOPHYSIOLOGY

Knowledge gained on the mechanisms by which NADPH oxidase normally kills microbes and resolves inflammation has broad relevance to understanding host-pathogen interactions (*figure 2*).

Deficient antimicrobial defence

Some oxygen metabolites generated by NADPH oxidase activation show direct cytotoxicity and antimicrobial properties.[10] NADPH oxidase can also mediate intracellular host defence indirectly by activation of microbicidal granule *proteases* in neutrophils as recently shown.[11] The influx of electrons into phagosomes is compensated

Figure 2. *Phagocyte NADPH-oxidase functions in host defence and inflammation*



NETs = neutrophil extracellular traps; PS = phosphatidylserine

by cation fluxes across the phagosomal membrane to maintain electrogenic neutrality. The increased ionic strength leads to solubilisation and activation of granule proteases, e.g. elastase and cathepsin G, bound to an anionic proteoglycan matrix stored in primary granules. In CGD neutrophils the granule matrix is not resolved after fusion of granules with the phagosome.

Neutrophils also release cytosolic and granule proteins as well as chromatine (DNA/histones), which mix to form neutrophil extracellular traps (NETs). These NETs bind and kill bacteria[12] and target fungi.[13] CGD neutrophils are deficient in NET formation,[14] which was reversed in one patient by gene therapy and was accompanied by resolution of his therapy-refractory pulmonary aspergillosis.[15] Current studies aim at understanding the relative contributions to antimicrobial host defence of each of the three ROS-dependent killing mechanisms: NADPH oxidase-generated ROS *vs* protease activation *vs* NET formation.

Excessive inflammation

There is new strong clinical and experimental evidence that the NADPH oxidase is critical for downregulation of inflammation. The recently recognised syndrome of 'mulch pneumonitis' in CGD patients exemplifies this role.[16] Two to three days after spreading mulch or clearing mouldy leaves (and inhaling *Aspergillus* spores) fever and dyspnoea manifest with diffuse interstitial infiltrates on chest radiographs and hypoxia. Successful treatment requires both antifungals and steroids, the latter to prevent extensive *Aspergillus*-triggered inflammation.

In CGD mice intratracheal instillation of zymosan (a fungal cell wall product of beta-glucans) elicits progressive pyogranulomata. Recent studies revealed a crucial role for a ROS-sensitive anti-inflammatory transcription factor, Nrf2. Consistent with these findings mononuclear blood cells from CGD patients stimulated by zymosan showed reduced Nrf2 activity.[17] In agreement with a proinflammatory state, monocytes from CGD patients reveal *increased* inflammasome activity as manifested by activation of caspase 1, followed by Il-1β production and release.[18,19]

Recent studies suggest an additional role of the NADPH oxidase in the process of macrophage mediated clearance of activated and infected neutrophils. Externalisation of the anionic phospolipid, phosphatidylserine (PS), on the neutrophil surface is recognised by PS receptors in macrophages and facilitates the uptake and degradation of such neutrophils.[20] CGD neutrophils are defective in ROS-dependent exposure of PS on the cell surface. In addition CGD macrophages have a reduced capacity for uptake of PS-positive target cells, thus impairing resolution of the inflammatory process.[21] PPARγ is known to upregulate proteins involved in both apoptotic cell recognition and digestion and has been found deficient in CGD macrophages. Pharmacological activation of PPARγ by proglitazone in CGD mice normalised uptake of CGD neutrophils by CGD macrophages. Proglitazone may thus be effective in the clinical treatment of CGD inflammation[22] and needs to be tested further.

Currently glucocorticoids are used as main anti-inflammatory agents in CGD. They prevent tumour necrosis factor-alpha-dependent multinucleated giant cell formation[23] and promote non-phlogistic phagocytosis of activated neutrophils by macrophages.[24] The ongoing molecular dissection of Nrf2 and PPARγ activation in CGD may hopefully yield novel targets for tailored pharmacological interventions in the near future, avoiding many of the steroid side effects.

RECURRENT INFECTIONS

Skin, lymph nodes, lung, and liver are the most frequent sites of infection in CGD. In North America and Europe five main groups of organisms persisting inside CGD neutrophils predominate:[5] *Staphylococcus aureus* (lymphadenitis, liver abscess), *Burkholderia* complex (necrotising pneumonia +/- sepsis), *Serratia marcescens* (sepsis +/- skin ulcers and osteomyelitis), *Nocardia and Aspergillus* spp (pneumonia +/- dissemination to brain and bone). The infections arise mostly from inescapable environmental exposure and intermittent compliance with long-term antimicrobial prophylaxis.

In other parts of the world different microbial agents predominate. Mycobacterial infections (due to *BCG* and *M. tuberculosis)* in CGD patients have been reported from China, Iran and Latin America.[25,26] Patients, however, develop severe localised (not disseminated) BCG infection and pulmonary (not miliary) tuberculosis. Infection-associated haemophagocytic syndrome in CGD patients triggered by *Leishmania* has been observed in the Mediterranean region.[27]

Recently, two additional chronic infections due to fastidious organisms were described in North American and European patients, requiring combined antibiotic therapy and surgery: *Granulibacter bethesdensis*, a gram-negative rod growing on charcoal yeast extract at 35° causing chronic multifocal necrotising lymphadenitis and requiring long-term ceftriaxone therapy,[28] and *Actinomyces* spp, gram-positive rods, growing anaerobically on sheep blood agar at 37° causing severe chronic actinomycosis and necessitating long-term penicillin G/V therapy.[29]

Diagnostic work-up of infections in CGD requires a vigorous microbiological diagnosis with full help from a specialist microbiology laboratory. Needle biopsies, ribosomal DNA-PCR analyses and susceptibility testing of difficult-to-grow organisms are important steps towards a tailored antimicrobial therapy. Whole body PET/CT scans

can be useful to localise occult infections for biopsy and to exclude dissemination.[30]

Prevention and treatment of infections

Common sense measures in reducing exposure to infectious agents can be downloaded from www.cgd.org.uk. Pulmonary infections can be prevented by avoiding sources of *Aspergillus* spores (e.g. farms, mulch, construction sites) and refraining from smoking. Patients should receive all routine immunisations, except BCG.

The cornerstone of clinical care is lifelong antibiotic and antifungal prophylaxis. Drugs of choice are the lipophilic co-trimoxazole (at 6 mg/kg/day of trimethoprim; during pregnancy replaced by cefuroxime) and itraconazole (at 5 mg/kg/day oral solution). Retrospective studies support long-term co-trimoxazole prophylaxis,[31,32] and a randomised, double-blind placebo-controlled study justifies routine administration of itraconazole in CGD.[33] Interferon-gamma prophylaxis is offered by most European physicians only in selected CGD cases. A small subgroup of variant X-CGD patients with splice site mutations has been shown to be responsive to interferon stimulation[34,35] through improved splicing. A significant clinical efficacy in preventing aspergillosis, however, has not been demonstrated yet.[36] In addition, the drug is expensive and requires repeated injections (2 x 50 ug/m^2/week subcutaneously).

Before culture results are available, empiric antibiotic therapy has to be based on the most likely infectious agents expected. Antibiotics should cover a broad range of bacteria including *S. aureus*, *Burkholderia*, *S. marcescens*, and *Nocardia*. Oral ciprofloxacin and intravenous meropenem are useful first-line agents. A course of oral ciprofloxacin can also be taken as reserve on holidays. In addition co-trimoxazole should be continued at a double dose (12 mg/kg/day trimethoprim) to compensate for possible intermittent compliance. In case of pneumonia, voriconazole needs to be added as antifungal agent (in children at 12 mg/kg/day). As infections often respond slowly, intravenous treatment must be followed by prolonged oral therapy. Treatment must be further extended if special organisms are isolated (eg. *Nocardia* spp and *Aspergillus* spp). A novel antibiotic, Linezolid, has proven effective second to high-dose co-trimoxazole (at 20 mg/kg/day trimethoprim) in nocardiosis with excellent penetration of the cerebrospinal fluid.[37] Posaconazole is effective salvage therapy against a broad spectrum of invasive fungal infections, including the difficult-to-treat infections of the central nervous system.[38]

Surgical procedures in CGD comprise drainage of abscesses, excision of a consolidated focal infection in lung or liver and relief of obstructions (e.g. hydronephrosis). Wounds and surgical sites in CGD heal very slowly and may form fistulas. Liver abscesses in CGD are dense and caseous. This is why larger liver abscesses require surgical excision and drainage in addition to a one- to two-month course of antibiotic therapy.[39] When surgery is contraindicated experimental approaches can be tried: percutaneous radiofrequency thermal ablation as used for treatment of liver cancer[40] or steroids at 1 mg/kg/day in addition to the antibiotic therapy.[41]

With the advent of potent new antifungal drugs the use of white cell transfusions has decreased considerably. Alloimmunisation to HLA class I antigens and transfer of cytomegalovirus (CMV) by infected neutrophils has complicated subsequent allogeneic stem cell transplantation. Therefore, white cell transfusions should now be reserved as a last resort.

INFLAMMATORY COMPLICATIONS

Persistent inflammation also occurs independently of infection. The inflammatory complications of CGD are most prominent in the gastrointestinal and urinary tracts. Gastric outlet obstruction is common.[42] About one third of patients are affected by granulomatous colitis mimicking Crohn's disease.[43,44] In the urinary tract the most common manifestation is inflammatory cystitis which can lead to obstruction of ureteric orifices and cause hydronephrosis. Imaging findings of infections and inflammatory complications often have unique features that can help to suggest a diagnosis of CGD.[45]

Treatment of exuberant inflammation

Cautious use of immunosuppressive therapy, namely corticosteroids, is required for acute granulomatous exacerbation in the lung, the bowel and urinary tract as well as for inflammatory bowel disease (IBD). Invasive lung aspergillosis and nocardiosis profit from initial addition of steroids (1 mg/kg/day for three days, then taper). Granulomatous cystitis quickly responds to corticosteroids (e.g. 0.5 to 1 mg/kg/day prednisone for the first week, to be tapered over six weeks). First-line therapy for IBD (*table 1*) in severe cases is prednisone (e.g. 1 mg/kg/day) with gradual tapering over several months. If high-dose steroids are administrated in the long term, antifungal prophylaxis should be switched from itraconazole to voriconazole, since itraconazole is a strong inhibitor of CYP3A4 increasing steroid levels threefold by blocking their degradation.[46] Faecal calprotectin is a good measure for follow-up of colitis activity.[47] Anti-TNF drugs (e.g. Remicade®) may be administered short term for remission induction in steroid-refractory patients. Long-term anti-TNF therapy combined with steroids however is contraindicated because of high risk of infections in an already genetically

Table 1. *CGD: drugs for treatment of granulotomatous colitis*

	Mildly/moderately active	Severely active	Perianal fistulas***
Topical treatments			
sulfasalazine oral (40-50 mg/kg/day)	+ (induction ± maintenance)	-	-
Systemic treatments			
prednisone oral (1 mg/kg/day, then taper)	+ (induction)		
iv. (1 mg/kg/day, then slow taper	-	+ (induction ± maintenance)	-
infliximab (5 mg/kg at 0, 2 6 weeks)*	-	+ (induction, if steroid refractory)	+ (induction)
azathioprine (2.5 mg/kg/day)**	-	+ (maintenance), if steroid dependent or refractory)	+ (maintenance)

***in CGD not for maintenance; **slow onset of action (3-4 mo); ***add metronidazole/ciprofloxacine.**

immunodeficient patient. Thalidomide may also be used as a successful treatment for refractory CGD colitis.[48] Steroid-dependent or refractory colitis can be cured by stem cell transplantation with rapid induction of remission (within two months),[49,50] avoiding the need for extensive colectomy and subsequent anastomosis complications.

CURE OF THE DISEASE

Haematopoietic stem cell transplantation

Conventional myeloablative marrow conditioning followed by transplantation of unmodified haematopoietic stem cells is a definite cure for CGD. In 2002 a European collaborative study reported the outcome of 27 mostly paediatric CGD patients receiving a busulfan-based regimen followed by an human leucocyte antigen (HLA) genoidentical marrow graft from a sibling donor (MSD).[49] Severe side effects from haematopoietic stem cell transplantation (HSCT), namely graft-*versus*-host disease and inflammatory flare-up, were exclusively seen in a subgroup of nine patients with ongoing infection, mainly aspergillosis. Overall survival was 85%, with 81% of patients cured. Most cured patients had >95% circulating donor myeloid cells. Pre-existing infections and chronic inflammatory lesions cleared in all engrafted survivors. Even children with severe lung restriction improved their lung function, albeit slowly.

These favourable results after myeloablation have recently been extended to matched unrelated donor (MUD) transplants. In a series from Ulm seven of nine MUD recipients are survivors[51] and 18 of 20 transplanted in Newcastle (from 10 MSD and 10 MUD) survived with most patients achieving normal neutrophil function, remission of colitis and catch-up-growth.[50]

The decision for or against HSCT should be made early in life based on the individual clinical course. HSCT may be most useful in patients with recurrent serious infections despite correct antimicrobial prophylaxis or with severe steroid-dependent inflammatory complications provided an HLA identical stem cell donor is available.[52]

Two other recent advances are noteworthy. The first is the development of a reduced intensity conditioning regimen (RIC) for adults and paediatric CGD patients over six years of age. RIC-HSCT using busulfan (8 to 10 mg/kg), adjusted with busulfan kinetics, fludarabine (180 mg/m^2), ATG-Fresenius (40 mg/kg) and HLA-matched donors (MSD=5, MUD=3) was performed in eight high-risk CGD patients in Zurich. All engrafted with full donor chimerism, without graft-*versus*-host disease and with resolution of active infections and inflammatory foci, except for one adult patient who had received a CMV negative MUD and died on day +150 from CMV pneumonitis.[52,53] RIC with subsequent HSCT is thus a promising treatment modality for fragile CGD patients with intractable infection or inflammation.

Second, in the era of pre-implantation genetic diagnosis, saviour siblings can be selected at a very early embryonic (8 cell) stage to screen for CGD and to determine HLA type, and thus can be chosen as potential umbilical cord blood donors for an affected older sibling lacking an HLA-identical stem cell donor. As the probability of a successful pregnancy, even in the most experienced *in vitro* fertilisation centres, is low (10%), and the treatment option requires the firm wish of the parents to have another healthy child, this treatment modality has to be approached with reserve and is not allowed in all countries.[54,55]

Stem cell gene therapy

Gene therapy for restoration of the NADPH oxidase is the ultimate goal. Experimental gene therapy for CGD in selected patients with very poor performance status has recently been undertaken in Frankfurt, London, Zurich, Seoul and the USA (at NIH) in 12 patients.[56-58] A transitory beneficial effect on pre-existing infections similar to the clinical response known of several white

cell transfusions from healthy donors has been observed in all. A major obstacle however remained: the lack of selective growth advantage of gene transduced cells. Despite submyeloablative chemotherapy long-term engraftment was only observed in three out of the 12 patients.[58] In these three patients limited expansion of gene-corrected cells was seen due to activating retroviral insertions in the MDS1/Evi1 oncogene. These three patients later developed a myelodysplastic syndrome with monosomy 7 and two received a myeloablative allogeneic (MUD) rescue transplant.[59] For safety reasons a new generation, self-inactivating (SIN) vector lacking the potent retroviral enhancer elements and showing much less transactivation potential has been developed. Transgene expression within this vector is driven by a myelospecific, cellular promoter, thus further reducing the probability of oncogene activation at the stem cell level.[58]

In summary a transitory gene therapy approach to CGD has finally become feasible to overcome life-threatening, therapy-resistant infections. Permanent cure of CGD by gene therapy, however, remains a more distant goal.

ACKNOWLEDGMENTS

My thanks go to Manuel Grez, Steven Holland, Dirk Roos and the members of our Paediatric Immunology/BMT team for helpful discussions and to Janine Reichenbach and Ulrich Siler for manuscript review.

REFERENCES

1. Holland SM. Chronic granulomatous disease. Clin Rev Allergy Immunol. 2010;38(1):3-10.
2. Seger RA. Modern management of chronic granulomatous disease. Br J Haematol. 2008;140(3):255-66.
3. Sumimoto H. Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species. Febs J. 2008;275(13):3249-77.
4. van den Berg JM, van Koppen E, Ahlin A, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009;4(4):e5234.
5. Winkelstein JA, Marino MC, Johnston RB, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine (Baltimore). 2000;79(3):155-69.
6. Matute JD, Arias AA, Wright NA, et al. A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40 phox and selective defects in neutrophil NADPH oxidase activity. Blood. 2009;114(15):3309-15.
7. Elloumi HZ, Holland SM. Diagnostic assays for chronic granulomatous disease and other neutrophil disorders. Methods Mol Biol. 2007;412:505-23.
8. Mauch L, Lun A, O'Gorman MR, et al. Chronic granulomatous disease (CGD) and complete myeloperoxidase deficiency both yield strongly reduced dihydrorhodamine 123 test signals but can be easily discerned in routine testing for CGD. Clin Chem. 2007;53(5):890-6.
9. Vowells SJ, Fleisher TA, Malech HL. Testing for chronic granulomatous disease. Lancet. 1996;347(9007):1048-9.
10. Rada B, Leto TL. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. Contrib Microbiol. 2008;15:164-87.
11. Reeves EP, Lu H, Jacobs HL, et al. Killing activity of neutrophils is mediated through activation of proteases by K+ flux. Nature. 2002;416(6878):291-7.
12. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303(5663):1532-5.
13. McCormick A, Heesemann L, Wagener J, et al. NETs formed by human neutrophils inhibit growth of the pathogenic mold Aspergillus fumigatus. Microbes Infect. 2010 Jul 16. [Epub ahead of print].
14. Fuchs TA, Abed U, Goosmann C, et al. Novel cell death program leads to neutrophil extracellular traps. J Cell Biol. 2007;176(2):231-41.
15. Bianchi M, Hakkim A, Brinkmann V, et al. Restoration of NET formation by gene therapy in CGD controls aspergillosis. Blood. 2009;114(13):2619-22.
16. Siddiqui S, Anderson VL, Hilligoss DM, et al. Fulminant mulch pneumonitis: an emergency presentation of chronic granulomatous disease. Clin Infect Dis. 2007;45(6):673-81.
17. Segal BH, Han W, Bushey JJ, et al. NADPH oxidase limits innate immune responses in the lungs in mice. PLoS One. 2010;5(3):e9631.
18. van de Veerdonk FL, Smeekens SP, Joosten LA, et al. Reactive oxygen species-independent activation of the IL-1beta inflammasome in cells from patients with chronic granulomatous disease. Proc Natl Acad Sci U S A. 2010;107(7):3030-3.
19. Meissner F, Seger RA, Moshous D, et al. Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. Blood. 2010 May 21. [Epub ahead of print].
20. Fadeel B, Xue D, Kagan V. Programmed cell clearance: molecular regulation of the elimination of apoptotic cell corpses and its role in the resolution of inflammation. Biochem Biophys Res Commun. 2010;396(1):7-10.
21. Sanmun D, Witasp E, Jitkaew S, et al. Involvement of a functional NADPH oxidase in neutrophils and macrophages during programmed cell clearance: implications for chronic granulomatous disease. Am J Physiol Cell Physiol. 2009;297(3):C621-31.
22. Fernandez-Boyanapalli R, Frasch SC, Riches DW, et al. PPAR{gamma} activation normalizes resolution of acute sterile inflammation in murine chronic granulomatous disease. Blood. 2010 Aug 6. [Epub ahead of print].
23. Maltesen HR, Nielsen CH, Dalboge CS, et al. Methylprednisolone prevents tumour necrosis factor-{alpha}-dependent multinucleated giant cell formation. Rheumatology (Oxford). 2010 Jul 15. [Epub ahead of print].
24. Liu Y, Cousin JM, Hughes J, et al. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. J Immunol. 1999;162(6):3639-46.
25. Lee PP, Chan KW, Jiang L, et al. Susceptibility to mycobacterial infections in children with X-linked chronic granulomatous disease: a review of 17 patients living in a region endemic for tuberculosis. Pediatr Infect Dis J. 2008;27(3):224-30.
26. Bustamante J, Aksu G, Vogt G, et al. BCG-osis and tuberculosis in a child with chronic granulomatous disease. J Allergy Clin Immunol. 2007;120(1):32-8.
27. Martin A, Marques L, Soler-Palacin P, et al. Visceral leishmaniasis associated hemophagocytic syndrome in patients with chronic granulomatous disease. Pediatr Infect Dis J. 2009;28(8):753-4.
28. Greenberg DE, Shoffner AR, Zelazny AM, et al. Recurrent Granulibacter bethesdensis infections and chronic granulomatous disease. Emerg Infect Dis. 2010;16(9):1341-8.
29. Reichenbach J, Lopatin U, Mahlaoui N, et al. Actinomyces in chronic granulomatous disease: an emerging and unanticipated pathogen. Clin Infect Dis. 2009;49(11):1703-10.
30. Gungor T, Engel-Bicik I, Eich G, et al. Diagnostic and therapeutic impact of whole body positron emission tomography using fluorine-18-fluoro-2-deoxy-D-glucose in children with chronic granulomatous disease. Arch Dis Child. 2001;85(4):341-5.
31. Weening RS, Kabel P, Pijman P, et al. Continuous therapy with sulfamethoxazole-trimethoprim in patients with chronic granulomatous disease. J Pediatr. 1983;103(1):127-30.
32. Margolis DM, Melnick DA, Alling DW, et al. Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease. J Infect Dis. 1990;162(3):723-6.

33. Gallin JI, Alling DW, Malech HL, et al. Itraconazole to prevent fungal infections in chronic granulomatous disease. N Engl J Med. 2003;348(24):2416-22.

34. Condino-Neto A, Newburger PE. Interferon-gamma improves splicing efficiency of CYBB gene transcripts in an interferon-responsive variant of chronic granulomatous disease due to a splice site consensus region mutation. Blood. 2000;95(11):3548-54.

35. Ishibashi F, Mizukami T, Kanegasaki S, et al. Improved superoxide-generating ability by interferon gamma due to splicing pattern change of transcripts in neutrophils from patients with a splice site mutation in CYBB gene. Blood. 2001;98(2):436-41.

36. Martire B, Rondelli R, Soresina A, et al. Clinical features, long-term follow-up and outcome of a large cohort of patients with Chronic Granulomatous Disease: an Italian multicenter study. Clin Immunol. 2008;126(2):155-64.

37. Moylett EH, Pacheco SE, Brown-Elliott BA, et al. Clinical experience with linezolid for the treatment of nocardia infection. Clin Infect Dis. 2003;36(3):313-8.

38. Segal BH, Barnhart LA, Anderson VL, et al. Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. Clin Infect Dis. 2005;40(11):1684-8.

39. Lublin M, Bartlett DL, Danforth DN, et al. Hepatic abscess in patients with chronic granulomatous disease. Ann Surg. 2002;235(3):383-91.

40. Haemmerich D, Wood BJ. Hepatic radiofrequency ablation at low frequencies preferentially heats tumour tissue. Int J Hyperthermia. 2006;22(7):563-74.

41. Yamazaki-Nakashimada MA, Stiehm ER, Pietropaolo-Cienfuegos D, et al. Corticosteroid therapy for refractory infections in chronic granulomatous disease: case reports and review of the literature. Ann Allergy Asthma Immunol. 2006;97(2):257-61.

42. Danziger RN, Goren AT, Becker J, et al. Outpatient management with oral corticosteroid therapy for obstructive conditions in chronic granulomatous disease. J Pediatr. 1993;122(2):303-5.

43. Marciano BE, Rosenzweig SD, Kleiner DE, et al. Gastrointestinal involvement in chronic granulomatous disease. Pediatrics. 2004;114(2):462-8.

44. Marks DJ, Miyagi K, Rahman FZ, et al. Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease. Am J Gastroenterol. 2009;104(1):117-24.

45. Towbin AJ, Chaves I. Chronic granulomatous disease. Pediatr Radiol. 2010;40(5):657-68.

46. Varis T, Kaukonen KM, Kivisto KT, et al. Plasma concentrations and effects of oral methylprednisolone are considerably increased by itraconazole. Clin Pharmacol Ther. 1998;64(4):363-8.

47. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. BMC Res Notes. 2009;2:221.

48. Sokol H, Suarez F, Meatchi T, et al. Thalidomide as a treatment for refractory CGD colitis. Am J Gastroenterol. 2009;104(4):1069.

49. Seger RA, Gungor T, Belohradsky BH, et al. Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hemopoietic allograft: a survey of the European experience, 1985-2000. Blood. 2002;100(13):4344-50.

50. Soncini E, Slatter MA, Jones LB, et al. Unrelated donor and HLA-identical sibling haematopoietic stem cell transplantation cure chronic granulomatous disease with good long-term outcome and growth. Br J Haematol. 2009;145(1):73-83.

51. Schuetz C, Hoenig M, Gatz S, et al. Hematopoietic stem cell transplantation from matched unrelated donors in chronic granulomatous disease. Immunol Res. 2009;44(1-3):35-41.

52. Seger RA. Hematopoietic stem cell transplantation for chronic granulomatous disease. Immunol Allergy Clin North Am. 2010;30(2):195-208.

53. Gungor T, Halter J, Klink A, et al. Successful low toxicity hematopoietic stem cell transplantation for high-risk adult chronic granulomatous disease patients. Transplantation. 2005;79(11):1596-606.

54. Reichenbach J, Van de Velde H, De Rycke M, et al. First successful bone marrow transplantation for X-linked chronic granulomatous disease by using preimplantation female gender typing and HLA matching. Pediatrics. 2008;122(3):e778-82.

55. Goussetis E, Konialis CP, Peristeri I, et al. Successful hematopoietic stem cell transplantation in 2 children with X-linked chronic granulomatous disease from their unaffected HLA-identical siblings selected using preimplantation genetic diagnosis combined with HLA typing. Biol Blood Marrow Transplant. 2010;16(3):344-9.

56. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med. 2006;12(4):401-9.

57. Kang EM, Choi U, Theobald N, et al. Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. Blood. 2010;115(4):783-91.

58. Grez M, Reichenbach J, Schwäble J, et al. Gene Therapy of CGD: The engraftment dilemma. Molecular Ther; in press

59. Stein S, Ott MG, Schultze-Strasser S, et al. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med. 2010;16(2):198-204.

Netherlands
The Journal of Medicine

REVIEW

Left-sided native valve *Staphylococcus aureus* endocarditis

M. Slabbekoorn[1]*, H.M. Horlings[2], J.T.M. van der Meer[3], A. Windhausen[4], J.A.P. van der Sloot[5], W.K. Lagrand[5]

[1]Department of Intensive Care Medicine, Medical Centre Haaglanden, the Hague, the Netherlands, Departments of [2]Pathology, [3]Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, [4]Cardiology, [5]Intensive Care Medicine, Amsterdam Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)70-330 21 19, fax: +31 (0)70-330 32 34, e-mail: m.slabbekoorn@hotmail.com

ABSTRACT

Despite improved diagnostic tools and expanded treatment options, left-sided native valve endocarditis caused by *Staphylococcus aureus* infection remains a serious and destructive disease. The high morbidity and mortality, however, can be reduced by early recognition, correct diagnosis, and appropriate treatment. In the following article, we discuss the clinical presentation, diagnostic workup and treatment of infective endocarditis, thereby reviewing the current guidelines. Blood cultures and echocardiography are the cornerstones of diagnosis in identifying infective endocarditis but are no substitute for clinical judgement. The modified Duke criteria may facilitate the diagnostic process, but clinical evaluation remains crucial.

KEYWORDS

Endocarditis, mitral valve, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is the leading cause of left-sided infective endocarditis and is associated with high morbidity and mortality.[1,2] Infective endocarditis caused by *S. aureus* is usually characterised by an acute presentation in which signs of severe sepsis are predominant and the classic physical findings of infective endocarditis are often absent. Embolism and infarction of various organs are frequently seen on presentation. For this reason (critical care) physicians are frequently confronted with the complications of advanced stages of the disease. We present a case of a typical presentation of *S. aureus* endocarditis of the mitral valve complicated by multiple systemic arterial embolisations and heart failure. The aim of this report is to discuss the early recognition, diagnostic workup and treatment of patients suspected for infective endocarditis, in particular *S. aureus* endocarditis, in conformity with the current guidelines.[1]

CASE REPORT

A 65-year-old man presented to our hospital after two days of fever and coughing. His presenting complaints were acute aphasia and hemiparesis of the right arm. His past medical history was significant for smoking for 40 years, alcohol abuse, and peripheral artery disease resulting in a femoro-popliteal bypass and amputation of his right lower leg.

Physical examination on presentation was significant for normal blood pressure, sinus tachycardia to 116 beats/min and a subfebrile core temperature of 37.9 °C. Auscultation of the heart and lungs revealed no abnormalities. Neurological examination confirmed an aphasia and hemiparesis of the right arm. Laboratory results included a high sedimentation rate (32 mm/h), an elevated white blood cell count of 14.6 x 10^9/l and thrombocytopenia of 82 x 10^9/l. Chest X-ray and computed tomography (CT) of the cerebrum were initially normal.

The patient was admitted to the neurology department and antibiotic treatment was initiated (amoxicillin/clavunalate and gentamicin) to treat his fever and haemodynamic deterioration in the absence of a primary focus. Three days later the patient was transferred to the

intensive care unit of our hospital because of progressive sepsis with haemodynamic instability, progressive need for positive inotropic support and deterioration of renal function necessitating renal replacement therapy. Because of declining consciousness (Glasgow Coma Scale <8/15) without sedative medication, the patient was intubated endotracheally and mechanical ventilation was started. On admission to the intensive care, his fingers, toes and legs showed peripheral stigmata of systemic embolism (*figures 1A* to *C*). Roth's spots were present on the retina of his right eye (figure not shown). Grade one decubitus wounds were observed on his buttocks. Transoesophageal echocardiography (TEE) detected large vegetations on the mitral valve with valvular perforation of the posterior leaflet (*figures 2A* and *B*). No other valvular abnormalities were observed. Left and right ventricular function were normal. On day two of his intensive care admission multiple separate blood cultures were positive for *S. aureus*. Antibiotic therapy was switched to flucloxacillin, dosed in accordance with the endocarditis guidelines (higher dose).[1] The diagnosis was considered 'definitive' according to the modified Duke, meeting two of the major criteria. A new CT scan of the cerebrum showed a left-sided, parieto-temporal-occipital lesion of low density with a midline shift. Emergency valvular surgery was considered but ultimately rejected due to the patient's high risk for intra-cerebral haemorrhage and his sombre prognosis. During his stay in the intensive care unit, the patient deteriorated clinically, requiring increasing inotropic and respiratory support. Because this deterioration occurred despite maximal treatment with no concomitant, treatable or potentially reversible factors, active treatment was suspended five days after ICU admission. The patient died soon afterwards.

Autopsy confirmed the diagnosis of endocarditis of the mitral valve. A vegetation of 20 mm was found on the mitral valve (*figure 3A*). Light microscopy of the mitral valve showed an acute infection with Gram positive cocci (*figure 3B* and *3C*). Septic emboli were observed by light microscopy in the left hemisphere, the spleen and kidneys.

Figure 2A. *Transoesophageal echocardiography showing vegetations of the mitral valve*

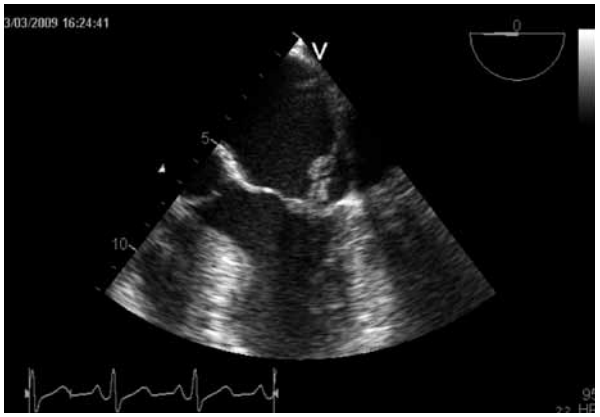


Figure 2B. *Transoesophageal echocardiography showing mitral valve regurgitation due to perforation of the mitral valve posterior leaflet*

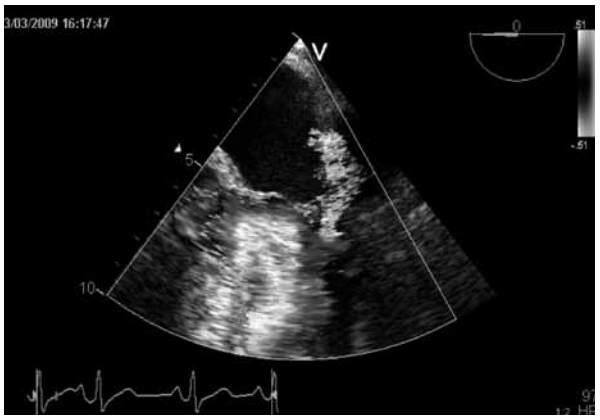


Figure 1A. *Janeway spots (fingers)*

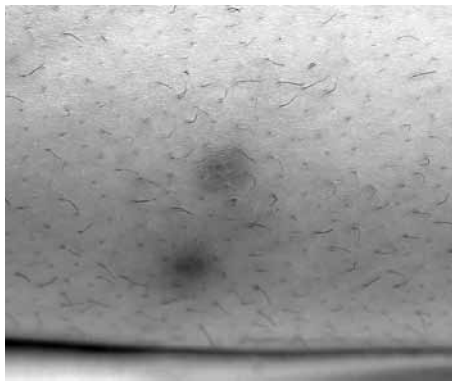


Figure 1B. *Embolic lesion (finger)*

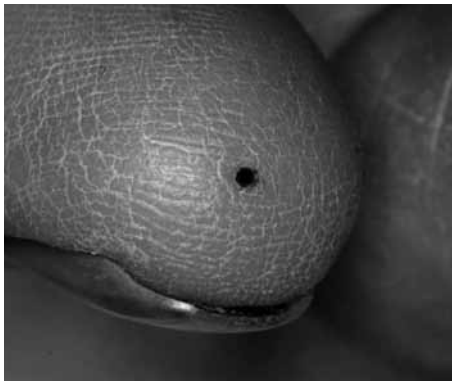


Figure 1C. *Splinter haemorrhages (toe)*

Figure 3A. *Macroscopy. Detail of vegetation of the mitral valve with valvular perforation (arrows)*

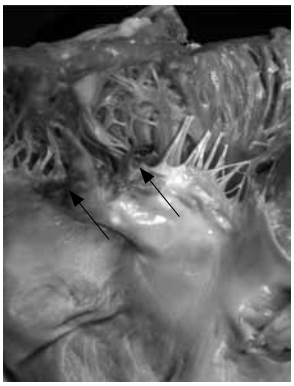


Figure 3B. *Microscopy. General view of vegetations of the mitral valve (Haematoxylin and Eosin staining)*

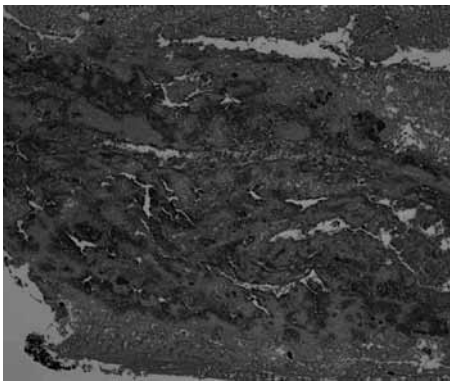
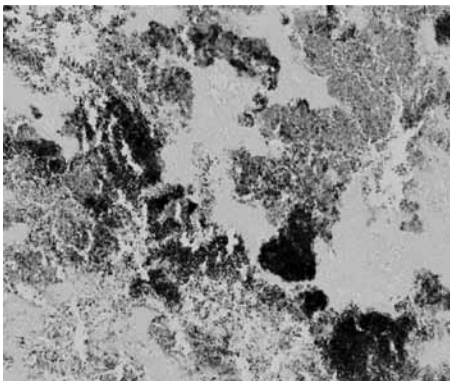


Figure 3C. *Microscopy. Bacterial colonies on mitral valve (Gram staining)*



DISCUSSION

Early recognition/ diagnosis

S. aureus endocarditis is a serious infection that is associated with high morbidity and mortality even in patients without risk factors for infective endocarditis.[1] Signs and findings which demand consideration of infective endocarditis are summarised in *table 1*. Our patient demonstrated the typical presentation of *S. aureus* endocarditis of the mitral valve with fever, systemic arterial embolisation to various organs, sepsis and heart failure.

Table 1. *Clinical presentation of infective endocarditis*

Infective endocarditis, in general, must be suspected in the following situations:

- New regurgitant heart murmur
- Embolic events of unknown origin
- Sepsis of unknown origin
- Fever: the most frequent sign of infective endocarditis

Infective endocarditis must be suspected if fever is associated with:

1. Intracardiac prosthetic material (e.g. prosthetic valve, pacemaker, implantable defibrillator, surgical baffle / conduit)
2. Previous history of infective endocarditis
3. Previous valvular or congenital heart disease
4. Other predisposition for infective endocarditis (e.g. immunocompromised state)
5. Predisposition and recent intervention with associated bacteraemia
6. Evidence of congestive heart failure
7. New conduction disturbance
8. Positive blood cultures with typical infective endocarditis causative organism or positive serology for chronic Q-fever
9. Vascular or immunological phenomena: embolic event, Roth's spots, splinter haemorrhages, Osler's nodes, Janeway lesion
10. Focal or non-specific neurological symptoms and signs
11. Peripheral abscesses (renal, splenic, cerebral, vertebral) of unknown cause

Embolism and infarction in various organs are frequently seen as first presentations of infective endocarditis. Systemic emboli involving the spleen and kidneys are mostly seen in cases of left-sided endocarditis.[3] Systemic embolisation in our patient was documented in cerebrum (resulting in cerebral infarction), spleen, and both kidneys. Peripheral embolic lesions were found in the right eye, fingers and toes.

Splinter haemorrhages, Janeway lesions, Osler's nodes and Roth's spots are uncommon findings (*figure 1*).[3,4] The origin of these findings remains a source of debate. Some authors have reported that microorganisms are directly involved in the genesis of these lesions and that the microorganisms can be cultured from these lesions. Others argue that the lesions result from immune-mediated vasculitis, irrespective of (local presence of) the microorganism.[1-3]

Embolic risk is higher in *S. aureus* endocarditis than in endocarditis caused by other microorganisms. It is also seen more frequently in left-sided endocarditis and with (mobile) vegetations >15 mm on echocardiography.[5] Cerebral embolism is seen most frequently (up to 18% of the patients, as reported by Nadji *et al.*)[6] The combination of embolisation to the central nervous system and to peripheral organs is seen in 10% of the patients.[3] More than half of these patients will not survive. Mortality is significantly higher in patients who have a neurological event compared with those who do not have any neurological events.

Blood cultures and echocardiography are the cornerstones in the diagnosis infective endocarditis. Confirming the diagnosis of 'infective endocarditis' clinically remains a difficult process and is a challenge for the (critical care) physician. The diagnostic process may be facilitated by the Duke criteria (*table 2*), which are based on strict clinical, microbiological and echocardiographic parameters.

Table 2. *Modified Duke criteria for diagnosis of infective endocarditis*[1]

Major criteria
Blood cultures
- ≥2 positive for typical infective endocarditis micro-organisms (*Streptococcus viridans* spp., *Streptococcus bovis*, HACEK group, *Staphyloccus aureus* or *Enterococci*) in absence of primary focus
- Persistently positive blood culture (2 sets drawn >12 hours apart, or ≥3 of 4 sets with first and last separated by >1 hour)
- 1 positive for *Coxiella burnettii* or IgG titre against phase 1>1:800

Endocardial involvement
- Positive TTE or TEE, i.e.:
  - Discrete, echogenic, oscillating intracardiac mass on valve or supporting structure, in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation (vegetation)
  - Periannular abscess or
  - New partial dehiscence of prosthetic valve
- New valvular regurgitation (worsening of or change in pre-existing heart murmur)

Minor criteria
Broader clinical findings
- Predisposing cardiac condition or intravenous drug use
- Fever (≥38 °C)
- Vascular phenomena (major arterial emboli, septic pulmonary infarct, mycotic aneurysm, intracranial haemorrhage, conjunctival haemorrhage, Janeway lesions)
- Immunological phenomena (glomerulonephritis, Osler's nodes, Roth's spots, positive rheumatoid factor);
- Microbiological findings of positive blood cultures not meeting major criteria or serological evidence of active infection with plausible microorganisms

TTE = transthoracic echocardiography; TEE = transoesophageal echocardiography.

Table 3. *Diagnosis*[1]

Definite	• Pathology or bacteriology of vegetations, major emboli, or intracardiac abscess specimen, or • 2 major criteria, or • 1 major and 3 minor criteria, or • 5 minor criteria
Possible	• 1 major and 1 minor criterion, or • 3 minor criteria
Rejected	• Firm alternative diagnosis, or • Resolution of syndrome after ≥4 days' antimicrobial therapy, or • No pathological evidence at surgery or autopsy after ≤4 days of antimicrobial therapy, or • Does not meet definite or possible criteria

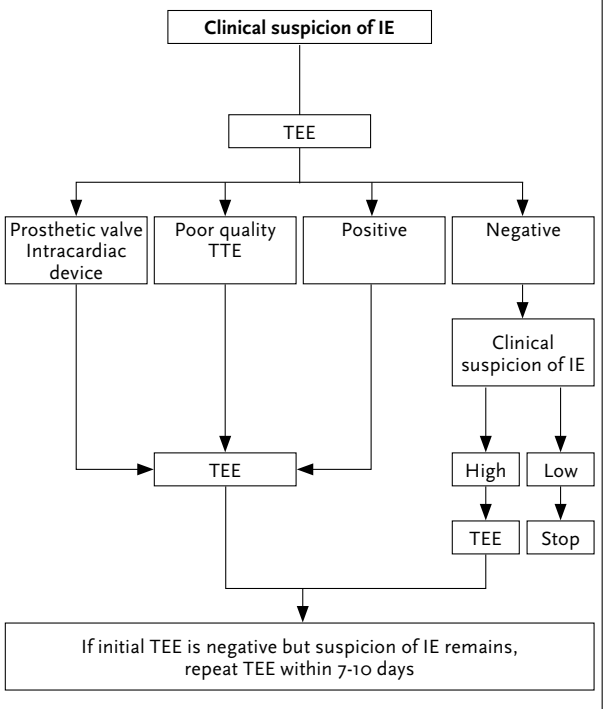
According to these criteria the diagnosis of infective endocarditis can be classified as definite, possible or rejected (*table 3*). It is to be noted, however, that these criteria were originally developed to compare clinical studies, yielding the highest possible specificity for infective endocarditis (but never 100%). This implies that sensitivity is not 100%. The criteria, therefore, may not be used to definitively exclude the diagnosis of 'infective endocarditis'.

In *S. aureus* bacteraemia a routine echocardiographic evaluation is justified because of the aspecific presentation of *S. aureus* endocarditis and the potentially devastating effects of intracardiac infection.[7] As in our case, acute valvular dysfunction due to perforation (*figure 2A* and *B*) results in acute severe heart failure. It is the most common cause of death in *S. aureus* endocarditis, especially in cases in which surgery is not, or can not be performed.

Echocardiography

Echocardiography plays an important role in the diagnostic workup of patients in whom infective endocarditis is suspected (*figure 4*). It must be noted, however, that the diagnosis of 'infective endocarditis' is not based solely on echocardiographic findings. Endocarditis is strictly a pathological-anatomical diagnosis in which the clinician is challenged to diagnose and treat the disease before autopsy is necessary to prove infective endocarditis as the final diagnosis. In the current guidelines the role of echocardiography is emphasised and well defined (*table 4*).[1] The modified Duke criteria may facilitate the diagnostic process, as discussed earlier (*tables 2* and *3*). Echocardiographic findings and the anatomically representative structures, are summarised in *table 5*. Of these, vegetation, abscess and dehiscence of a prosthetic valve

Figure 4. *Echocardiographic workup algorithm for patients suspected for infective endocarditis*



IE = Infective endocarditis; TTE = Transthoracic echocardiography; TEE = Transoesophageal echocardiography

Table 4. *Role of echocardiography in infective endocarditis*[1]

Recommendations for echocardiography	Class	Level
A Diagnosis		
1. TTE is recommended as the first-line imaging modality in suspected infective endocarditis	I	B
2. TEE is recommended in patients with high clinical suspicion of infective endocarditis and a normal TTE	I	B
3. Repeat TTE / TEE within 7-10 days is recommended in the case of an initially negative examination when clinical suspicion of infective endocarditis remains high	I	B
4. TEE should be considered in the majority of adult patients with suspected infective endocarditis, even in cases with positive TTE, owing to its better sensitivity and specificity, particularly for the diagnosis of abscesses and measurement of vegetation size	IIa	C
5. TEE is not indicated in patients with good quality negative TTE and low clinical suspicion of infective endocarditis	III	C
B Follow-up under medical therapy		
1. Repeat TTE and TEE are recommended as soon as a new complication of infective endocarditis is suspected (new murmur, embolism, persisting fever, heart failure, abscess, atrioventricular block	I	B
2. Repeat TTE and TEE should be considered during follow-up of uncomplicated infective endocarditis, in order to detect new silent complications and monitoring vegetation size. The timing and mode (TTE or TEE) of repeat examination depend on the initial findings, type of micro-organism, and initial response to therapy	IIa	B
C Intra-operative echocardiography		
Intra-operative echocardiography is recommended in all cases of infective endocarditis requiring surgery	I	C
D Following completion of therapy		
TTE is recommended at completion of antibiotic therapy for valuation of cardiac and valve morphology and function	I	C

TTE = transthoracic echocardiography; TEE = transoesophageal echocardiography.

Table 5. *Anatomic and echocardiographic definitions*[1]

	Surgery / necropsy	Echocardiography
Vegetation	Infected mass attached to an endocardial structure, or on implanted intracardiac material	Oscillating or non-oscillating mass on valve or other endocardial structures, or on implanted intracardiac material
Abscess	Perivalvular cavity with necrosis and purulent material not communicating with the cardio-vascular lumen	Thickened, non-homogenous perivalvular area with echodense or echolucent appearance
Pseudoaneurysm	Perivalvular cavity communicating with the cardiovascular lumen	Pulsatile perivalvular echo-free space, with colour-Doppler flow detected
Perforation	Interruption of endocardial tissue continuity	Interruption of endocardial tissue continuity traversed by colour-Doppler flow
Fistula	Communication between two neighbouring cavities through a perforation	Colour-Doppler communication between two neighbouring cavities through a perforation
Valve aneurysm	Saccular outpouching of valvular tissue	Saccular bulging of valvular tissue
Dehiscence of a prosthetic valve	Dehiscence of the prosthesis	Paravalvular regurgitation identified by TTE / TEE, with or without rocking motion of the prosthesis

TTE = transthoracic echocardiography; TEE = transoesophageal echocardiography.

are major Duke criteria. TTE has a low sensitivity for the detection of cardiac vegetations, and a negative TTE cannot exclude infective endocarditis in patients with *S. aureus* bacteraemia or in patients with a high clinical suspicion for infective endocarditis.[8] TEE, however, is useful in detecting even small vegetations as well as cardiac complications such as (small) abscess formation, pseudoaneurysm, fistula and valvular perforations and is, therefore, indicated when suspicion is high or in patients with prosthetic valves or intracardiac devices (*figure 4*).[1]

Treatment

The therapy of infective endocarditis consists of elimination of the infection focus, antimicrobial treatment and, if necessary and if possible, early surgical treatment. Antiplatelet and antithrombotic therapy are extremely controversial, should be individualised, and should be discontinued when infective endocarditis is complicated by cerebral embolism, infarction or haemorrhage.[9,10]

Antimicrobial therapy

The optimal antibiotic therapy for a methicillin-susceptible *S. aureus* is intravenous flucloxacillin, preferably given continuously.[1,11] No randomised controlled trials have determined the optimal duration of therapy, but based on current literature left-sided endocarditis should be treated for at least six weeks. Based on *in vitro* and animal study evidence of synergistic bactericidal activity, combination therapy is used widely. In *S. aureus* endocarditis, animal and human studies suggest that flucloxacillin, when

combined with a cell-wall active agent such as an aminoglycoside during the initial three to five days of therapy, can lead to a shortened duration of bacteraemia, although mortality and morbidity remained unchanged.[12,13] In right-sided *S. aureus* endocarditis, however, addition of aminoglycosides did not result in additional benefit.[14] Additional administration of low-dose aminoglycosides in left-sided infective endocarditis, even when given for a short duration, was found to result in a significantly higher incidence of nephrotoxicity.[15]

Before admission to the intensive care unit, our patient had been treated with gentamicin for three days, and we therefore continued flucloxacillin monotherapy.

The port of entry or focus from which the infective endocarditis originates is generally not obvious. Our patient only had minimal signs of decubitus wounds which might have been the source for his bacteraemia.

Surgical therapy

Surgical therapy, even in the early active phase, should be a treatment consideration in order to avoid progressive heart failure, to control infection or to prevent (further) systemic embolism (*table 6*). Age per se is not a contraindication to surgery. Early consultation with the cardiothoracic surgeon is recommended to secure the optimal therapeutic approach, especially in *S. aureus* endocarditis.

In our patient surgery was considered immediately because of the presence of haemodynamically significant left-sided valvular regurgitation with perforation, the large and highly mobile vegetation, and the evidence of systemic embolisation.[16] Appropriately timed and performed surgical therapy of the infected heart valve substantially reduces early and late mortality.[17] However, as evidenced by CT-scan evaluation, our patient already had severe neurological complications at the time of diagnosis. As valve replacement is relatively contraindicated in the presence of large cerebral embolic events, additional diagnostic imaging examinations are necessary to determine the cause, location and extent of the lesion.[3] Most surgeons would like to delay operative intervention for at least 7-14 days to lesson likelihood of, or worsening of, the neurological deficit with the knowledge that subsequent anticoagulant therapy, during and after surgery, is usually necessary. Also, in the early stages of endocarditis the infected heart tissue is soft, making surgical repair technically difficult ('like sewing in butter'). Delaying surgery, on the other hand, also entails high risks. The decision to operate in cases of infective endocarditis, therefore, depends on the clinical situation and should be individualised.

In this case CT scanning showed a large lesion of the left parieto-temporal-occipital region which was why surgery was postponed. In the following days the patient deteriorated haemodynamically due to his sepsis and progressive heart failure. Because of this clinical course, in combination with a poor neurological prognosis, it was decided to suspend further treatment.

Prognosis

The six-month mortality rate of complicated left-sided native valve endocarditis is approximately 25%, but

Table 6. *Indications and timing of surgery in left-sided native valve infective endocarditis*[1]

		Timing	Class	Level
A	**Heart failure**			
•	Aortic or mitral infective endocarditis with severe acute regurgitation or valve obstruction causing refractory pulmonary oedema or cardiogenic shock	Emergency	I	B
•	Aortic or mitral infective endocarditis with fistula into a cardiac chamber or pericardium causing refractory pulmonary oedema or cardiogenic shock	Emergency	I	B
•	Aortic or mitral infective endocarditis with severe acute regurgitation or valve obstruction and persisting heart failure or echocardiographic signs of poor haemodynamic tolerance (early mitral closure or pulmonary hypertension)	Urgent	I	B
•	Aortic or mitral infective endocarditis with severe regurgitation and no heart failure	Elective	IIa	B
B	**Uncontrolled Infection**			
•	Locally uncontrolled infection (abscess, false aneurysm, fistula, enlarging vegetation)	Urgent	I	B
•	Persisting fever and positive blood cultures >7-10 days	Urgent	I	B
•	Infection caused by fungi or multiresistant organisms	Urgent / elective	I	B
C	**Prevention of embolism**			
•	Aortic or mitral infective endocarditis with large vegetations (>10 mm) following one or more embolic episodes despite appropriate antibiotic treatment	Urgent	I	B
•	Aortic or mitral infective endocarditis with large vegetations (>10 mm) and other predictors of complicated course (heart failure, persistent infection, abscess)	Urgent	I	C
•	Isolated very large vegetations (>15 mm)	Urgent	IIb	C

patients with infective endocarditis comprise an extremely heterogeneous group.[10]

Hasbun and colleagues derived a prognostic classification system for patients with infective endocarditis.[18] Five clinical features were significantly associated with six-month mortality: i.e. abnormal mental status, moderate to severe heart failure, causative organism other than *Streptococcus viridans* spp., medical therapy without valve surgery and increased Charlson comorbidity score (in which the one-year mortality risk is based on 19 categories of comorbidities). A weighted scoring system was made by use of these prognostic features. Patients were classified into four groups with progressively increasing six-month mortality risks, ranging from 5 to 59%.[12] In our patient all five prognostic features of this scoring system were present, resulting in a calculated six-month mortality risk of (at least) 59%.

CONCLUSION

The presented case illustrates the acute presentation of *S. aureus* endocarditis of the mitral valve in which the signs of severe sepsis, systemic embolisation and heart failure were predominant. Blood cultures and echocardiography are cornerstones of diagnosis in infectious endocarditis but clinical judgement remains crucial. The modified Duke criteria facilitate the diagnostic process, but do not replace clinical evaluation. Early recognition, diagnosis and treatment according to current guidelines, remain extremely important to reduce morbidity and mortality in this disease.

REFERENCES

1. Habib G, Hoen B, Tornos P, et al. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Eur Heart J. 2009;30(19):2369-413.
2. Mylonakis E, Calderwood SB. Infective endocarditis in adults. N Engl J Med. 2001;345(18):1318-30.
3. Murray RJ. Staphylococcus aureus infective endocarditis: diagnosis and management guidelines. Intern Med J. 2005;35(Suppl 2):S25-S44.
4. Espersen F, Frimodt-Moller N. Staphylococcus aureus endocarditis. A review of 119 cases. Arch Intern Med. 1986;146(6):1118-21.
5. Fabri J, Jr, Issa VS, Pomerantzeff PM, Grinberg M, Barretto AC, Mansur AJ. Time-related distribution, risk factors and prognostic influence of embolism in patients with left-sided infective endocarditis. Int J Cardiol. 2006;110(3):334-9.
6. Nadji G, Remadi JP, Coviaux F, et al. Comparison of clinical and morphological characteristics of Staphylococcus aureus endocarditis with endocarditis caused by other pathogens. Heart. 2005;91(7):932-7.
7. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30(4):633-8.
8. Shively BK, Gurule FT, Roldan CA, Leggett JH, Schiller NB. Diagnostic value of transesophageal compared with transthoracic echocardiography in infective endocarditis. J Am Coll Cardiol. 1991;18(2):391-7.
9. Tornos P, Almirante B, Mirabet S, Permanyer G, Pahissa A, Soler-Soler J. Infective endocarditis due to Staphylococcus aureus: deleterious effect of anticoagulant therapy. Arch Intern Med. 1999;159(5):473-5.
10. Haldar SM, O'Gara PT. Infective endocarditis: diagnosis and management. Nat Clin Pract Cardiovasc Med. 2006;3(6):310-7.
11. Leder K, Turnidge JD, Korman TM, Grayson ML. The clinical efficacy of continuous-infusion flucloxacillin in serious staphylococcal sepsis. J Antimicrob Chemother. 1999;43(1):113-8.
12. Korzeniowski O, Sande MA. Combination antimicrobial therapy for Staphylococcus aureus endocarditis in patients addicted to parenteral drugs and in nonaddicts: A prospective study. Ann Intern Med. 1982;97(4):496-503.
13. Drinkovic D, Morris AJ, Pottumarthy S, MacCulloch D, West T. Bacteriological outcome of combination versus single-agent treatment for staphylococcal endocarditis. J Antimicrob Chemother. 2003;52(5):820-5.
14. Ribera E, Gomez-Jimenez J, Cortes E, et al. Effectiveness of cloxacillin with and without gentamicin in short-term therapy for right-sided Staphylococcus aureus endocarditis. A randomized, controlled trial. Ann Intern Med. 1996;125(12):969-74.
15. Cosgrove SE, Vigliani GA, Fowler VG, Jr, et al. Initial low-dose gentamicin for Staphylococcus aureus bacteremia and endocarditis is nephrotoxic. Clin Infect Dis. 2009;48(6):713-21.
16. Hendren WG, Morris AS, Rosenkranz ER, et al. Mitral valve repair for bacterial endocarditis. J Thorac Cardiovasc Surg. 1992;103(1):124-8.
17. Yamaguchi H, Eishi K. Surgical treatment of active infective mitral valve endocarditis. Ann Thorac Cardiovasc Surg. 2007;13(3):150-5.
18. Hasbun R, Vikram HR, Barakat LA, Buenconsejo J, Quagliarello VJ. Complicated left-sided native valve endocarditis in adults: risk classification for mortality. JAMA. 2003;289(15):1933-40.

Netherlands
The Journal of Medicine

REVIEW

Infectious disease as aetiological factor in the pathogenesis of systemic sclerosis

M. Radić[1]*, D. Martinović Kaliterna[1], J. Radić[2]

Departments of [1]Rheumatology, [2]Nephrology, Split University Hospital, Split, Croatia,
*corresponding author: e-mail: mislavradic@gmail.com

ABSTRACT

Systemic sclerosis is an autoimmune disease characterised by vascular obliteration, excessive extracellular matrix deposition and fibrosis of the connective tissues of the skin, lungs, gastrointestinal tract, heart, and kidneys. The pathogenesis of systemic sclerosis is extremely complex; at present, no single unifying hypothesis explains all aspects. Over the last 20 years increasing evidence has accumulated to implicate infectious agents in the aetiology of systemic sclerosis. Increased antibody titres, a preponderance of specific strains in patients with systemic sclerosis, and evidence of molecular mimicry inducing autoimmune responses suggest mechanisms by which infectious agents may contribute to the development and progression of systemic sclerosis. Here we review the current state of knowledge of infectious risk factors in systemic sclerosis and the possible mechanisms by which infectious exposures might induce pathologic processes.

KEYWORDS

Systemic sclerosis, pathogenesis, infections

INTRODUCTION

The cause of systemic sclerosis (SSc) has remained elusive despite intense investigations. Although the disease is not inherited in a classical Mendelian pattern, there is strong evidence that genetic factors contribute to its development and clinical manifestations, as discussed in more detail below. However, it has become apparent that environmental agents play a crucial and more important role than genetic influences. One study reported a remarkably low concordance in the development of SSc among homozygous twins, indicating that the heritability component of the disease was very low and that the most important factor was of an environmental or acquired origin.[1] Many agents have been postulated as being involved in the cause of the disease. The hypothesis that infectious agents may cause systemic sclerosis has been studied extensively. Some researchers have suggested that the production of specific autoantibodies in SSc is the result of an antigen-driven response caused by molecular mimicry. Molecular mimicry was originally defined as the theoretical possibility that sequence similarities between foreign and self-peptides are sufficient to result in the cross activation of autoreactive T cells by virus-derived peptides.[2] Escaping the process of clonal deletion, certain populations of autoreactive T cells are known to persist in normal individuals and require only an appropriate stimulus to initiate self-directed immune responses and potentially autoimmune disease.[3] The concept of molecular mimicry proposes that antibodies against self-antigens are produced because these antigens contain epitopes that share structural similarities with viral or bacterial proteins. In the immunopathogenesis of SSc, herpesviruses, retroviruses, and human cytomegalovirus (CMV) infections, among others, have been suggested as possible causative agents. Evidence supporting the role of retroviruses includes the demonstration of sequence homologies between certain retroviral proteins and the topoisomerase I antigen, which is the target of anti-Scl 70 antibodies in patients with systemic sclerosis.[4] In addition, it has been shown that the induced expression of retroviral proteins in normal human dermal fibroblasts results in the acquisition of a SSc-like phenotype in the production of extracellular matrix proteins.[4] Furthermore, antibodies to retroviral proteins have been detected in serum specimens from patients with SSc.[5] Another hypothesis has suggested that human cytomegalovirus may be involved in the initial events of SSc. This hypothesis is supported by the observations of a higher prevalence of IgA antihuman cytomegalovirus antibodies in patients with SSc, which

are capable of inducing apoptosis in human endothelial cells; the increased prevalence of anticytomegalovirus IgA antibodies in patients positive for Scl-70 autoantibodies; and the severe fibroproliferative vascular changes and the increased occurrence of antinuclear antibodies with an immunofluorescence pattern similar to that present in serum specimens from patients with SSc in human cytomegalovirus infections.[6,7] Despite intensive study, however, there is no definitive evidence to conclude that SSc has a viral origin.

PARVOVIRUS B19

Parvovirus B19 has been proposed as a causative agent in rheumatoid disease and other vascular injury syndromes, including Wegener's granulomatosis,[8] microscopic polyarteritis nodosa,[9] Henoch Schonlein purpura,[10] and dermatomyositis[11] in SSc.

Ferri *et al.* first suggested the possible involvement of parvovirus B19 in SSc. They found that antiparvovirus B19 NS-1, which could be a marker of persistent parvovirus B19 infection, was frequently detected in the serum of patients with SSc.[12] However, circulating parvovirus B19 DNA was detected in only 4% of SSc patients. They subsequently showed parvovirus B19 infection of bone marrow in SSc.[13] The presence of parvovirus B19 DNA was demonstrated in a significant percentage of bone marrow biopsies from SSc patients and was never detected in the control group. SSc patients with bone marrow parvovirus B19 infection showed a shorter mean disease duration than parvovirus B19-negative patients. These patients showed the most severe active endothelial injury and perivascular inflammation. The historical record was reported to be consistent with the finding of parvovirus B19 infection of bone marrow in SSc patients.[14] Ray *et al.* showed that incubation with parvovirus B19-containing serum induced an invasive phenotype in normal human synovial fibroblasts.[15] A direct correlation between the extent of degenerative endothelial cell alterations and the degree of B19 RNA expression suggested a causal role of B19 in the propagation of the endothelial cell dysfunction.[16] Endothelial injury in patients infected with B19 likely reflects a combination of direct viral cytotoxicity and humoral immunity. It has been shown that B19 exerts a cytotoxic effect on infected cells through a non-structural protein designated NS-1.[17] The ability of parvovirus B19 to persistently infect SSc fibroblasts might be responsible for important cell alterations, as suggested by phenotypic changes observed in normal human synovial infected *in vitro* by parvovirus B19.[18,19] Zakrzewska *et al.* showed some differences in the rate of persistence of B19V DNA, in the simultaneous persistence of two genotypes and in the pattern of viral expression among SSc patients and controls.[20]

CYTOMEGALOVIRUS

CMV infection may play a part in SSc pathogenesis due to its ability to infect both endothelial and monocyte/macrophage cells and through the upregulation of fibrogenic cytokines and induction of immune dysregulation.[21,22] It has been proposed as accelerating factor in autoimmune vasculopathy, allograft rejection and coronary restenosis.[22] CMV infects vascular endothelium, and this infection is characterised by latency, reactivation, and shedding of the virus to distal tissues. In both rat and mouse models, it was shown that infection with CMV leads to the development of intimal lesions.[23-25] In addition, indirect evidence of a role for CMV in SSc includes an association between increased serum levels of CMV-specific antibodies and the prevalence of SSc-related autoantibodies in patients with SSc.[26,27]

The appearance of SSc shortly after an acute episode of viral infection suggested CMV as a possible trigger for SSc.[28] CMV infection is characterised by latency, reactivation, and downstream shedding of the virus.[24] Infection of endothelial cells selectively alters the expression of integrins, downregulating $\alpha 5\beta 1$ and $\alpha 2\beta 1$ and upregulating $\alpha 6\beta 1$ and $\alpha 3\beta 1$ integrins.[29] It also induces the expression of fibrogenic cytokines and contributes to immune dysregulation and possibly to autoimmunity against selective epitopes such as centromere, Scl-70, RNP, and anti-RNA polymerases.[30] The most direct evidence of a link between CMV and SSc is the presence of high-titre IgG antibodies to the polyglycine motifs of CMV.[31]

One difficulty in making a clear association between CMV and SSc is the fact that 60 to 90% of adults show serological evidence of past CMV infection, yet SSc affects at most three in 10,000 people. Recent identification of genes controlling CMV susceptibility in mice through genetic analysis may be able to shed light on this issue by facilitating studies of genetic susceptibility to CMV in humans. In the immune-compromised mouse model of CMV-induced neointimal formation, genetically resistant mice (the C57BL/6 strain) do not develop any vascular pathology in response to the viral infection, whereas CMV-susceptible mice (129 interferon-γR-/-) form reproducible neointimal lesions in a dose-dependent response to CMV infection.[23] Another possible mechanism that may explain sporadic development of SSc in response to CMV infection and the 8:1 ratio of SSc-affected women to SSc-affected men is the microchimerism hypothesis. Microchimerism refers to prolonged survival of allotypic lymphocytes (foetal T cells acquired during pregnancy or cells received by blood transfusion or organ transplant) usually in circulating blood. Microchimeric T cells of foetal origin were verified 27 years after giving birth in one woman and have been shown to be more common

and more numerous in women with SSc than in healthy, age-matched controls.[32] The pathogenic effects of these cells are not known, but microchimeric cells were also found in the cellular infiltrate of sclerodermatous lesions.[33] The engraftment and survival of these cells are dependent on the complex relationships between the tissue antigens of the mother and offspring (or host and donor) and are highly variable as a result.[34] Support for the idea that CMV may induce the proliferation of microchimeric cells comes from *in vitro* studies that show that T cells exposed to allotypic endothelial cells become more highly activated and proliferate to a greater extent if the endothelial cells are infected with CMV.[35] Therefore, in people with circulating microchimeric T cells, the vascular endothelium represents an allotypic stimulus to those cells. However, if the endothelium is infected with CMV, proliferation and cytokine expression may be amplified, possibly triggering a cascade of endothelial activation, vascular inflammation, and neointimal formation in the same fashion as transplanted T cells do in graft-*versus*-host disease.

HELICOBACTER PYLORI

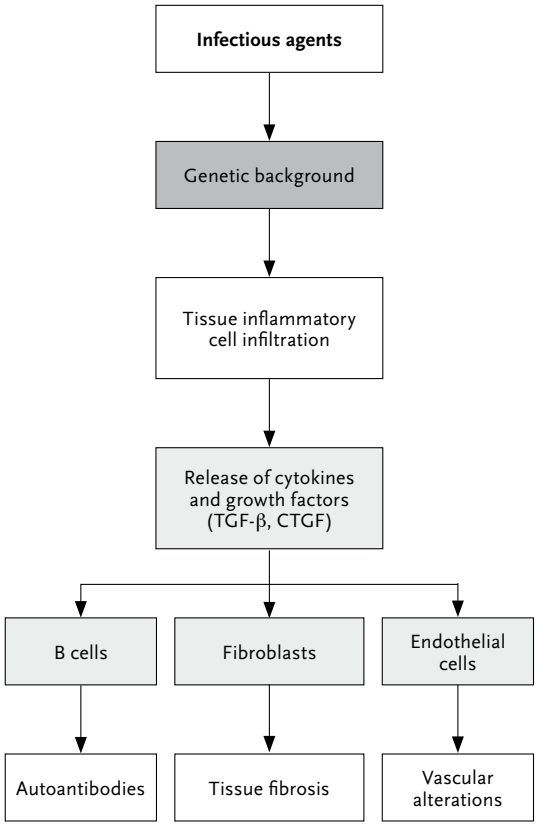
The most recent research on the involvement of bacterial infections in the pathogenesis of SSc focuses on *Helicobacter pylori* (*H. pylori*) which has been implicated in other vascular diseases.[36] Studies have investigated *H. pylori* infections for an association with Raynaud's phenomenon, Sjögren syndrome, and SSc. In a study of patients with primary Raynaud's phenomenon, eradication of *H. pylori* infection was associated with complete disappearance of the episodes of Raynaud's phenomenon in 17% of treated patients and a reduction in symptoms in an additional 72%.[37] Although this study was not double blinded, it is intriguing that symptoms of Raynaud's phenomenon did not improve in those patients in whom eradication of *H. pylori* failed. A more recent trial of comparable design reported very similar results.[38] One study identified higher incidence rates of serological evidence of *H. pylori* infection in patients with rheumatological diseases, including SSc.[39] In contrast, three larger studies found no difference in *H. pylori* infection rates between patients with SSc with Raynaud's phenomenon compared with healthy controls.[40-42] However, even if it were true that *H. pylori* infection rates do not correlate with SSc, this does not necessarily rule out its involvement in SSc. A recent study[43] indicated that, despite the absence of a difference in *H. pylori* infection rates between SSc patients and control subjects, 90% of patients with SSc were infected with the virulent CagA strain compared with only 37% of the infected control subjects. Therefore, confounding factors such as coinfections, differences in *H. pylori* strains, and immunological and genetic host factors will have to be further identified and controlled in order to understand the role of *H. pylori* in Raynaud's phenomenon, SSc, and other vascular phenomena. The association between *H. pylori* infection and Raynaud's syndrome[41,43] has been attributed to increased levels of cytokines and acute phase reactants, such as C-reactive protein and fibrinogen, resulting in vasospasm and platelet aggregation. Kalabay *et al.*,[44] who found a high prevalence of *H. pylori* infection in patients with systemic sclerosis (78%) (n=55), attempted to explain the preferential occurrence of *H. pylori* infection in SSc in two ways. First, an increased prevalence of *H. pylori* infection might be favoured by the disturbed gastrointestinal motility, a clinical phenomenon well known in patients with SSc. The second explanation may be that *H. pylori* infection and the immunological mechanisms operative in the course of SSc may be related to each other. We recently performed a study aiming to evaluate the possible association between *H. pylori* infection with disease activity, biochemical and serological data.[45] Our preliminary results suggest that *H. pylori* infection is implicated in activity of SSc, especially in skin involvement of this disease. This study may indicate *H. pylori* infection as a possible cofactor in the development of SSc. Clinical trials are still necessary to define the pathogenesis and confirm the increase in association between *H. pylori* and SSc.

DISCUSSION

An increasing body of evidence suggests that there are many potential environmental triggers for SSc and that host factors determine the susceptibility of the host to disease in response to these triggers.[46] Infectious agents, both bacterial and viral, have long been suspected as a contributing factor in the development and progression of SSc. The rationale for this infection hypothesis is that many SSc-like symptoms are transiently elicited by infectious agents in otherwise healthy individuals. There are two general lines of evidence implicating bacterial infections in the pathogenesis of SSc. One is anecdotal evidence that treatment with antibiotics relieves SSc symptoms in some patients. The other is that graft-*versus*-host disease, which is recognised as having many similarities to SSc, cannot be induced in germ-free animals and is significantly reduced in children pre-treated with antibiotics to eradicate their normal bacterial flora.[47] Arson *et al.* recently assessed serological reactivity against various infectious agents in patients with SSc and compared them with healthy controls.[48] Serological samples obtained from 80 patients with SSc were compared with 296 compatible healthy controls; all samples were tested for the presence of antibodies directed against hepatitis B virus, hepatitis C virus, toxoplasmosis, rubella, CMV, Epstein-Barr virus (EBV), and *Treponema*

pallidum. The results of this study demonstrate that antibodies against CMV, HBV, and toxoplasmosis were detected more often in patients with SSc. This association confirms that infectious agents might have a role in disease pathogenesis and expression. In *figure 1*, it is hypothesised that the infectious agents, both bacterial and viral, are the incising factor that acts on a genetically predisposed host and results in the subsequent recruitment and homing of macrophages and T cells to the affected tissues. The most prominent clinical manifestations of systemic sclerosis are caused by the exaggerated accumulation of collagen and other connective tissue components in the affected organs. The inflammatory cells would undergo selective proliferation and expansion, perhaps because of an antigen-driven response, and then release cytokines and growth factors that initiate the process of tissue and vascular fibrosis. Infectious agents cause a profound phenotypic change in various target cells of different lineages (immune cells, fibroblasts, and endothelial and vascular smooth-muscle cells). This phenotypic change could be caused by integration of genetic material (for example, of retroviral origin) within the genetic sequence of the target cells that through unknown mechanisms would induce the expression of specific regulatory genes, altering the function and behaviour of the target cells. These alterations are manifested by increased collagen and extracellular matrix production in fibroblasts, generation of autoantibodies and cellular immune abnormalities in lymphocytes, and severe fibroproliferative and prothrombotic alterations in endothelial cells. The target cell effects cytokines and growth factors, particularly transforming growth factor-β and connective tissue growth factor. Molecular mimicry is a mechanism that may explain the pathogenicity of antibodies against viral proteins in SSc. Infection with HCMV may generate a host-antiviral response that is self-reactive toward autoantigens and endothelial cells. Self-reactive antibodies against a virus may induce endothelial cell apoptosis through interaction with the integrin α3β1 and α6β1 NAG-proteins complex.[49] Endothelial injury represents one of the first steps in the pathogenesis of SSc. Endothelial cells may be infected by bacteria or viruses that may be instrumental in inducing vasculitis. After a few days of viral infection, endothelial abnormalities are followed by necrosis.[48] Antibodies against endothelial cells (AECA) induce both upregulation of adhesion molecules with consequent mononuclear cell adhesion and endothelial cell apoptosis.[50] Microbial superantigens could initiate an immediate T cell while it has been shown that B cell response may bind to microbial superantigens to surface class II major histocompatibility complex molecules and become a target of T-helper lymphocytes. Superantigens are proteins that are expressed endogenously in the organism or that are derived exogenously by bacteria.[51]

Figure 1. *Infection hypothesis of the pathogenesis of systemic sclerosis*



CTGF = connective tissue growth factor; TGF-β = transforming growth factor-β.

Numerous infectious agents have been proposed as possible triggering factors in SSc but very few infections are as rare as SSc. Therefore, development of SSc is unlikely to depend exclusively on an infectious agent. Instead, it likely occurs as a result of interactions between the infectious agent and a cascade of host-specific factors and events. This is not surprising because immune response to infection is highly individual. It is controlled by multiple genes, age, and the route of infection. It may even be different in the same individual from one day to the next owing to a number of factors, including co-infections, stress, and pregnancy. In addition, polymorphisms in genes unrelated to immunity may cause an infectious agent to induce disease through molecular mimicry in one person and not another. Therefore, in a disease as varied, complex, and rare as SSc, infection prevalence alone should not be expected to provide sufficient evidence for or against a pathological role in the disease. Despite intensive studies there is no definitive evidence to conclude that SSc has a viral or bacterial origin. In SSc, some viral or bacterial products could synergise with other factors in the microenvironment predisposing to SSc development. We definitely need some new studies or experimental

(animal) models to define precise mechanisms by which an infectious agent contributes to the disease process because a direct association is still missing.

REFERENCES

1. Feghali Bostwick C, Medsger TA Jr, Wright TM. Analysis of systemic sclerosis in twins reveals low concordance for disease and high concordance for the presence of antinuclear antibodies. Arthritis Rheum. 2003;48:1956-63.
2. Fujinami RS, Oldstone MB. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science. 1985;230:1043-5.
3. Kreuwel HT, Sherman LA. The T-cell repertoire available for recognition of self-antigens. Curr Opin Immunol. 2001;13(6):639-43.
4. Jimenez SA, Diaz A, Khalili K. Retroviruses and the pathogenesis of systemic sclerosis. Int Rev Immunol. 1995;12:159-75.
5. Dang H, Dauphinee MJ, Talal N, et al. Serum antibody to retroviral gag proteins in systemic sclerosis. Arthritis Rheum. 1991;34:1336-7.
6. Pandey JP, LeRoy EC. Human cytomegalovirus and the vasculopathies of autoimmune diseases (especially scleroderma), allograft rejection, and coronary restenosis. Arthritis Rheum. 1998;41:10-5.
7. Neidhart M, Kuchen S, Distler O, et al. Increased serum levels of antibodies against human cytomegalovirus and prevalence of autoantibodies in systemic sclerosis. Arthritis Rheum. 1999;42:389-92.
8. Nikkari S, Mertsola J, Korvenranta H. Wegener's granulomatosis and parvovirus B19 infection. Arthritis Rheum. 1994;37:1707-8.
9. Finkel TH, Torok TJ, Ferguson PJ, et al. Chronic parvovirus B19 infection and systemic necrotising vasculitis: opportunistic infection or aetiological agent. Lancet. 1994;343:1255-8.
10. Veraldi S, Mancuso R, Rizzitelli G, et al. Henoch Schonlein syndrome associated with human parvovirus B19 infection. Eur J Dermatol. 1999;9:232-3.
11. Crowson AN, Magro CM, Dawood MR. A casual role for parvovirus B19 infection in adult dermatomyositis and other autoimmune syndromes. J Cutan Pathol. 2000;27:505-15.
12. Ferri C, Longombardo G, Azzi A, Zakrzewska K. Parvovirus B19 and systemic sclerosis. Clin Exp Rheumatol. 1999;17:267-8.
13. Ferri C, Zakrzewska K, Longombardo G, et al. Parvovirus B19 infection of bone marrow in systemic sclerosis patients. Clin Exp Rheumatol. 1999;17:718-20.
14. Altschuler EL. The historical record is consistent with the recent finding of parvovirus B19 infection of bone marrow in systemic sclerosis patients. Clin Exp Rheumatol. 2001;19:228.
15. Ray NB, Nieva DR, Seftor EA, et al. Induction of an invasive phenotype by human parvovirus B19 in normal human synovial fibroblasts. Arthritis Rheum. 2001;44:1582-6.
16. Magro CM, Nuovo GJ, Ferri C, Crowson AN, Giuggioli D, Sebastiani M. Parvoviral infection of endothelial cells and stromal fibroblasts: a possible pathogenetic role in scleroderma. J Cutan Pathol. 2004;31:43-50.
17. Sol N, Le Junter J, Vassias I, et al. Possible interactions between the NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. J Virol. 1999;73:8762-70.
18. Ferri C, Giuggioli D, Sebastian M, et al. Parvovirus B19 infection of cultured skin fibroblasts from systemic sclerosis patients. Arthritis Rheum. 2002;46:2262-3.
19. Ray NB, Nieva DRC, Seftor EA, et al. Induction of an invasive phenotype by human parvovirus B19 in normal human synovial fibroblasts. Arthritis Rheum. 2001;44:1582-6.
20. Zakrzewska K, Corcioli F, Carlsen KM, et al. Human parvovirus B19 (B19V) infection in systemic sclerosis patients. Intervirology. 2009;52:279-82
21. Vaughan JH, Shaw PX, NguyenMD, et al. Evidence of activation of 2 herpesvirus, Epstein–Barr virus and cytomegalovirus, in systemic sclerosis and normal skins. J Rheumatol. 2000;27:821-3.
22. Pandey P, LeRoy EC. Human cytomegalovirus and the vasculopathies of autoimmune diseases (especially scleroderma), allograft rejection, and coronary restenosis. Arthritis Rheum. 1998;41:10-5.
23. Hamamdzic D, Harley RA, Hazen-Martin D, LeRoy EC. Neointimal vascular lesion associated with CMV infection. BMC Musculoskeletal Disorders. 2001; 2:3.
24. Zhou YF, Shou M, Harrell RF, Yu ZX, Unger EF, Epstein SE. Chronic non-vascular cytomegalovirus infection: effects on the neointimal response to experimental vascular injury. Cardiovasc Res. 2000;45:1019-25.
25. Presti RM, Pollock AJ, Dal Canto AK, et al. Interferon γ regulates acute and latent murine cytomegalovirus infection and chronic disease of the great vessels. J Exp Med. 1998;188:577-88.
26. Vaughan JH, Shaw PX, Nguyen MD, et al. Evidence of activation of 2 herpesviruses, Epstein-Barr virus and cytomegalovirus, in systemic sclerosis and normal skin. J Rheumatol. 2000;27:821-4.
27. Neidhart M, Kuchen S, Distler O, et al. Increased serum levels of antibodies against human cytomegalovirus and prevalence of autoantibodies in systemic sclerosis. Arthritis Rheum. 1999;42:389-92.
28. Ferri C, Cazzato M, Giuggioli D, Sebastiani M, Magro CM. Systemic sclerosis following human cytomegalovirus infection. Ann Rheum Dis. 2002;61:937-8.
29. Shahgasempour S, Woodroffe SB, Sullivan-Tailyour G, Garnet HM. Alteration in the expression of endothelial cell integrin receptors alpha5beta1 and alpha2beta1 and alpha6beta1 after in vitro infection with a clinical isolate of human cytomegalovirus. Arch Virol. 1997;142:125–38.
30. Pandey P, LeRoy EC. Human cytomegalovirus and the vasculopathies of autoimmune diseases (especially scleroderma), allograft rejection, and coronary restenosis. Arthritis Rheum. 1998;41:10–5.
31. Vaughan JH, Smith EA, LeRoy EC, Arnett FC, Wright TA, Medsger TJ. An autoantibody in systemic scleroderma that reacts with glycine rich sequences. Arthritis Rheum. 1995;38(Suppl 9):255.
32. Artlett CM, Cox LA, Jimenez SA. Detection of cellular microchimerism of male or female origin in systemic sclerosis patients by polymerase chain reaction analysis of HLA-Cw antigens. Arthritis Rheum. 2000;43:1062-7.
33. Artlett CM, Smith JB, Jimenez SA. Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. N Engl J Med. 1998;338:1186–91.
34. Nelson JL, Furst DE, Maloney S, et al. Microchimerism and HLA-compatible relationships of pregnancy in scleroderma. Lancet. 1998;351:559-62.
35. Waldman WJ. Cytomegalovirus as a perturbing factor in graft/host equilibrium: havoc at the endothelial interface. In CMV-Related Immunopathology. Monographs in Virology, Volume 21. Edited by Scholz M, Rabenau HF, Doerr HW. Basel: Karger; 1998, 54-66.
36. Ando T, Minami M, Ishiguro K, et al. Changes in biochemical parameters related to atherosclerosis after Helicobacter pylori eradication. Aliment Pharmacol Ther. 2006;24(Suppl 4):58-64.
37. Gasbarrini A, Massari I, Serricchio M, et al. Helicobacter pylori eradication ameliorates primary Raynaud's phenomenon. Dig Dis Sci. 1998;43:1641-5.
38. Csiki Z, Gal I, Sebesi J, Szegedi G. Raynaud syndrome and eradication of Helicobacter pylori. Orv Hetil. 2000;141:2827-9.
39. Aragona P, Magazzu G, Macchia G, et al. Presence of antibodies against Helicobacter pylori and its heat-shock protein 60 in the serum of patients with Sjogren's syndrome. J Rheumatol. 1999;26:1306-11.
40. Savarino V, Sulli A, Zentilin P, Raffaella Mele M, Cutolo M. No evidence of an association between Helicobacter pylori infection and Raynaud phenomenon. Scand J Gastroenterol. 2000;35:1251-4.
41. Sulli A, Seriolo B, Savarino V, Cutolo M. Lack of correlation between gastric Helicobacter pylori infection and primary or secondary Raynaud's phenomenon in patients with systemic sclerosis. J Rheumatol. 2000;27:1820-1.

42. Herve F, Cailleux N, Benhamou Y, et al. Helicobacter pylori prevalence in Raynaud's disease. Rev Med Interne. 2006;27:736-41.

43. Danese S, Zoli A, Cremonini F, Gasbarrini A. High prevalence of Helicobacter pylori type I virulent strains in patients with systemic sclerosis. J Rheumatol. 2000;27:1568-9.

44. Kalabay L, Fekete B, Czirjak L, et al. Helicobacter pylori infection in connective tissue disorders is associated with high levels of antibodies to mycobacterial hsp65 but not to human hsp60. Helicobacter. 2002;7:250-6.

45. Radić M, Kaliterna DM, Bonacin D, Morović Vergles J, Radić J. Correlation between Helicobacter pylori infection and systemic sclerosis activity. Rheumatology (Oxford). 2010 (in press).

46. Ebert EC. Gastric and enteric involvement in progressive systemic sclerosis. J Clin Gastroenterol. 2008;42:5-12.

47. Hamamdzic D, Kasman LM, LeRoy EC. The role of infectious agents in the pathogenesis of systemic sclerosis. Curr Opin Rheumatol. 2002;14:694-8.

48. Arnson Y, Amital H, Guiducci S, Matucci-Cerinic M, Valentini G, Barzilai O, et al. The role of infections in the immunopathogensis of systemic sclerosis--evidence from serological studies. Ann N Y Acad Sci. 2009;1173:627-32.

49. Tachibana I, Bodorova J, Berditchevski F, Zutter MM, Helmer ME. NAG-2 a novel transmembrane-4 superfamily (TM4SF) protein that complexes with integrins and other TM4SF proteins. J Biol Chem. 1997;272:29181-9.

50. Bordron A, Dueymes M, Levy Y, et al. The binding of some human antiendothelial cell antibodies induce endothelial cell apoptosis. J Clin Invest. 1998;101:2029-35.

51. Lobo-Yeo A, Lamb JR. Superantigens as immunogens and tolerogens. Clin Exp Rheumatol. 1993;11:17-21.

Netherlands
The Journal of Medicine

REVIEW

Hereditary persistence of alpha-fetoprotein (HPAFP): review of the literature

A.C. Houwert[1], J.C. Giltay[2], E.G.W.M. Lentjes[3], M.T.W.T. Lock[1,4]*

Departments of [1]Urology, [2] Medical Genetics, [3]Clinical Chemistry and Haematology, University Medical Center, Utrecht, the Netherlands,[4]Central Military Hospital, Utrecht, the Netherlands, *corresponding author: tel.: +31 (0) 88-755 38 54, fax: +31 (088) -254 05 32, e-mail: M.T.W.T.Lock@umcutrecht.nl

ABSTRACT

Alpha-fetoprotein (AFP) serum levels are raised in several clinical conditions, ranging from non-pathological conditions to malignancies. Hereditary persistence of alpha-fetoprotein (HPAFP) is a rare benign disorder with elevated AFP levels. HPAFP is described as a benign autosomal dominantly inherited condition which is not associated with any clinical disability or additional symptoms. In the past 28 years, only 19 families have been described; due to this unfamiliarity with HPAFP, elevated AFP levels are never attributed to HPAFP. However, undiagnosed HPAFP can result in inappropriate and unnecessary treatment decisions. Therefore, HPAFP should be taken into consideration in patients with unexplained elevated AFP levels, and especially in patients with urological disorders.

KEYWORDS

Alpha-fetoprotein (AFP), gene transcription, hereditary persistence of alpha fetoprotein (HPAFP), tumour marker

INTRODUCTION

The serum protein alpha-fetoprotein (AFP) was first detected in 1956.[1,2] It is produced in the foetus by the yolk sac and the foetal liver, and is, to a lesser extent, also produced in the gastrointestinal tract.[3]

Foetal serum shows measurable AFP levels 29 days post conception; production of the foetal serum AFP increases until week 30 to 32, and subsequently levels fall. This decrease in the foetal serum concentration is due to both foetal growth and volume expansion. These aspects are discussed in detail in the review by Thomas *et al.*[4] Infants reach the same AFP levels as adults at approximately eight months of age.[5] These adult levels vary from 1 to 5 ng/ml; 99% of normal adults have levels lower than 10 ng/ml.[6] The main properties of AFP are: drug conjugation, cytotoxicity induction, growth control, ligand binding and ligand transport.[7]

Elevated AFP levels may result from a variety of clinical conditions: from normal conditions such as pregnancy to disorders such as liver cirrhosis and malignancies.[8] AFP's role as an important tumour marker has been well established.[9] In addition, AFP can also be falsely increased due to heterophilic antibodies. Heterophilic antibodies are antibodies in serum that can interfere in two-site immunoassays and which can be responsible for falsely high AFP levels.[10,11]

Another cause of elevated AFP is hereditary persistence of alpha-fetoprotein (HPAFP). In 1983 the first case of HPAFP was described; it was identified in an antenatal screening programme for spina bifida. HPAFP is a rare benign disorder in which serum AFP levels are persistently elevated without any clinical disability or additional symptoms. When family members of the patient described were tested, they were also found to have elevated levels of serum AFP. The trait was described as autosomal dominantly inherited and as not associated with any clinical disability or symptoms.[12]

The objective of the present review is to give an overview of all the described cases of HPAFP, to describe the genetics and pathophysiology of HPAFP and to describe the clinical conditions causing elevated AFP levels.

LITERATURE SEARCH

On 12 April 2010 a systematic search was conducted in the bibliographic databases PubMed, EMBASE, Cochrane and the Cumulative Index to Nursing and Allied Health Literature

(CINAHL). *Table 1* presents the search strategy used. The search term yielded 17 articles in PubMed and six articles in EMBASE. No articles were retrieved from the Cochrane and CINAHL databases. Twelve articles were excluded because they failed to report on any new cases of HPAFP. The reference lists and related articles of the studies yielded by this search were checked for additional relevant articles, and this resulted in five additional articles. In the end, the search revealed 16 articles describing 19 new cases of HPAFP.

RESULTS

Since the first family with HPAFP was described in 1983, 18 additional families have been reported (*table 2*). The diagnosis HPAFP was based on family studies which showed elevated AFP levels in family members. The families described were from all over the world, from Scotland to Japan.

Of the 19 families with HPAFP, eight index patients showed higher AFP levels after they had presented with urological disorders: four patients with a seminoma testis, one patient with a non-seminoma testis, one patient with a benign testicular cyst, one patient with a testicular nodule and one patient with testicular pain.[13-19] Index patients had serum AFP levels varying between 15 and 3564 ng/ml after treatment of the initial diagnosis (*tables 2* and *3*). Serum AFP levels of family members varied from 9 to 1260 ng/ml. Patients presenting with urological disorders revealed serum AFP levels ranging from about 9 to 65 ng/ml, with peaks of up to a maximum of 159 ng/ml.[13-19] To date, in six of the 19 families a point mutation has been reported; five families showed a -119 G > A substitution and one family showed a -55 C > A substitution.[20-23] In two families DNA sequencing was performed; however, the results failed

Table 1. *Search strategy (12 April 2010)*

No.	Synonyms
#1	hereditary AND persistence
#2	alpha OR alfa
#3	fetoprotein OR foetoprotein OR fetoproteine OR foetoproteine
#4	#2 AND #3
#5	(#4 AND #1) [Title/Abstract]

Table 3. *Conversion table for alpha-fetoprotein* [41]

Amount	Unit
1.0000	µg/l
1.0000	ng/ml
1.2100	kU/l

Table 2. *Described families*

Year	Author	Primary condition	Range serum AFP index patient	Range serum AFP family	Mutation	Unnecessary treatment
1983	Ferguson Smith[12]	Pregnancy	'Grossly elevated'	'High serum levels AFP'	-119 G>A	None
1986	Staples[13]	Hereditary spherocytosisand seminoma	14-21 kU/l	'Elevated'	-	None
1990	Greenberg[14]	Testicular cyst	30-46 ng/ml	17-64 ng/ml	-	None
1993	Tokoro[25]	Idiopathic familial basal ganglia calcification and ganglioglioma	290 ng/ml	245 ng/ml	-	Chemotherapy
1994	Feng[39]	Check up	21-129 ng/ml	46-198 ng/ml	-	None
1997	Mal[40]	Tuberculosis	200-800 ng/ml	637-1080 ng/ml	-	None
1998	Schefer[15]	Testicular nodule	20-24 ug/ml♦	22-51 ug/ml♦	-	Surgery
1999	Cochran[16]	Testicular germ cell tumour	61.3-152.9 ng/ml	21.4-27.7 ng/ml	*	Chemotherapy
2001	Flechon[17]	Seminoma testis	31.9-42.5 ng/ml	9-65 ng/ml	-	None
2001	Flechon[17]	Testicular pain	15 ng/ml	12.5 ng/ml	-	None
2002	Platini[18]	Seminoma testis	35-43 ng/ml	21-159 ng/ml	-	Chemotherapy
2003	Blesa[21]	Asthenia	1500-3564 ng/ml	240-881 ng/ml	-119 G>A	None
2004	Alj[22]	Pleurisy	900 U/ml	500-880 U/ml	-119 G>A	None
2004	Alj[22]	Dorsal pain	180 U/ml	420-580 U/ml	-55 C>A	None
2004	Yeh[24]	Check up	143 ng/ml	66-322 ng/ml	*	None
2004	Klümpen[19]	Seminoma testis	20-51 ug/l	26 ug/l	-	Surgery
2005	Nagata[23]	Check up	516 ng/ml	292-465 ng/ml	-119 G >A	None
2005	Nagata[23]	Check up	1200 ng/ml	1260 ng/ml	-119 G >A	None
2008	Xiaxin Li[8]	Endocrine evaluation	55-88 ng/ml	19-39 ng/ml	-	None

- = not known; ♦ = the unit is expressed in ug/ml; we assume this is not well reported; * = not found.

to reveal the previously described mutations associated with HPAFP.[16,24] In 26% of the index patients (5/19), unnecessary treatment was administered, namely surgery (two patients) and chemotherapy (three patients).[15,16,18,19,25] Of these five patients who underwent unnecessary treatment, 80% (four patients) had originally presented with urological disorders.[15,16,18,19]

DISCUSSION

Our literature review revealed that little is known about HPAFP. Therefore, it is necessary to describe the genetics and pathophysiology of HPAFP and other disorders that may lead to elevated AFP levels (*table 4*). Subsequently, an overview of our findings on HPAFP will be presented.

Genetics and pathophysiology of HPAFP

The AFP gene has been localised on chromosome 4 within the q11-13 region.[26,27] The expression of a gene is regulated by three elements: enhancers, promoters and silencers. These elements are characteristic DNA sequences usually located in the proximal part of the gene, the 5'-end. Concerning AFP, the 5'-end of the AFP region is exceedingly important in regulating the gene transcription because it contains all the three elements that provide a precisely regulated AFP gene transcription. These elements contain sequences that are specific to the binding of transcriptional factors. These transcriptional factors determine whether the rate of transcription will be increased or decreased. The transcriptional factors participating in the AFP gene regulation are a hepatocyte nuclear factor (HNF-1), which can bind both proximally and distally on the 5'-end region, and a non-tissue-specific factor (NF-1).[9]

HNF-1 stimulates the AFP gene activation. By contrast, NF-1 suppresses the activity in a high concentration, whereas in a low concentration it weakly stimulates AFP gene activation.[28,29] So far, two specific point mutations have been identified in the HNF-1 binding sites of the AFP gene promoter, which are associated with an increased gene transcription causing HPAFP. These point mutations are a -55 C> A substitution in the proximal HNF-1 binding site and a -119 G > A substitution in the distal HNF-1 binding site.[20,22] These point mutations lead to increased binding of HNF-1; as a result, AFP gene transcription is increased, which results in elevated AFP levels. Interestingly, the HNF-1 binding site partially overlaps the recognition site for NF-1; therefore, an increase in HNF-1 will cause a decrease in NF-1 binding, and will also result in elevated AFP levels. In a low concentration, NF-1 weakly stimulates AFP gene activation. In short, these two point mutations lead to an increase in AFP gene transcription, resulting in elevated AFP levels.[9]

Table 4. *Clinical conditions with elevated alpha-fetoprotein*

Condition		Serum AFP ng/ml	References
HPAFP		9-3564	*Table 2*
	Urological disorders	9-159	*Table 2*
Normal conditions		1-5	6
	Pregnancy:		42
	• 1st trimester	9-18	
	• 2nd trimester	53-79	
	• 3rd trimester (first half)	142-283	
	• 3rd trimester (second half)	99-192	
Congenital disorders	MSAFP	100-500	31
	Neural tube defects • MSAFP between 9-28 weeks of gestation	20-655	30
Non-malignant conditions	Non-hepatic diseases	<40	32,33
	Liver cirrhosis	30-460	35
		40-500	32
		<500	34
	Drug-induced liver damage	<500	33
	Acute and chronic hepatitis	40-500	32,33
		<500	34
Malignancies	Hepatoblastoma	500-10,000	34
	Testicular carcinoma	40-3000	32
		<50	34
	Germ cell tumours	<1,000	34
	Cancers in biliary tract and pancreas	20-557	38
		40-3000	32
	Other cancers: • gastric • cancer – colonic • cancer – lung cancer	40-3000	32
	Hepatocellular carcinoma	44-5,000,000	32
		30-7080	35
		20-9961	38

HPAFP = hereditary persistence of alpha-fetoprotein; MSAFP = maternal serum alpha-fetoprotein.

Elevated serum AFP

There are several causes of an elevated serum AFP: congenital disorders, non-malignant conditions and malignancies (*table 4*).

Congenital disorders

In 1974 Brock *et al.* described 13 cases of anencephaly and spina bifida. They measured the maternal serum AFP (MSAFP) between nine and 28 weeks of gestation and concluded that a form of prenatal diagnosis would be useful for both neural tube defects.[30] Mizejewski *et al.* stated in their review that the MSAFP levels associated with foetal defects are abnormally high: between 100 to 500 ng/ml. Their review also includes a clear overview

of all the congenital disorders causing elevated AFP levels.[31]

Non-malignant conditions

Non-malignant conditions causing elevated AFP levels can be divided into non-hepatic disorders and hepatic disorders. In non-hepatic disorders such as benign breast disease, ulcerative diseases and chronic lung diseases, serum AFP levels do not exceed 40 ng/ml; the same is true for blood bank donors. In liver cirrhosis, acute and chronic hepatitis and drug-induced liver damage, AFP levels varied between 40 and 500 ng/ml.[32-35] Serum AFP levels can also be elevated due to hepatic regeneration, since AFP is synthesised by the developing foetal liver.[33,36]

Malignancies

AFP has been associated with malignancy since Abelev *et al*. demonstrated in 1963 that AFP was detectable in transplantable hepatomas in mice.[37] In hepatoblastoma, serum AFP levels range from 500 to 10,000 ng/ml.[34] Testicular carcinoma, including testicular teratocarcinoma or germ cell tumours of the testis, showed AFP concentrations of over 40 ng/ml but less than 3000 ng/ml, with peaks of up to 8438 ng/ml.[32,34] Patients with germ cell tumours showed serum AFP levels of less than 1000 ng/ml.[34] Non-hepatic derived tumours, such as pancreatic cancer, gastric cancer, colonic cancer and lung cancer, had positive AFP levels with values between 40 and 3000 ng/ml.[32,38] Patients with hepatocellular carcinoma (HCC) had elevated AFP levels with a range in serum concentration of 44 to 5,000,000 ng/ml.[32,38] In 1993 Sato *et al*. described 33 patients with HCC and liver cirrhosis. Their patients had serum AFP levels of 30 to 7080 ng/ml at the time of tumour detection.[35]

Overview

Our literature search has revealed that little is known about HPAFP: in all, only 19 families have been described. In eight out of 19 patients, HPAFP was diagnosed because of urological disorders. In these patients, the high serum AFP levels were initially linked to the urological disorder and ultimately to HPAFP. These cases show that HPAFP is a disorder which mostly remains unrecognised, and this results in unnecessary diagnostic evaluation and inappropriate treatment decisions (e.g. radiation, chemotherapy and surgery).

We found that in non-hepatic disorders serum AFP levels do not exceed levels of 40 ng/ml, in benign hepatic disorders they do not exceed 500 ng/ml, and in malignancies serum AFP levels can be as high as thousands of ng/ml (*table 4*). Although there is a clear line of demarcation between the non-malignant conditions and malignancies, serum AFP levels can vary greatly. Malignancies cannot be ruled out if AFP is within normal levels. It remains unclear why there is such a wide variation in serum AFP levels.

In patients with elevated AFP levels, HPAFP can be a confounding factor, especially in HPAFP patients with a urological disorder. These patients can present with serum AFP levels ranging from 9 to 65 ng/ml, with peaks of up to a maximum of 159 ng/ml.[13-19] Consequently, it is difficult to differentiate between a urological malignancy or a recurrence of such a malignancy and the benign condition HPAFP.

However, not all elevated levels of serum AFP are linked to a clinical condition. In the Netherlands, several different methods are used to measure AFP. These methods show a great inter-method variability, due to the binding of the antibodies to different epitopes on the AFP molecule. This may have consequences for patients with values just above the reference range. Moreover, the number of check-ups has grown substantially. As a result, increased AFP levels are found more frequently.

CONCLUSIONS

The hereditary condition HPAFP should be taken into consideration in patients with an unexplained persistent elevation of AFP levels, and especially in patients with malignant or non-malignant urological disorders. Persistent high serum AFP levels may lead to inappropriate diagnostic evaluations and inappropriate treatment decisions, which may easily result in unnecessary treatment such as radiation, chemotherapy and surgery.

RECOMMENDATIONS

From this review it has become evident that HPAFP should be taken into consideration in patients with unexplained elevated AFP levels. Kashyap *et al*. recommend that all patients with AFP levels higher than 20 ng/ml should undergo a thorough examination so as to eliminate malignancies.[38] However, consensus has not been reached on the AFP level that would rule out a malignancy. Therefore our advice for patients with an unexplained persistent AFP elevation is a complete physical examination and a biochemical screening, including blood cell counts, renal function (creatinine), liver enzymes, coagulation status, hepatitis B surface antigen (HBsAG), antibody to hepatitis B surface antigen (anti-HBs), hepatitis C virus antibody (anti-HCV), and an ultrasonographic examination of the liver. All these tests are necessary to exclude a liver disorder as the cause of the elevated AFP. If all these tests are negative, it is useful to take HPAFP into consideration and to perform a family study in which AFP levels are determined in first-degree relatives. Alternatively

HPAFP could be confirmed by identifying a mutation in the 5'- regulatory region of the AFP gene in the index patient and possibly in other family members as well.

REFERENCES

1. Halbrecht I, Klibanski C. Identification of a new normal embryonic haemoglobin. Nature. 1956;178(4537):794-5.
2. Bergstrand CG, Czar B. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest. 1956;8(2):174.
3. Gitlin D, Perricelli A, Gitlin GM. Synthesis of -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. Cancer Res. 1972;32(5):979-82.
4. Thomas RL, Blakemore KJ. Evaluation of elevations in maternal serum alpha-fetoprotein: a review. Obstet Gynecol Surv. 1990;45(5):269-83.
5. Wu JT, Book L, Sudar K. Serum alpha fetoprotein (AFP) levels in normal infants. Pediatr Res. 1981;15(1):50-2.
6. Ball D, Rose E, Alpert E. Alpha-fetoprotein levels in normal adults. Am J Med Sci. 1992;303(3):157-9.
7. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med (Maywood). 2001;226(5):377-408.
8. Li X, Alexander S. Hereditary persistence of alpha-fetoprotein. Pediatr Blood Cancer. 2009;52(3):403-5.
9. Lazarevich NL. Molecular mechanisms of alpha-fetoprotein gene expression. Biochemistry (Mosc). 2000;65(1):117-33.
10. Dahlmann N, Hartlapp JH. False positivity with one-step monoclonal assay for alpha-fetoprotein. Lancet. 1988;1:1172-3.
11. Dahlmann N, Hartlapp JH. Chemotherapy of a patient because of spuriously elevated alpha-fetoprotein levels. Identification of the responsible factor. Klin Wochenschr. 1989;67(7):408-12.
12. Ferguson-Smith M, May HM, O' Hare E, Aitken DA. Hereditary persistence of alphafetoprotein: a new autosomal dominant trait identified in an antenatal screening programme for spina bifida. Abstract. J Med Genet. 1983;20(6):454-8.
13. Staples J. Alpha-fetoprotein, cancer, and benign conditions. Lancet. 1986;2:1277.
14. Greenberg F, Rose E, Alpert E. Hereditary persistence of alpha-fetoprotein. Gastroenterology. 1990;98(4):1083-5.
15. Schefer H, Mattmann S, Joss RA. Hereditary persistence of alpha-fetoprotein. Case report and review of the literature. Ann Oncol. 1998;9(6):667-72.
16. Cochran PK, Chauvenet AR, Hart PS, de Graaf SS, Cushing B, Kroovand L, et al. Hereditary persistence of alpha-fetoprotein in a child with testicular germ cell tumor. Med Pediatr Oncol. 1999;32(6):436-7.
17. Flechon A, Droz JP. Hereditary persistence of alpha-fetoprotein in testis disease. J Urol. 2001;165:2004.
18. Platini C. Hereditary persistence of alpha-fetoprotein: report of a case. Rev Med Interne. 2002;23:182-8.
19. Klumpen HJ, Westermann AM. Diagnostic dilemma in a man with seminoma. Lancet. 2004;364:2230.
20. McVey JH, Michaelides K, Hansen LP, Ferguson-Smith M, Tilghman S, Krumlauf R, et al. A G-->A substitution in an HNF I binding site in the human alpha-fetoprotein gene is associated with hereditary persistence of alpha-fetoprotein (HPAFP). Hum Mol Genet. 1993;2(4):379-84.
21. Blesa JR, Giner-Duran R, Vidal J, Lacalle ML, Catalan I, Bixquert M, et al. Report of hereditary persistence of alpha-fetoprotein in a Spanish family: molecular basis and clinical concerns. J Hepatol. 2003;38(4):541-4.
22. Alj Y, Georgiakaki M, Savouret JF, Mal F, Attali P, Pelletier G, et al. Hereditary persistence of alpha-fetoprotein is due to both proximal and distal hepatocyte nuclear factor-1 site mutations. Gastroenterology. 2004;126(1):308-317.
23. Nagata-Tsubouchi Y, Ido A, Uto H, Numata M, Moriuchi A, Kim I, et al. Molecular mechanisms of hereditary persistence of alpha-fetoprotein (AFP) in two Japanese families A hepatocyte nuclear factor-1 site mutation leads to induction of the AFP gene expression in adult livers. Hepatol Res. 2005;31(2):79-87.
24. Yeh SH, Kao JH, Chen PJ. Heterogeneity of hereditary persistence of alpha-fetoprotein. Gastroenterology. 2004;127(2):687; author reply 688.
25. Tokoro K, Chiba Y, Ohtani T, Abe H, Yagishita S. Pineal ganglioglioma in a patient with familial basal ganglia calcification and elevated serum alpha-fetoprotein: case report. Neurosurgery. 1993;33(3):506-11; discussion 511.
26. Minghetti PP, Harper ME, Alpert E, Dugaiczyk A. Chromosomal structure and localization of the human alpha-fetoprotein gene. Ann NY Acad Sci. 1983;417:1-12.
27. Harper ME, Dugaiczyk A. Linkage of the evolutionarily-related serum albumin and alpha-fetoprotein genes within q11-22 of human chromosome 4. Am J Hum Genet. 1983;35(4):565-72.
28. Feuerman MH, Godbout R, Ingram RS, Tilghman SM. Tissue-specific transcription of the mouse alpha-fetoprotein gene promoter is dependent on HNF-1. Mol Cell Biol. 1989;9(10):4204-12.
29. Bois-Joyeux B, Danan JL. Members of the CAAT/enhancer-binding protein, hepatocyte nuclear factor-1 and nuclear factor-1 families can differentially modulate the activities of the rat alpha-fetoprotein promoter and enhancer. Biochem J. 1994;301:49-55.
30. Brock DJ, Bolton AE, Scrimgeour JB. Prenatal diagnosis of spina bifida and anencephaly through maternal plasma-alpha-fetoprotein measurement. Lancet. 1974;1:767-9.
31. Mizejewski GJ. Levels of alpha-fetoprotein during pregnancy and early infancy in normal and disease states. Obstet Gynecol Surv. 2003;58(12):804-26.
32. Waldmann TA, McIntire KR. The use of a radioimmunoassay for alpha-fetoprotein in the diagnosis of malignancy. Cancer. 1974;34(4 Suppl):1510-5.
33. Bloomer JR, Waldmann TA, McIntire KR, Klatskin G. Alpha-Fetoprotein in noneoplastic hepatic disorders. JAMA. 1975;233(1):38-41.
34. Abelev GI, Eraiser TL. Cellular aspects of alpha-fetoprotein reexpression in tumors. Semin Cancer Biol. 1999;9(2):95-107.
35. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med. 1993;328(25):1802-6.
36. Taketa K. Alpha-fetoprotein: reevaluation in hepatology. Hepatology. 1990;12(6):1420-32.
37. Abelev GI, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS. Production of embryonal alpha-globulin by transplantable mouse hepatomas. Transplantation. 1963;1:174-80.
38. Kashyap R, Jain A, Nalesnik M, Carr B, Barnes J, Vargas HE, et al. Clinical significance of elevated alpha-fetoprotein in adults and children. Dig Dis Sci. 2001;46(8):1709-13.
39. Feng YZ, Liu J, Zhou XR. Hereditary persistence of alpha-fetoprotein. Chin J Cancer Res. 1994;6(3):232-4.
40. Mal F, Girard P, Gayet B. Congenital high increase of serum alpha-fetoprotein: case of a family. Gastroenterol Clin Biol. 1997;21(11):903.
41. Federale overheidsdienst (FOD) volksgezondheid, veiligheid van de voedselketen en leefmilieu, commissie voor klinische biologie, Dienst voor laboratoria van klinische biologie, comite van deskundigen. Globaal rapport; Externe kwaliteitsevaluatie voor analyseren klinische biologie; Immunoassays. 2008.
42. Gonzalez-Bugatto F, Foncubierta E, Bailen Mde L, Illanes S, Hervias-Vivancos B, Bartha JL. Maternal and fetal serum transformed alpha-fetoprotein levels in normal pregnancy. J Obstet Gynaecol Res. 2009;35(2):271-6.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Pronounced weight gain in insulin-treated patients with type 2 diabetes mellitus is associated with an unfavourable cardiometabolic risk profile

H.J. Jansen[1*], G. Vervoort[1], M. van der Graaf[2], C.J. Tack[1]

Departments of [1]Internal Medicine and [2]Radiology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24-361 38 89, fax: +31 (0)24-354 00 22, e-mail: H.Jansen@aig.umcn.nl

ABSTRACT

Pronounced weight gain after start of insulin therapy in patients with type 2 diabetes mellitus (T2DM) may offset beneficial effects conferred by the improvement of glycaemic control. This hypothesis was tested by comparing the cardiometabolic risk profile of a group of type 2 diabetes patients with a marked increase in body weight ('gainers') after the start of insulin treatment and a similar group without any or only minimal weight gain ('non-gainers').

In a cross-sectional study, we compared two predefined groups of patients with T2DM who had been on insulin therapy for a mean of 4.0 years: 'gainers' *vs* 'non-gainers'. Cardiometabolic risk was assessed by measuring fat content and distribution (physical examination, bioelectrical impedance analysis, dual energy X-ray absorption, and magnetic resonance imaging), liver fat content (magnetic resonance spectroscopy), physical activity levels (Sensewear® armband) and plasma markers.

Each subgroup consisted of 14 patients. Gainers had significantly more total body and trunk fat (especially subcutaneous fat) compared with non-gainers. Gainers had similar liver fat content, and slightly higher levels of fat hormones. Furthermore, gainers performed significantly less physical activity. Lastly, gainers had higher total cholesterol, low-density lipoprotein cholesterol, and alanine aminotransferase levels with similar cholesterol-lowering treatment.

Patients with T2DM who show pronounced weight gain during insulin therapy have a less favourable cardiometabolic risk profile compared with patients who show no or minimal weight gain.

KEYWORDS

Insulin-associated weight gain, type 2 diabetes mellitus, cardiometabolic risk profile

INTRODUCTION

Insulin therapy is frequently needed to achieve adequate glycaemic control in patients with type 2 diabetes mellitus (T2DM), but often at the expense of weight gain. Although values differ between studies, and studies are generally of limited duration, the estimated weight gain during the first year of insulin therapy ranges from approximately 2 to 6 kg.[1] This weight gain shows large inter-individual differences, with some patients experiencing substantial insulin-associated weight gain, while others do not show any weight gain at all or even lose weight.

The determinants of insulin-associated weight gain are not entirely elucidated; most authors view the improvement in glycaemic control as the major determinant of weight gain. However, the level of improvement in glycaemic control is only weakly correlated with the increase in body weight.[2,3] A frequently mentioned clinical experience is that a subset of patients exists that shows a persistent and continuous increase in body weight over time even when stable glycaemic control has been obtained.

It is obvious that weight gain in an already overweight population is undesirable. Weight gain will deter further optimisation of insulin therapy[4] and in itself will adversely influence the cardiometabolic risk profile.[5] Little is known about the effects of insulin-associated weight gain on cardiometabolic risk in patients with T2DM. One may hypothesise that the benefits of insulin treatment

conferred by the improvement of glycaemic control may be offset by the disadvantages associated with pronounced weight gain. An increased fat mass may cause aggravation of insulin resistance, dyslipidaemia and hypertension and may increase the levels of inflammatory markers and the propensity for thrombotic events.[6] Indeed, in type 1 diabetes, patients who experienced pronounced weight gain during intensive insulin therapy showed a less favourable cardiovascular risk profile.[7] In the ACCORD trial,[8] the intensively treated group with T2DM showed increased mortality. In this group, more than 75% of the patients used insulin therapy in combination with several oral drugs. More than 25% of the patients treated in the intensive-therapy group showed a mean weight gain of >10 kg during follow-up. Although the study did not reveal any direct effect of the exaggerated weight gain on cardiovascular events, extensive weight gain might have had a negative influence on cardiometabolic risk.

In the present study, we hypothesised that pronounced weight gain during insulin therapy would be associated with an unfavourable cardiometabolic risk profile. This hypothesis was tested by comparing the cardiometabolic risk profile of insulin-treated patients with T2DM who showed weight gain at the extreme ends of the spectrum ('gainers' *vs* 'non-gainers').

RESEARCH DESIGN AND METHODS

Patient groups

Patients were selected out of a cohort of patients with T2DM who started insulin therapy in our University Diabetes Clinic between 2001 and 2006. To prevent confounding with respect to influences of different types of insulin on body weight we only included patients who started and continued on biphasic insulin (NovoMix® or Mixtard® insulin), twice-daily. Patients were selected based on the weight gain after starting insulin. We defined a 'gainer' as a patient who showed an increase in body weight of ≥0.5 kg/month within the first 18 months after starting insulin therapy and ≥5% weight gain at total follow-up (i.e. at the time of cross-sectional measurement, which was different for each patient). We defined a 'non-gainer' as a patient with a maximum weight gain ≤2.5 kg at follow-up. These criteria were derived from a historical insulin-treated group (n=140), and represent the upper and lower subgroups of weight gain. Assessment at follow-up of the cardiometabolic risk profile between the two groups (gainers *vs* non-gainers) was performed. All selected patients had a minimal follow-up of 18 months. Exclusion criteria were: heart failure (NYHA class III-IV), liver or renal disease (defined by chronic renal disease stage ≥III), hypoalbuminaemia, use of alcohol of more than 2 units/day, drug abuse, use of thiazolidinedione derivatives or prednisone, and pregnancy or the intention to become pregnant during the study. Eligible patients were not on anti-obesity medications and acarbose treatment. Also weight loss surgery patients or patients who followed any other weight management program were excluded.

The inclusion and exclusion criteria were reviewed at a screening visit, where patients underwent history taking and a complete physical examination. The study protocol was approved by the local ethics committee. All patients provided written informed consent.

Cardiometabolic risk assessment

Cardiometabolic risk profile at follow-up was assessed by the following: 1) body fat distribution (weight, height, waist and hip circumference, bioelectrical impedance analysis (BIA), dual energy X-ray absorption (DEXA), and MRI),[9,10] 2) liver fat content (LFAT) by magnetic resonance spectroscopy (MRS),[11] 3) physical activity levels,[12] 4) classical risk factors, other biochemical cardiometabolic markers.[13]

Patients were tested after overnight fasting and with an empty bladder. To determine body mass index (BMI), weight (kg) was divided by height in metres, squared. Weight was measured with subjects wearing light underwear only. Scales were calibrated annually. Waist circumference was measured midway between the lower rib margin and the iliac crest at expiration, and hip circumference over the greater trochanter; waist-to-hip ratio (WHR) was calculated.

To assess fat distribution three different methods were used: BIA, DEXA and MRI. BIA was carried out using an Akern soft tissue analyser (BIA Quatum/S Body Composition Analyser model no. BIA-101, Akern Srl, Pontassieve (Florence), Italy). BIA was performed to assess total body water (TBWa) and fat-free mass (FFM). Patients rested in a supine position for approximately five minutes to equalise fluid compartments. Four surface electrodes were applied (two each to an arm and a leg). Phase sensitive sensors separated the components of the modulus into Reactance and Resistance.

Total-body DEXA scanning was performed using a Hologic QDR 4500 densitometer (Hologic Inc., Bedford, USA) to determine fat mass (total fat mass and trunk fat) and lean mass. To assess non-trunk fat, the trunk-to-leg ratio (trunk mass divided by leg mass) was calculated for each patient.

MRI measurements were performed on a Tim-Trio MR system (Siemens, Erlangen, Germany). A series of T1-weighted (flash 2D) axial MR images was acquired from a region extending from 4 cm above to 4 cm below the fourth to fifth lumbar interspace. Visceral and subcutaneous fat areas were determined based on signal intensity. Proton MR spectra (STEAM; TE/TR:20/3000ms) were obtained without water suppression from a 8-ml voxel positioned in the liver during breath holding. The water signal intensity (S_{water}) and the methylene lipid signal

intensitiy (S_{fat}) were used to calculate the percentage of liver fat by the following formula: $((S_{fat})/(S_{fat} + S_{water})) \times 100\%)$.[14] Total MR examination time was 30 minutes. Patients with pacemakers, implantable cardioverter defibrillators, metal implants, and claustrophobia were included, but did not undergo magnetic resonance imaging (MRI). In 11 gainers and 12 non-gainers MRI/MRS was performed. The remaining patients experienced claustrophobia during the MRI/MRS scan although they were not known with claustrophobia at inclusion.

Physical activity was measured using a SenseWear Pro Armband™ (Body Media, Pittsburgh, PA, USA).[15,16] The device was placed on the right upper arm over the triceps muscle for five consecutive days. Measurements were only used for calculations if >90% of data were available. SenseWear Innerview professional software 6.1 was used to analyse the data.

Classical risk factors as blood pressure, smoking habits, lipids, renal function and albumin excretion ratio (AER) were determined. Blood pressure was measured at the right arm with the patient in a supine position after a minimum of five minutes rest. All patients had already taken their antihypertensive drugs. Blood pressure was determined twice by a manual sphygmomanometer. The average blood pressure (mean systolic and diastolic blood pressure) was calculated. Furthermore, fasting blood samples were drawn to assess: HbA_{1c}, lipids, alanine aminotransferase (ALAT), and creatinine (all determined by standard laboratory methods). Renal function expressed as glomerular filtration rate (GFR) was calculated by the modified diet in renal disease (MDRD) formula.[17] The adipocytokines, adiponectin and leptin were determined by using DuoSet ELISA development system kits (R&D systems, Minneapolis, USA), free fatty acids (FFA) using Cobas Mira Plus® (Roche Diagnostics Ltd., Basal, Switzerland), and the inflammatory markers high-sensitive C-reactive protein (hsCRP) by Dako ELISA (Glastrup, Denmark), IL-6 and 18 by Luminex® Corporation assay (Austin, Texas, USA)).

Statistical analyses

Differences between groups were analysed by unpaired Student's *t*-test and Mann-Whitney U test as appropriate. For comparing dichotomous variables the χ^2 test was used. All calculations were made using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Data are expressed as means ± SD, unless otherwise indicated. A p value <0.05 was considered significant in all statistical comparisons.

RESULTS

A total of 14 patients were included in each group. *Table 1* shows the characteristics of the patients. All patients were Caucasian. The two groups (gainers *vs* non-gainers) were compared after a mean of 4.0±1.6 years insulin therapy (i.e. mean follow-up); as per protocol, all patients were still on biphasic insulin. Gainers were on insulin longer than non-gainers (4.6±1.7 *vs* 3.7±2.1 year, p=0.03).

Table 1. *Patient characteristics after a mean of four years of insulin therapy*

	Gainers	Non-gainers
Gender (male/female)	8/6	10/4
Age (year)	63±7	65±7
Diabetes duration (years)	9 (2-25)	12 (2-22)
Insulin therapy (years)	4.6±1.7	3.7±2.1 *
Medication (n)		
• Insulin alone	7	5
• Insulin + Metformin	6	7
• Insulin + SU	0	1
• Insulin + Metformin + SU	1	1
Insulin dose (U/day)	68±37	60±37
Insulin dose (U/kg)	0.7±0.4	0.7±0.4

Data are means±SD or median (range); *P<0.05; SU = sulfonylurea derivative.

Change in weight and HbA_{1c} after start of insulin therapy

As per definition, gainers showed a substantially larger weight gain (+11 kg [range +5.2 to +19.6 kg]) compared with the non-gainers (-1.2 kg [range -7.6 to +2.5 kg], *figure 1*). Of note, body weight at the start of insulin therapy was slightly lower in gainers (83± 5 *vs* 87±13 kg, p=NS). After four years of insulin therapy, BMI, waist circumference and WHR were not significantly different between the two groups (BMI 32.3±5.6 *vs* 29.0±4.5 kg/m^2, waist circumference 110±15 *vs* 106±13 cm, WHR 1.05±0.12 *vs* 1.03±0.10, for gainers and non-gainers, respectively, p=NS). Mean HbA_{1c} decreased from 9.9±2.6 to 7.2±0.7 in gainers *vs* 8.9±1.3 to 7.4±0.9 % in non-gainers (p=NS for the difference in gainers *vs* the difference in non-gainers).

Cardiometabolic risk profile at follow-up

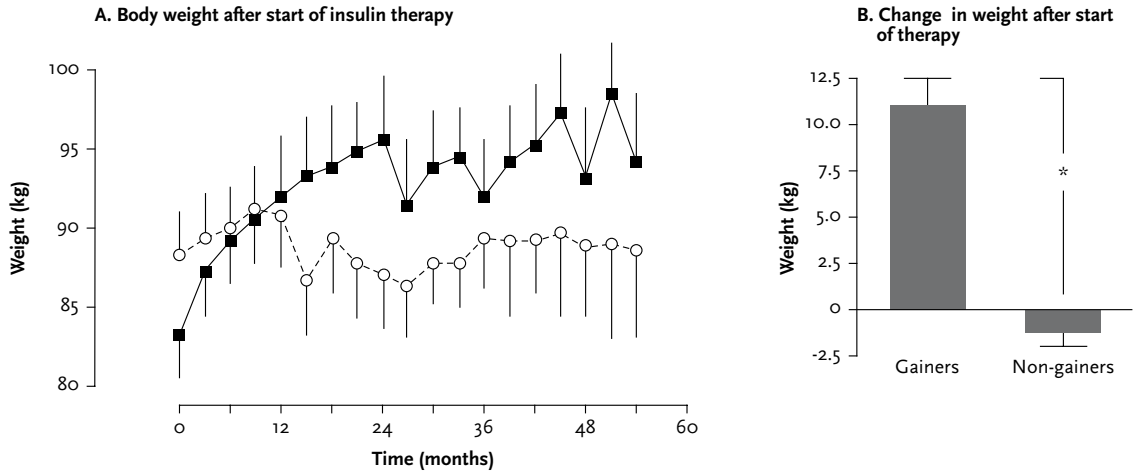
Fat distribution

Gainers had significantly more total body fat (32.4±9.4 *vs* 24.6±7.7 kg, p=0.03) and more trunk fat compared with non-gainers (18.3±5.5 *vs* 14.1±4.2 kg, p=0.04). Gainers had slightly higher TBWa compared with non-gainers (37.7±7.5 *vs* 35.9±7.1 litres, p=NS). FFM was comparable in gainers and non-gainers as measured by DEXA (58.4±9.1 *vs* 59.6±8.7 kg, p=NS) and by BIA. In both groups the trunk-to-leg ratio was similar.

As measured by MRI, gainers had significantly higher subcutaneous fat than non-gainers (2.5±0.8 *vs* 1.8±0.8 litres, p=0.04), while visceral fat was similar (1.7±0.7 *vs* 1.5±0.7 litres, p=NS).

Sixteen patients (70%) had LFAT levels above the upper reference value of 5.5%.[18] Gainers and non-gainers had similar LFAT (9.6±2.7 *vs* 9.3±1.6 %, respectively, p=NS).

Figure 1. *A. (left panel) Observed change in weight after start of insulin therapy (t=0) comparing gainers (closed squares) and non-gainers (open circles). B. (right panel) Absolute change in weight after start of insulin therapy. * P<0.05.*



Physical activity levels

The average total energy expenditure at follow-up was significantly lower in gainers than in non-gainers (2275±385 *vs* 2632±734 kcal/day, p=0.005). Physical activity duration expressed as metabolic equivalent (MET) ≥3, which is consuming ≥3 kcal/kg of body weight per hour, tended to be lower in gainers compared with non-gainers (56±51 *vs* 83±80 min/day, p=0.06). Also the amount of vigorous activity (MET >6) was lower in gainers than in non-gainers (2.4±3.4 *vs* 6.5±5.5 minutes, p=0.03). The amount of sedentary activity (MET 0-3) and moderate activity (MET 3-6) was similar between the two groups, as was the number of steps per day (5416±3543 *vs* 5282±3681), and the total duration of rest (514±139 *vs* 484±148 minutes) and sleep (409±137 *vs* 394±135 minutes), all gainers *vs* non-gainers, respectively.

Cardiometabolic markers

Prior to the start of insulin therapy the classical cardiometabolic risk markers (i.e. BMI, blood pressure, lipid profiles, smoking, GFR and AER) were similar in the two groups (*table 2*).

The cardiometabolic risk markers of the two groups measured in the present study are shown in *table 3*. Blood pressure was similar in gainers compared with non-gainers, as was the average number (2.1 *vs* 2.2) and dose of antihypertensive medication. Total cholesterol and low-density lipoprotein cholesterol (LDL-cholesterol) were significantly higher in gainers than in non-gainers, as was the level of high-density lipoprotein cholesterol (HDL-cholesterol), despite similar use of statins at equipotent doses.

There were no differences between the two groups with respect to smoking habits. Creatinine was slightly lower

Table 2. *Baseline cardiometabolic risk markers comparing gainers and non-gainers*

	Gainers	Non-gainers	p-value
Classical risk factors			
SBP (mmHg)	148±26	153±26	NS
DBP (mmHg)	85±12	88±8	NS
Total cholesterol (mmol/l)	5.1±1.0	4.5±0.9	NS
LDL-cholesterol (mmol/l)	3.0±1.0	2.3±0.9	NS
HDL-cholesterol (mmol/l)	1.2±0.2	0.9±0.2 *	0.01
Triglycerides (mmol/l)	2.0±1.1	3.3±2.4	NS
Smoking (n)	11	12	NS
Creatinine (µmol/l)	92.6±29.7	99.5±28.9	NS
GFR (MDRD); ml/min/1.73m²	79.0±34.6	64.5±0.19	NS
Albumin excretion ratio (µg/min)	29.8±24.6	39.1±53.4	NS
Liver enzymes			
ALAT (U/l)	36.8±18.4	36.2±20.4	NS

Data are means ± SD; SBP = systolic blood pressure; DBP = diastolic blood pressure; LDL = low-density lipoprotein; HDL = high-density lipoprotein; GRF = glomerular filtration rate; MDRD = modified diet in renal disease; ALAT = alanine aminotransferase. All data were analysed by Student *t*-test, except for the albumin excretion ratio (AER) data, which were analysed with Mann-Whitney *U*-test. * P<0.05.

Table 3. *Cardiometabolic risk markers comparing gainers and non-gainers at follow-up*

	Gainers	Non-gainers	P-value
Classical risk factors			
SBP (mmHg)	150±23	146±31	NS
DBP (mmHg)	81±10	80±11	NS
Total cholesterol (mmol/l)	4.8±0.9	3.8±0.8 *	0.001
LDL-cholesterol (mmol/l)	2.9±0.8	2.1±0.4 *	0.006
HDL-cholesterol (mmol/l)	1.2±0.2	1.0±0.2 *	0.03
Triglycerides (mmol/l)	2.1±1.4	1.7±0.6	NS
Smoking (n)	2	2	NS
Creatinine (µmol/l)	85±29	97±38	NS
GFR (MDRD; ml/min/1.73 m²)	110±28	85±33 *	0.04
AER (µmol/min)	113±228	74±143	NS
FFA (µmol/l)	0.6±0.2	0.6±0.3	NS
Fat hormones			
Leptin (ng/ml)	43.3±26.7	29.7±21.3	NS
Adiponectin (µg/ml)	3.0±1.4	2.1±1.0	NS
Inflammatory markers			
hsCRP (pmol/ml)	4.8±3.3	3.7±4.2	NS
IL-6 (pg/ml)	11.5±18.3	5.9±2.9	NS
IL-18 (pg/ml)	139±39	133±46	NS
Liver enzymes			
ALAT (U/l)	33.1±11.4	23.8±9.1 *	0.04

Data are means ± SD. SBP = systolic blood pressure, DBP = diastolic blood pressure, LDL = low-density lipoprotein, HDL = high-density lipoprotein, GRF = glomerular filtration rate; MDRD = modified diet in renal disease; AER = albumin excretion ratio; ALAT = alanine aminotransferase. All data were analysed by Student *t*-test, except for the data on AER, leptin, hsCRP, IL-6 and 18 which were analysed by nonparametric Mann-Whitney *U* test. * P<0.05.

in gainers than in non-gainers and urinary albumin excretion appeared quantitatively higher in gainers, but the differences were not significant. Calculated GFR was significantly higher in the gainers group. FFA and adiponectin levels were similar between the two groups. Leptin, and the inflammatory cytokines were slightly higher in gainers than in non-gainers. ALAT levels were significantly higher in gainers.

DISCUSSION

The main finding of this cross-sectional pilot study is that patients who develop pronounced weight gain after long-term insulin therapy have more total, trunk and subcutaneous fat, perform less physical activity and show slightly higher cholesterol and ALAT levels and GFR compared with those who do not gain weight. All together these findings suggest that this group of 'gainers' may have an unfavourable cardiometabolic risk profile compared with 'non-gainers'.

So far, hardly any studies have investigated the effects of long-term insulin therapy on body weight as a primary endpoint. Follow-up in most studies is limited to 6 to 12 months, with a reported increase in body weight of approximately 2 to 6 kg.[1] Whether ongoing weight increase occurs in insulin-treated patients while stable glycaemic control has been obtained is less clear. Recently, Aas *et al.*[19] reported a mean weight gain approaching 4 kg over three years in insulin-treated participants of the DIGAMI study. Approximately 30% of the weight gain took place beyond one year of therapy. Also Kooy *et al.*[20] found ongoing weight increase in a group of insulin-treated patients who were followed for over four years. The limited findings from literature match with general clinical experience and suggest that – at least in a subset of patients – weight continues to increase during insulin treatment without further improvement in glycaemic control. The 'gainers' selected in the present study may represent this group.

The two groups seemed to differ with respect to body weight before the start of insulin, with the gainers starting at a lower weight, although this difference was statistically not significant. This may suggest that gainers had lost more weight before the onset of insulin and thus simply regained more weight after starting insulin, as has been suggested before.[21] As the initial HbA_{1c} level was also slightly higher in the gainers group (not statistically significant), a relative contribution from 'initial regain' cannot be fully excluded. Indeed, gainers suffered a mean of 4.3 kg±6.4 kg weight loss within 12 months prior to the start of insulin therapy. However, the observed sustained increase in body weight during long-term insulin treatment cannot be attributable to exaggerated weight loss before therapy.

The adverse effect of a sustained increase in body weight during insulin treatment as found in the present study is supported by previous findings. Yki-Järvinen *et al.*[4] reported higher blood pressure and lipid levels in patients with an exaggerated weight increase. In a recent report, initiation of insulin treatment after myocardial infarction

was associated with a significant increase in weight and incidence of re-infarction, although the latter was not clearly explained by the increased weight.[19] In addition, elevated levels of adipokines were found in a group of patients treated with insulin who gained weight compared with a group treated by lifestyle intervention that lost weight, despite similar glycaemic levels. All together these data, though limited, suggest that insulin-associated weight gain may indeed negatively affect cardiometabolic risk profile.

We found that the group of subjects with pronounced weight increase had higher total, trunk and subcutaneous fat. Long-term effects of insulin-associated weight gain on body composition have not been reported, but in short-term studies, insulin treatment showed an increase in fat mass but also FFM, in line with the anabolic effect of insulin.[22] The present study did not reveal a difference in lean body mass between the two groups. Although this does not exclude a beneficial effect of insulin treatment in itself on lean body mass, it does show that the exaggerated weight increase is explained by an increase in fat only.

Most studies suggest that visceral rather than subcutaneous fat is associated with insulin-resistance and may confer increased cardiometabolic risk.[23-25] From this point of view, the currently reported increase in subcutaneous fat mass may not necessarily incur to a strongly elevated cardiometabolic risk, but may still contribute to an adverse cardiometabolic risk profile.[26] It could be hypothesised that gainers exhibit higher levels of adipocytokines (e.g. leptin, IL-6) at the level of subcutaneous adipose tissue compared with non-gainers. Furthermore, it could be argued that dietary content (e.g. ceramide intake) might influence body weight and metabolic effects on adipose tissue.[27] Unfortunately, we did not perform subcutaneous fat biopsies in the two groups and took a standardised questionnaire survey for assessing patients dietary habits.

It is known that hepatic fat accumulation is associated with (hepatic) insulin resistance in non-alcoholic fatty liver disease and is also a predictor of cardiometabolic disease.[27] Juurinen *et al.*[28] showed that after seven months of insulin therapy (basal insulin) patients had improvement of hepatic insulin sensitivity and reduction of hepatic fat content. LFAT content after longer periods of insulin treatment has not been studied. The present results found no statistically significant differences between the two groups with respect to LFAT content as measured by MRS. Both groups had substantial percentages of LFAT (~10%), which is in line with results reported in literature.[29] The number of subjects in whom LFAT measurements were successful in the study was relatively low and thus the lack of a difference may represent a power problem, especially as the slightly higher ALAT levels suggest that the group of gainers may have had slightly higher LFAT. Alternatively, higher ALAT levels may confer an elevated cardiovascular risk, independent of LFAT.[30,31]

MDRD-GFR was higher in the gainers, which was associated with a tendency towards lower serum creatinine and an increased albumin excretion rate. Together these results suggest the existence of glomerular hyperfiltration, which in itself has been listed as a cardiovascular risk marker.[32]

(Low-grade) inflammation and adipocytokines (i.e. leptin) are associated with obesity and cardiovascular disease.[33-35] In line with this association, leptin, IL-6, IL-18 and hsCRP tended to be higher in gainers. Adiponectin levels are negatively associated with obesity and with cardiovascular endpoints. Adiponectin levels in both groups were similar, suggesting that insulin-associated weight gain does not necessarily translate in a (further) decrease in adiponectin levels.

Physical activity is a strong predictor of future cardiovascular disease and a determinant of body weight.[36] Gainers had lower levels of total energy expenditure compared with non-gainers, and performed less vigorous exercise compared with non-gainers. Due to the cross-sectional design of our study, it cannot be determined whether the decreased level of physical exercise is the cause of the exaggerated insulin-associated weight gain or the consequence. However, no matter the cause or consequence, a low physical exercise level remains a cardiovascular risk factor. It can be speculated, for instance, that (pronounced) weight gain in insulin-treated patients and change in physical activity is associated with a decrease in mood or tendency towards depression. In this study we did not assess (changes in) mood or depression score. Further prospective work is warranted in order to investigate the relationship between insulin-associated weight gain and level of physical activity.

The study has a number of limitations. The cross-sectional comparison cannot determine whether the unfavourable cardiometabolic risk profile observed in the gainers is the direct consequence of insulin-associated weight gain. The study cannot determine whether part of the observed weight gain is due to the 'natural' course of body weight associated with ageing; this would require a control group of either matched non-diabetic subjects, or subjects with T2DM on oral medication. The study also has a number of strengths. There is a rather homogenous population, similarly treated patients from a single centre, all on biphasic insulin. We used a set of sophisticated techniques to quantify body fat distribution and physical activity.

The results of our study may have clinical implications. As it seems that pronounced weight gain during (long-term) insulin therapy is associated with a less favourable cardiometabolic risk profile, it may be important to determine which patients are most at risk for weight gain. This would require assessment of predictive factors and

lifestyle characteristics before onset of insulin treatment, which, however, are largely unknown. Most authors view the change in glycaemic control (i.e. change in HbA_{1c}) as the major determinant of insulin-associated weight gain.[2,3] Although part of the short-term insulin-associated weight gain may be explained by change in HbA_{1c} in this study, this cannot explain sustained weight gain after long-term insulin therapy when stable or even increased HbA_{1c} is observed. Further prospective studies are needed to improve identification of patients who are at risk for extensive body weight increase and develop interventions to prevent the weight gain.

In conclusion, the present study suggests that pronounced weight gain during (long-term) insulin therapy in patients with T2DM is associated with an unfavourable cardiometabolic risk profile. Further work is required to determine the individual risk factors for exaggerated weight increase, to assess long-term consequences and to develop potential interventions.

ACKNOWLEDGEMENTS

We would like to thank Kristine van Doesum for the analyses of the MRI slides of each patient, and we thank Vincenzo Positano (IFC-CNR, Pisa, Italy) for kindly providing the software package HippoFat to us. We also thank Marjo van de Ven who performed the DEXA scans of each patient.

REFERENCES

1. Holman RR, Thorne KI, Farmer AJ, et al. Addition of biphasic, prandial, or basal insulin to oral therapy in type 2 diabetes. N Engl J Med. 2007;357(17):1716-30.
2. Salle A, Ryan M, Guilloteau G, Bouhanick B, Berrut G, Ritz P. 'Glucose control-related' and 'non-glucose control-related' effects of insulin on weight gain in newly insulin-treated type 2 diabetic patients. Br J Nutr. 2005;94(6):931-7.
3. Jacob AN, Salinas K, Adams-Huet B, Raskin P. Weight gain in type 2 diabetes mellitus. Diabetes Obes Metab. 2007;9(3):386-93.
4. Yki-Järvinen H, Ryysy L, Kauppila M, et al. Effect of obesity on the response to insulin therapy in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab. 1997;82(12):4037-43.
5. Anderson JW, Kendall CW, Jenkins DJ. Importance of weight management in type 2 diabetes: review with meta-analysis of clinical studies. J Am Coll Nutr. 2003;22(5):331-9.
6. Aas AM, Seljeflot I, Torjesen PA, Diep LM, Thorsby PM, Birkeland KI. Blood glucose lowering by means of lifestyle intervention has different effects on adipokines as compared with insulin treatment in subjects with type 2 diabetes. Diabetologia. 2006;49(5):872-80.
7. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Diabetes Control and Complications Trial. JAMA. 1998;280(2):140-6.
8. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med. 2008;358(24):2545-59.
9. Wilson PW, Bozeman SR, Burton TM, Hoaglin DC, Ben-Joseph R, Pashos CL. Prediction of first events of coronary heart disease and stroke with consideration of adiposity. Circulation. 2008;118(2):124-30.
10. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L. Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J (Clin Res Ed). 1984;289(6454):1257-61.
11. Kotronen A, Juurinen L, Tiikkainen M, Vehkavaara S, Yki-Jarvinen H. Increased liver fat, impaired insulin clearance, and hepatic and adipose tissue insulin resistance in type 2 diabetes. Gastroenterology. 2008;135(1):122-30.
12. Sesso HD, Paffenbarger RS, Jr, Lee IM. Physical activity and coronary heart disease in men: The Harvard Alumni Health Study. Circulation. 2000;102(9):975-80.
13. Qasim A, Mehta NN, Tadesse MG, et al. Adipokines, insulin resistance, and coronary artery calcification. J Am Coll Cardiol. 2008;52(3):231-6.
14. Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol. 1999;276:E977-89.
15. St-Onge M, Mignault D, Allison DB, Rabasa-Lhoret R. Evaluation of a portable device to measure daily energy expenditure in free-living adults. Am J Clin Nutr. 2007;85(3):742-9.
16. Mignault D, St-Onge M, Karelis AD, Allison DB, Rabasa-Lhoret R. Evaluation of the Portable HealthWear Armband: a device to measure total daily energy expenditure in free-living type 2 diabetic individuals. Diabetes Care. 2005;28(1):225-7.
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130(6):461-70.
18. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab. 2005;288(2):E462-8.
19. Aas AM, Ohrvik J, Malmberg K, Ryden L, Birkeland KI. Insulin-induced weight gain and cardiovascular events in patients with type 2 diabetes. A report from the DIGAMI 2 study. Diabetes Obes Metab. 2009;11(4):323-9.
20. Kooy A, de Jager J, Lehert P, et al. Long-term effects of metformin on metabolism and microvascular and macrovascular disease in patients with type 2 diabetes mellitus. Arch Intern Med. 2009;169(6):616-25.
21. Larger E, Rufat P, Dubois-Laforgue D, Ledoux S. Insulin therapy does not itself induce weight gain in patients with type 2 diabetes. Diabetes Care. 2001;24(10):1849-50.
22. Wolfe RR. Effects of insulin on muscle tissue. Curr Opin Clin Nutr Metab Care. 2000;3(1):67-71.
23. Kuk JL, Church TS, Blair SN, Ross R. Does measurement site for visceral and abdominal subcutaneous adipose tissue alter associations with the metabolic syndrome? Diabetes Care. 2006;29(3):679-84.
24. Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. Diabetes. 2004;53(8):2087-94.
25. Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation. 2007;116(1):39-48.
26. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30(5):1212-8.
27. Kolak M, Westerbacka J, Velagapudi VR, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. Diabetes. 2007;56(8):1960-8.
28. Juurinen L, Tiikkainen M, Hakkinen AM, Hakkarainen A, Yki-Jarvinen H. Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes. Am J Physiol Endocrinol Metab. 2007;292(3):E829-35.

29. Gastaldelli A, Cusi K, Pettiti M, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology. 2007;133(2):496-506.

30. Targher G, Arcaro G. Non-alcoholic fatty liver disease and increased risk of cardiovascular disease. Atherosclerosis. 2007;191(2):235-40.

31. Kim HC, Nam CM, Jee SH, Han KH, Oh DK, Suh I. Normal serum aminotransferase concentration and risk of mortality from liver diseases: prospective cohort study. BMJ. 2004;328(7446):983.

32. Tomaszewski M, Charchar FJ, Maric C, et al. Glomerular hyperfiltration: a new marker of metabolic risk 1. Kidney Int. 2007;71(8):816-21.

33. Koenig W, Sund M, Frohlich M, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation. 1999;99(2):237-42.

34. Engeli S, Feldpausch M, Gorzelniak K, et al. Association between adiponectin and mediators of inflammation in obese women. Diabetes. 2003;52:942-7.

35. Reilly MP, Iqbal N, Schutta M, et al. Plasma leptin levels are associated with coronary atherosclerosis in type 2 diabetes. J Clin Endocrinol Metab. 2004;89:3872-8.

36. Mora S, Lee IM, Buring JE, Ridker PM. Association of physical activity and body mass index with novel and traditional cardiovascular biomarkers in women. JAMA. 2006;295:1412-9.

Netherlands
The Journal of Medicine

CASE REPORT

A rare cause of abdominal pain: eosinophilic gastroenteritis

N.A.F. Verheijden*, S.A.M. Ennecker-Jans

Amphia Hospital, 4818CK Breda, *corresponding author: tel.: +31 (0)76-595 15 55, +31 (0)76-595 11 24, e-mail: NVerheijden@amphia.nl

ABSTRACT

Eosinophilic gastroenteritis is a disease that is characterised by an eosinophil-driven inflammation of the digestive tract, presenting with non-specific symptoms, including abdominal pain, nausea, and diarrhoea. The diagnosis is established by histopathological analysis revealing eosinophilic infiltration of the lamina propria. The disease is relatively rare but a proper diagnosis is important, since specific treatment may limit the disease severity and progression.

KEYWORDS

Eosinophilic gastroenteritis, abdominal pain, eosinophilic infiltration

What was known on this topic?
Eosinophilic gastroenteritis is a blood disorder, which damages the mucosa of the gastrointestinal tract. Symptoms are related to the layer of bowel mucosa involved. Peripheral eosinophilic counts and serum IgE should be performed in patients with nonspecific abdominal complaints. When elevated, endoscopic investigation of the upper gastrointestinal tract should be performed and biopsies of both abnormal and normal mucosa should be taken.

What does this add?
Symptoms of EG may mimic irritable bowel disease. Proper treatment can prevent further mucosal damage and progression to severe malabsorption and malnutrition. Therefore, it is important to recognise EG. Several forms of treatment are described, including diet restriction and treatment with oral corticosteroids. There have been no prospective, randomised therapeutic clinical trials. Thus, treatment is empiric and based upon the severity of clinical symptoms.

INTRODUCTION

Eosinophilic gastroenteritis (EG) is a rare condition, first described in 1937 by Kaijser *et al*. It is defined as a disorder primarily affecting the gastrointestinal tract with eosinophil-rich inflammation, in the absence of known causes of eosinophilia (e.g. drug reactions, parasitic infections or malignancy).[1] Three different forms of EG can be distinguished: mucosal disease, muscle layer disease and subserosal disease.
The symptoms of EG are related to the layer involved. Mucosal disease is the most common form and presents with nonspecific symptoms such as abdominal pain, nausea, vomiting, diarrhoea or malabsorption. The second form, muscle layer disease, is a more serious form that presents with symptoms due to intestinal obstruction. The third form, subserosal disease, is uncommon and presents with ascites. Incidence in the USA is approximately 2.5 per 100,000 adults.[2,3] EG has been diagnosed with increasing frequency in recent years.[4-6] This is most likely due to increased awareness. Nevertheless, we underline the importance of recognising EG, since proper treatment can prevent further mucosal damage and progress to severe malabsorption and malnutrition.

CASE REPORT

A 61-year-old man, with a history of myocardial infarction, atrial fibrillation, and asthmatic rhinitis, presented at the Emergency Room with diffuse abdominal pain. He did not complain of nausea, vomiting or diarrhoea. The pain started four days ago. His stools did not change and he did not have fever. A year ago he had an episode with the same symptoms. Gastroscopic investigation did not show any abnormalities at that time. It was performed,

however, a few weeks after spontaneous disappearance of his complaints.

Laboratory investigations showed elevated peripheral eosinophil counts and an elevated serum IgE (*table 1*). Further imaging did not show any pathology, especially no signs of ischaemia. Faecal examination was negative.

The patient was admitted. Since the abdominal pain persisted, we performed gastroscopic investigation. This showed polypoid gastric mucosa with erythema. Furthermore erosions of both gastric and duodenal mucosa were seen(*figure 1*).

Biopsies were taken from the abnormal appearing mucosa. These biopsies revealed distinct eosinophilic infiltration (*figure 2*). Therefore the diagnosis of eosinophilic gastroenteritis was confirmed.

Because of the serious symptoms we immediately started treatment with prednisone 20 mg for two weeks, before performing skin prick tests. There is some evidence that avoidance of food allergens can improve disease activity. The symptoms declined rapidly after starting treatment. Moreover, there was a remarkable decline in the peripheral eosinophil counts. Prednisone was tapered during six weeks. Skin prick tests were performed afterwards, and were negative.

Table 1. *Laboratory findings in our patient suffering from eosinophilic gastroenteritis*

Laboratory findings	Normal	At diagnosis	6 weeks after treatment with Prednisone
Peripheral eosinophil counts	0.5	18.04	0.38
Serum IgE	<100	2238	507

Figure 1. *Macroscopic picture of the affected duodenal mucosa, showing erosions and duodenal folds*

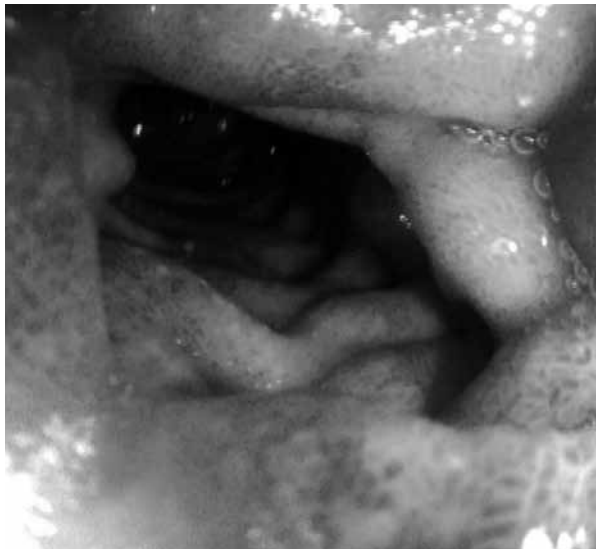
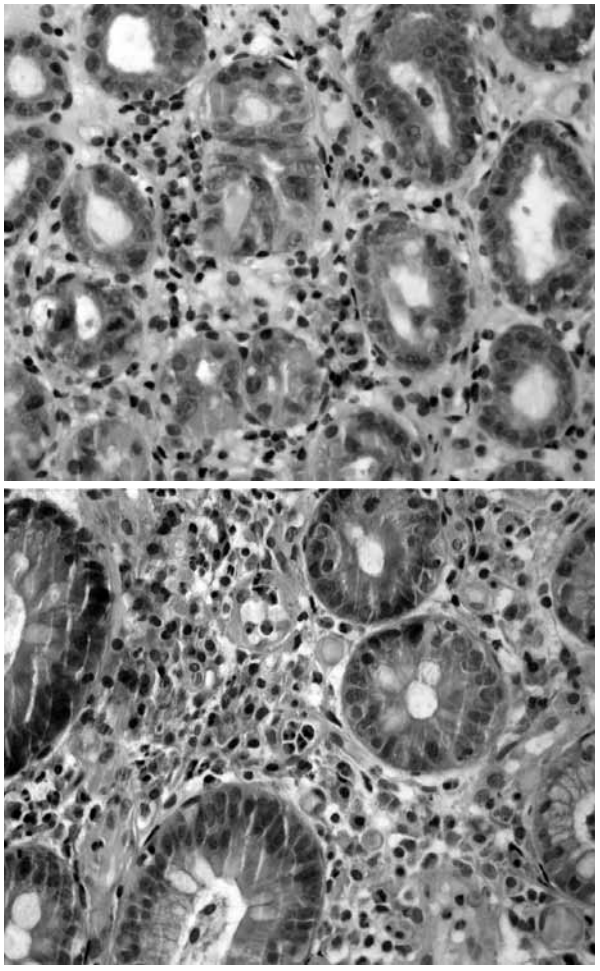


Figure 2. *Biopsies of gastric mucosa showing prominent eosinophilic infiltration of the lamina propria, consisting of >20 eosinophilic granulocytes per high power field*



DISCUSSION

Diagnosis

Since EG presents with nonspecific symptoms, it is not easy to diagnose and may be confused with irritable bowel disease. Because treatment can prevent further mucosal damage, it is important to recognise EG. Our patient had suffered the same symptoms previously, but investigations at that time did not reveal a diagnosis. History and laboratory evaluation of our patient showed several features of EG. For example, the patient had a history of asthmatic rhinitis and a previous episode of abdominal symptoms. Besides, peripheral eosinophil counts were elevated. The sensitivity of this test is about 80%. In one study 23% of patients lacked peripheral eosinophilia. Furthermore, serum IgE levels were elevated.

Patients with EG may suffer from malabsorption with hypoalbuminaemia or anaemia. Up to 50% of patients had a history of food allergy or intolerance.[7,8] To confirm

diagnosis, a biopsy of gastric or duodenal mucosa is necessary. Endoscopic appearance in eosinophilic gastroenteritis is nonspecific and includes mucosal folds, hyperaemia or ulceration. Because of mucosal sparing, it is recommended to obtain at least six biopsy specimens from both normal and abnormal areas of the bowel. Histopathology will reveal eosinophilic infiltration of the lamina propria. Diagnostic criteria vary from >20 to >50 eosinophils per high power field.[8,9]

Treatment

The literature shows scarce data on the proper treatment in EG. According to different studies, the first step in treating EG is diet restriction.[10] It is recommended to perform skin prick testing to identify any food allergies. If present, patients should start a restricted diet. Referral to a dietician will make this approach more successful.

Although there is an evident association between food allergy and EG, results of elimination diets are often poor. When improvement of symptoms with diet restriction is poor or not feasible, the following step in treatment of EG is the use of steroids. An effective relief of symptoms usually occurs within two weeks. Several studies report good results with steroids in dosages from 20 to 40 mg/day, for six to eight weeks.[11-14]

Successful treatment with budesonide has been described in certain studies as well. It inhibits both eosinophilic activation and survival.[1,14-16] The main advantage of budesonide is its high metabolism, and therefore a lower risk of side effects such as adrenal suppression.[14] Other less common drugs have also been found to be effective in the treatment of EG. These therapies focus at the assumed allergy component in the pathogenesis of EG. Cromolyn prevents the release of mast cell mediators and eosinophil mediator release.[17] A positive response with oral cromolyn was seen in some[18,19] but not all case reports.[8] There was a variation in results with Montelukast, an antagonist of the leukotriene receptor Cys-LT1. Activation of this receptor results in contraction of smooth muscle, oedema and eosinophil migration. Montelukast was effective and steroid sparing in some reported cases[20-22] but less successful in others.[23] Recently a review in this journal described promising effects of several new therapies, including imatinib, in hypereosinophilic syndromes.[24] A single case report showed a rapid decline of symptoms with imatinib in a patient suffering from chronic eosinophilic leukaemia with gastrointestinal involvement.[25] So several forms of treatment are described. There have been no prospective, randomised therapeutic clinical trials. Thus, treatment is empiric and based upon the severity of clinical symptoms. The subsequent course is variable. Patients may experience periodic flares months to years after the first episode.

REFERENCES

1. Rothenberg ME. Eosinophilic gastrointestinal disorders (EGID). J Allergy Clin Immunol. 2004;113(1):11-28.
2. Guajardo JR, Plotnick LM, Fende JM, Collins MH, Putnam PE, Rothenberg ME. Eosinophil-associated gastrointestinal disorders: a world-wide-web based registry. J Pediatr. 2002;141(4):576-81.
3. Noel RJ, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. N Engl J Med. 2004;351(9):940-1.
4. Chang F, Anderson S. Clinical and pathological features of eosinophilic oesophagitis: a review. Pathology. 2008;40(1):3-8.
5. Cherian S, Smith NM, Forbes DA. Rapidly increasing prevalence of eosinophilic oesophagitis in Western Australia. Arch Dis Child. 2006;91(12):1000-4.
6. Straumann A, Simon HU. Eosinophilic esophagitis: escalating epidemiology? J Allergy Clin Immunol. 2005;115(2):418-9.
7. Klein NC, Hargrove RL, Sleisenger MH, Jeffries GH. Eosinophilic gastroenteritis. Medicine (Baltimore). 1970;49(4):299-319.
8. Talley NJ, Shorter RG, Phillips SF, Zinsmeister AR. Eosinophilic gastroenteritis: a clinicopathological study of patients with disease of the mucosa, muscle layer, and subserosal tissues. Gut. 1990;31(1):54-8.
9. Baig MA, Qadir A, Rasheed J. A review of eosinophilic gastroenteritis. J Natl Med Assoc. 2006;98(10):1616-9.
10. Justinich C, Katz A, Gurbindo C, Lepage G, Chad Z, Bouthillier L, et al. Elemental diet improves steroid-dependent eosinophilic gastroenteritis and reverses growth failure. J Pediatr Gastroenterol Nutr. 1996;23(1):81-5.
11. Chen MJ, Chu CH, Lin SC, Shih SC, Wang TE. Eosinophilic gastroenteritis: clinical experience with 15 patients. World J Gastroenterol. 2003;9(12):2813-6.
12. Furuta GT, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, et al. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. Gastroenterology. 2007;133(4):1342-63.
13. Lee CM, Changchien CS, Chen PC, Lin DY, Sheen IS, Wang CS, et al. Eosinophilic gastroenteritis: 10 years experience. Am J Gastroenterol. 1993;88(1):70-4.
14. Tan AC, Kruimel JW, Naber TH. Eosinophilic gastroenteritis treated with non-enteric-coated budesonide tablets. Eur J Gastroenterol Hepatol. 2001;13(4):425-7.
15. Elsing C, Placke J, Gross-Weege W. Budesonide for the treatment of obstructive eosinophilic jejunitis. Z Gastroenterol. 2007;45(2):187-9.
16. Siewert E, Lammert F, Koppitz P, Schmidt T, Matern S. Eosinophilic gastroenteritis with severe protein-losing enteropathy: successful treatment with budesonide. Dig Liver Dis. 2006;38(1):55-9.
17. Sprenger RA, Arends JW, Poley JW, Kuipers EJ, ter Borg F. Eosinophilic oesophagitis: an enigmatic, emerging disease. Neth J Med. 2009;67(1):8-12.
18. Perez-Millan A, Martin-Lorente JL, Lopez-Morante A, Yuguero L, Saez-Royuela F. Subserosal eosinophilic gastroenteritis treated efficaciously with sodium cromoglycate. Dig Dis Sci. 1997;42(2):342-4.
19. Van Dellen RG, Lewis JC. Oral administration of cromolyn in a patient with protein-losing enteropathy, food allergy, and eosinophilic gastroenteritis. Mayo Clin Proc. 1994;69(5):441-4.
20. Neustrom MR, Friesen C. Treatment of eosinophilic gastroenteritis with montelukast. J Allergy Clin Immunol. 1999;104:506.
21. Quack I, Sellin L, Buchner NJ, Theegarten D, Rump LC, Henning BF. Eosinophilic gastroenteritis in a young girl--long term remission under Montelukast. BMC Gastroenterol. 2005;5:24.
22. Schwartz DA, Pardi DS, Murray JA. Use of montelukast as steroid-sparing agent for recurrent eosinophilic gastroenteritis. Dig Dis Sci. 2001;46(8):1787-90.
23. Daikh BE, Ryan CK, Schwartz RH. Montelukast reduces peripheral blood eosinophilia but not tissue eosinophilia or symptoms in a patient with eosinophilic gastroenteritis and esophageal stricture. Ann Allergy Asthma Immunol. 2003;90(1):23-7.
24. Antoniu SA. Novel therapies for hypereosinophilic syndromes. Neth J Med. 2010;68(1):304-10.
25. Pitini V, Sturniolo G, Cavallari V, Arrigo C. Rapid reversion of eosinophilic gastroenteritis associated with FIP1L1-PDGFRA fusion after targeted therapy with imatinib. Br J Haematol. 2006;132(2):123.

Netherlands
The Journal of Medicine

PHOTO QUIZ

A diffuse painful desquamating rash

N.F. Abraham[1*], S.E. Mercer[2], S. Pandey[2], V.J. LaBombardi[2], R.G. Phelps[1,2]

Departments of [1]Dermatology and [2]Division of Dermatopathology, Mount Sinai School of Medicine, New York, NY, United States of America, *corresponding author: tel.: +1 (212) 241-9148, fax: +1 (212) 241-7832, ninafaryl@gmail.com

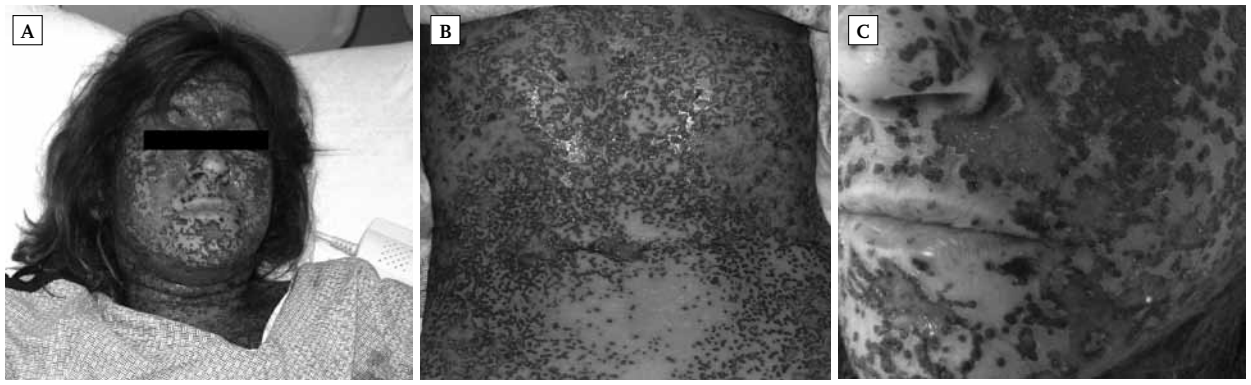
CASE REPORT

A 44-year-old woman was transferred to our hospital service for evaluation and treatment of a generalised, painful rash, with fever and lymphadenopathy present for ten days (*figure 1*). Prior to transfer, our patient spent a week at an outside hospital where she was treated with multiple antibiotics, including bactrim, vancomycin, nafcillin, and ceftazadine. She also had received intravenous solumedrol. Despite treatment, the patient's rash, initially localised to the face, rapidly progressed. On admission to our hospital, we performed a punch biopsy of skin from the patient's trunk and submitted it for frozen and permanent sections. Our patient's past medical history included severe atopic dermatitis treated with prednisone 10 mg daily and multiple MRSA infections of the soft tissue and lung.

WHAT IS YOUR DIAGNOSIS?

See page 374 for the answer to this photo quiz.

Figure 1. *Clinical appearance of the patient*



The lesions initially involved the face and consisted of erythematous vesicles and ulcers. B) The process rapidly spread to her trunk and proximal extremities and became confluent, resulting in large areas of desquamation. C) Representative lesions on the face demonstrated a mixture of discrete crusted ulcers and confluent desquamation.

PHOTO QUIZ

Just an open book?

A.M.F. Rutten[1*], D.H.C. Burger[2], G.A.M. van der Nat[1], H.M. Teijgeler[2]

Departments of [1]Intensive Care Medicine, [2]Surgery, St Elisabeth Hospital, Tilburg, the Netherlands,
[*]corresponding author: tel.: +31 (0)13-539 13 13, e-mail: a.rutten2@elisabeth.nl

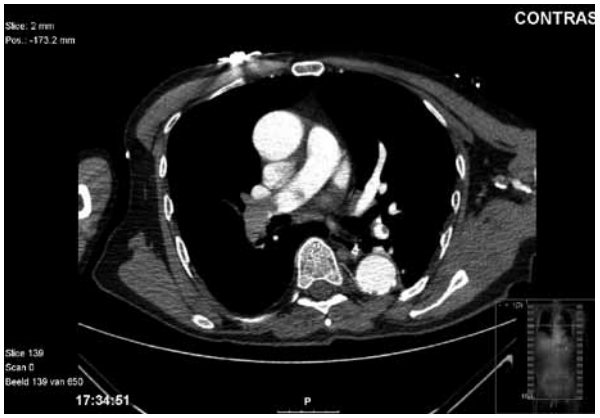
CASE REPORT

A 68-year-old man was admitted to our hospital with an open-book pelvic fracture. He was injured when he fell off a horse two days earlier. His medical history revealed hypertension. The day after admission he developed a left-sided hemiparalysis and aphasia. The diagnosis cerebrovascular accident (CVA) was suspected. A computed tomography of the brain showed a media infarction in the right frontotemporal regon and no haemorrhage. Treatment with thrombocyte aggregation inhibitors was started. No thrombolysis was given, because of the risk of haemorrhage from the pelvic region. The duplex of the carotid arteries showed no significant stenosis. In the following days he made a full neurological recovery. On day 13 of admission the patient had an acute drop in consciousness on the ward. The patient was unresponsive and hypoxic with a saturation of 73% but haemodynamically stable. His pupils were unresponsive and dilated for a short period. His right arm felt cold. He was intubated, after which a computed tomography of the brain showed no new CVA or haemorrhage. The computed tomography of the thorax and abdomen revealed filling defects in the left femoral vein, pulmonary arteries (*figure 1*), aorta descendens and renal arteries. There was little perfusion of the kidneys.

WHAT IS YOUR DIAGNOSIS?

See page 375 for the answer to this photo quiz.

Figure 1. *Filling defects in the pulmonary arteries*



Netherlands
The Journal of Medicine

PHOTO QUIZ

The electrocardiogram of a man found in the forest

M.K. Lam[1]*, L.S. Hofstra[2]

[1]University Medical Center Groningen, Groningen, the Netherlands, [2]Intensive Care Unit, Scheper Hospital, Emmen, the Netherlands, *corresponding author: tel.: +31 (0)6-234 38366, fax: +31 (0)59-169 12 34, e-mail: mingkai_lam@hotmail.com

CASE REPORT

A 65-year-old man left his home drunk and fell asleep somewhere in the forest nearby. He survived the cold and rainy night and was found by villagers the next morning. On arrival at the emergency department a blood pressure of 178/89 mmHg, regular heart rate of 58 beats/min and body temperature of 28.1°C were measured. Despite a somnolent impression, the patient scored a maximum Glasgow Coma Score.

Abnormal laboratory analyses were troponin-T 0.15 Ig/l (normal <0.1 Ig/l), glucose 2.4 mmol/l (normal 5 mmol/l) and ethanol 1.6 g/l (normal 0 g/l). Sodium, potassium and calcium were within normal ranges. Arterial blood gas showed respiratory acidosis: pH 7.25, P_{co2} 6.78 kPa, P_{o2} 14.76 kPa, HCO_3 22 mmol/l, and saturation 97%. *Figure 1* shows the electrocardiogram (ECG) during severe hypothermia. Patient was rewarmed using external and internal rewarming techniques on the intensive care unit and gained a temperature of 36.9 °C after approximately eight hours. A new ECG was taken (*figure 2*).

WHAT IS YOUR DIAGNOSIS?

See page 376 for the answer to this photo quiz.

Figure 1.

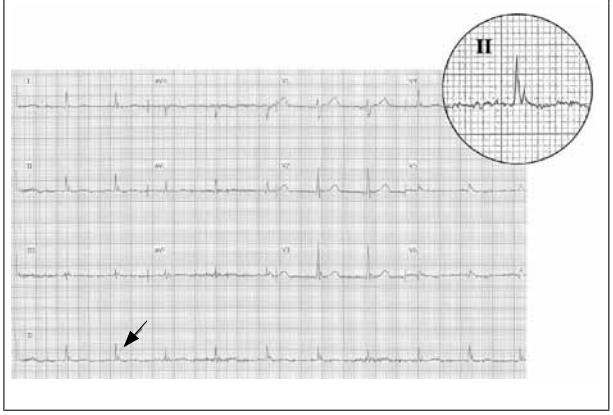
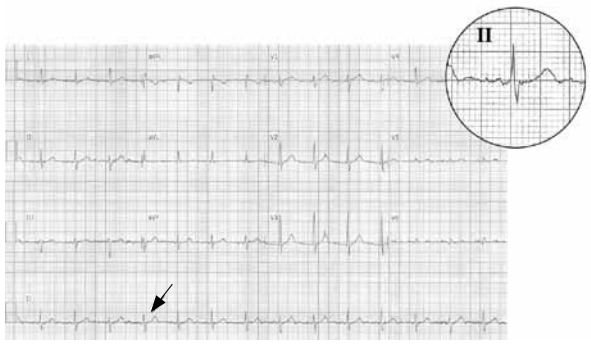


Figure 2.



Netherlands
The Journal of Medicine

PHOTO QUIZ

A 33-year-old man presenting with rectal ulceration and nephrotic syndrome

K. Boslooper[1], G.D. Laverman[2], H. van der Heide[3], A.T.M.G. Tiebosch[4], W.M.T. Janssen[1*]

[1]Departments of Internal Medicine, [3]Gastroenterology, [4]Pathology, Martini Hospital, Groningen, the Netherlands. [2]Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, Groningen, the Netherlands, *corresponding author: tel.: +31 (050)-524 58 70, fax: +31 (50)-524 58 89, e-mail: W.M.T.Janssen@mzh.nl

CASE REPORT

A previously healthy 33-year-old heterosexual male with rectal pain and bleeding and profuse nocturnal sweating was admitted to the gastroenterology department of our hospital. He appeared moderately ill with a normal temperature of 37.0 °C, a blood pressure of 120/70 mmHg and a regular pulse of 70 beats/min. Subtle periorbital oedema and a firm rectal mass, suspicious for carcinoma, were the only remarkable clinical findings. Relevant laboratory tests were as follows: erythrocyte sedimentation rate 75 mm/hour (<15), haemoglobin 7.7 mmol/l (8.7 to 10.9), creatinine 59 µmol/l (70 to 110), total protein 58 g/l (60 to 80), albumin 21 g/l (34 to 48), cholesterol 6.60 mmol/l (3.0 to 6.4) and urine was dipstick positive for protein. A suspected nephrotic syndrome was confirmed by a 24-hour urine collection with 19 grams of protein and a selectivity index of 12, indicating selective proteinuria. A kidney biopsy was performed. Light microscopy revealed sporadic irregularity of the glomerular basement membrane consistent with spikes (*figure 1*). Furthermore, an abundant accumulation of silver positive granules in the tubular cells were seen. Immunofluorescence showed a diffuse, weak, deposition of IgG in a granular pattern and to a lesser extent of IgM and C3 (*figure 2*). According to the Churg classification, a mild membranous glomerulopathy stage I was present.

Meanwhile colonoscopy had been performed and an ulcer was seen at 5 cm, of which the biopsies showed granulomatous inflammation (*figure 3*). Computer tomography of the abdomen showed, in addition to a localised rectal lesion, perirectal, presacral and iliacal lymphadenopathy.

WHAT IS YOUR DIAGNOSIS?

See page 377 for the answer to this photo quiz.

Figure 1. *Renal biopsy showing one glomerulus with sporadic irregularity of the glomerular basement membrane consistent with spikes and accumulation of silver positive granules in the tubular cells (haematoxylin-eosin staining, original magnification x 40)*

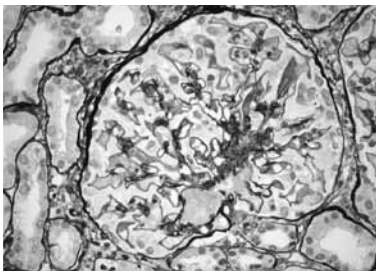


Figure 2. *Immunofluorescence staining showing a diffuse, weak, deposition of IgG in a granular pattern (original magnification x 40)*

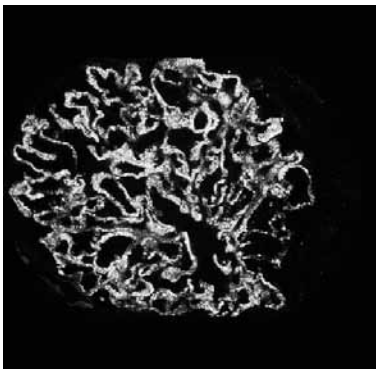
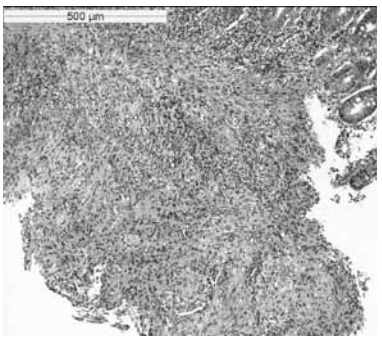


Figure 3. *Rectal biopsy showing chronic partly granulomatous inflammation with ulceration and spirochetosis (haematoxylin-eosin staining, original magnification x 20)*



ANSWER TO PHOTO QUIZ (PAGE 370)

A DIFFUSE PAINFUL DESQUAMATING RASH

DIAGNOSIS

Our patient's history of antibiotic use and a rapidly progressive rash with large eroded areas prompted us to submit tissue for frozen section to rule out Stevens-Johnson syndrome. Examination of the frozen section slides revealed massive epidermal necrosis and multinucleated keratinocytes with nuclear chromatin margination and moulding (*figure 2A and B*) consistent with a diagnosis of eczema herpeticum (EH). Treatment with intravenous acyclovir was initiated and the patient dramatically improved over the course of the next week. She was also treated with intravenous clindamycin for any potential bacterial superinfection and topically with emollients and vaseline-impregnated gauze. Our initial diagnosis was subsequently confirmed by direct fluorescent antibody, which was positive for herpes simplex virus type 1 *(figure 2C)*.

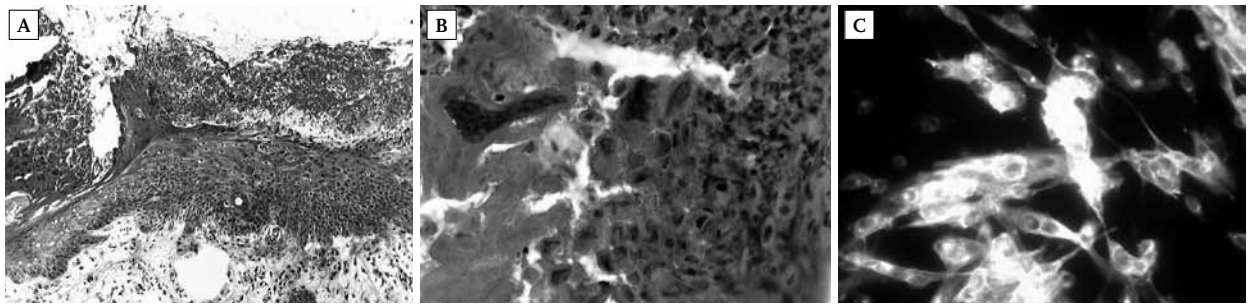
Patients with eczematous skin disease or atopic dermatitis are prone to the development of viral skin infections. Among the most commonly recognised is eczema herpeticum. EH is caused by dissemination of herpes simplex virus, typically herpes simplex type 1.[1] It often presents in the first three decades of life. According to a study of 100 EH patients by Wollenberg *et al.,* 20% of the cases were related to primary infection, 26% were caused by secondary infection and the remaining 54 cases could not be characterised.[2] Rates of recurrence have been reported to be as low as 16% to as high as 50%.[1,2]

Clinically, the disease is characterised by monomorphic, dome-shaped vesicles that develop into punched-out, crusted erosions. The eruption disseminates over 7-10 days most commonly affecting the head, neck and upper body. Patients may also experience fever, malaise and lymphadenopathy. Feared complications include keratoconjunctivitis, viraemia, meningitis, and encephalitis.[1,3] The diagnosis of EH is based on the clinical presentation and a high clinical suspicion should prompt immediate treatment with IV acyclovir or oral valtrex for seven days.[4] The diagnosis should be confirmed by viral detection. Methods of detection include Tzank test, viral culture, light or electron microscopy, direct fluorescent antibody and PCR.[3] This case highlights the importance of early recognition of eczema herpeticum and the utility of submitting tissue for frozen section to facilitate a rapid diagnosis.

REFERENCES

1. Beck LA, Boguniewicz M, Hata T, Schneider LC, Hanifin J, Gallo R, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. J Allergy Clin Immunol. 2009;124(2):260-9, 269.e1-7.
2. Wollenberg A, Zoch C, Wetzel S, Plewig G, Przybilla B. Predisposing factors and clinical features of eczema herpeticum: a retrospective analysis of 100 cases. J Am Acad Dermatol. 2003;49(2):198-205.
3. Bussmann C, Peng WM, Bieber T, Novak N. Molecular pathogenesis and clinical implications of eczema herpeticum. Expert Rev Mol Med. 2008;14;10:e21.
4. Wollenberg A, Wetzel S, Burgdorf WH, Haas J. Viral infections in atopic dermatitis: pathogenic aspects and clinical management. J Allergy Clin Immunol. 2003;112(4):667-74.

Figure 2. *Histopathological features of a frozen section of a punch biopsy from the flank*



Scanning magnification revealed massive necrosis of the epidermis (H&E, 100x). B) Higher power perspective demonstrated nuclear changes diagnostic of herpes infection, including margination of chromatin, multinucleation of keratinocytes, and moulding of keratinocyte nuclei (H&E, 400x). C) Direct fluorescence assay with a monoclonal antibody labelled with fluorescein isothyocyanate confirmed the diagnosis of eczema herpeticum and identified the virus subtype as HSV-1 (400x). H&E, haematoxylin and eosin.

ANSWER TO PHOTO QUIZ (PAGE 371)

JUST AN OPEN BOOK?

DIAGNOSIS

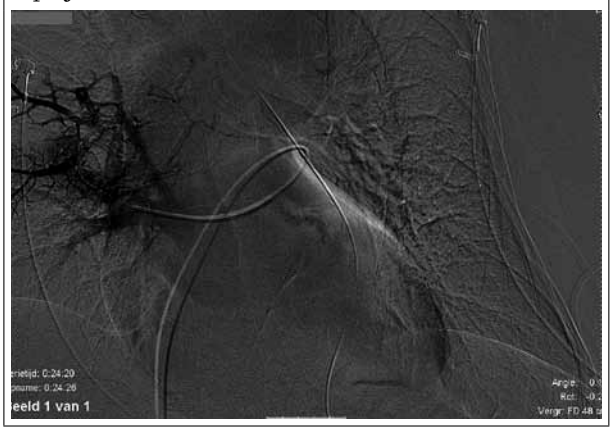
The diagnosis of deep venous thrombosis with multiple embolisms in the pulmonary and renal arteries and aorta descendens was made. A patent foramen ovale was suspected. Echocardiography revealed high right ventricle pressures and a leftward shift of the septum, a patent foramen ovale was suspected but not proven. Thrombolysis was given because the patient had an increasing hypoxaemia despite maximal oxygen supply and ventilatory support. This improved the perfusion of his arm, but the saturation difficulties remained. Although haemodynamic support through fluid therapy and vasopressors was started the haemodynamic situation deteriorated. A fragmentation of the thrombus in the pulmonary arteries through femoral vein catherisation was performed. The diagnosis of patent foramen ovale was confirmed when the catheter for fragmentation reached the left atrium (*figure 2*). After the procedure the oxygen saturation improved and inotropic support was reduced. Because of the renal failure the patient required continuous haemofiltration for seven days.

Unfortunately after cessation of sedation the patient remained unresponsive. A computed tomography of the brain was made which showed extensive ischaemic regions in the left and right hemisphere, probably due to multiple embolisms to the brain. This was not considered to be compatible with a reasonable quality of life.

The family was informed after which the joint decision was made to cease respiratory support. The patient died shortly after.

A patent foramen ovale is a common condition and has a prevalence of 15 to 25%.[1-3] This may be a case any doctor can encounter in his career and thus should be aware of. In retrospect, in the work up of the initial stroke a foramen ovale should have been suspected, but in this case it was not. The echocardiogram could not determine the foramen ovale, even when the patient had clinical signs of a considerable shunt through the foramen ovale. Early recognition in patients with arterial thrombosis (e.g. a stroke) is essential to prevent further possibly devastating clinical deterioration.

Figure 2. *Catheter for fragmentation reached though the left atrium the pulmonary arteries and an angiography is performed*



REFERENCES

1. Di Tullio MR, Sacco RL, Sciacca RR, Jin Z, Homma S. Patent foramen ovale and the risk of ischemic stroke in a multiethnic population. J Am Coll Cardiol. 2007;49:797-802.
2. Hagen PT, Scholz DG, Edwards WD. Incidence and size of patent foramen ovale during the first 10 decades of life: an autopsy study of 965 normal hearts. Mayo Clin Proc. 1984;59:17-20.
3. Meissner I, Whisnant JP, Khanderia BK, et al. Prevalence of potential risk factors for stroke assessed by transesophageal echocardiography and carotid ultrasonography: the SPARC study Stroke Prevention: Assessment of Risk in a Community. Mayo Clin Proc. 1999;74:862-69.

ANSWER TO PHOTO QUIZ (PAGE 372)

THE ELECTROCARDIOGRAM OF A MAN FOUND IN THE FOREST

DIAGNOSIS

The ECG shows Osborn waves due to severe hypothermia. The term Osborn waves refers to a deflection immediately following the QRS complex and in the same direction as the R wave. Osborn waves are mainly observed in hypothermic conditions.[1] Other conditions such as hypercalcaemia and brain injuries, including haemorrhage, have also been reported to cause Osborn waves. Osborn waves are attributed to a disturbance in 4-aminopyridine sensitive transient outward current between epicardium and endocardium. This imbalance produces a transmural voltage gradient during ventricular action that manifests as a Osborn wave on the ECG.[2]

The clinical significance of Osborn waves is unclear. Osborn waves observed in patients who have suffered from hypercalcaemia and neurological disorders are usually not accompanied by arrhythmias. Due to the lack of evidence, it is unclear whether Osborn waves can be regarded as a predictive sign for ventricular arrhythmias during hypothermic conditions. Still, in most cases the appearance of Osborn waves on the ECG is accompanied with a serious underlying disorder and therefore requires further diagnostic investigation and treatment.

REFERENCES

1. Osborn JJ. Experimental hypothermia; respiratory and blood pH changes in relation to cardiac function. Am J Physiol. 1953;175(3):389-98.
2. van Noord T, Ligtenberg JJ, Zijlstra JG. Diagnostic image (209). A man after submersion in water. Ned Tijdschr Geneeskd. 2004;148(40):1966

DIAGNOSIS

The diagnosis of nephrotic syndrome associated with secondary syphilis was made.

Serological tests were positive, while no other sexually transmitted diseases (HIV, lymphogranuloma venereum) were found (*table 1*). A causal relationship was strongly supported by complete resolution of proteinuria within one week after initiating benzylpenicillin.

Before the penicillin-era renal involvement was a well-recognised complication of syphilis, with an incidence varying from 0.3 to 8%.[1] Nephropathy usually occurs in the secondary stage, four to ten weeks after the initial chancre.[4] Since syphilis became a well-treatable disease, advanced stages of syphilis – and thus associated renal involvement – have become rare. However, since 2001 a rising incidence of syphilis has been reported in Western countries, often accompanied with HIV co-infection.

Proteinuria is usually found and may vary from transient mild albuminuria to a fulminant nephrotic syndrome.[1] Presentation with acute nephritic syndrome, acute renal failure[1] or salt-losing nephropathy[2] are less commonly reported. Membranous nephropathy as histopathological substrate is frequently reported, but minimal change nephropathy, rapidly progressive glomerulonephritis with formation of crescents[3] or interstitial nephritis[2] may also occur in association with syphilis. Immunofluorescence studies show granular deposition of immunoglobulins, mainly IgG, and C3, along the basement membrane and at electron microscopy subepithelial electron dense deposits are seen.[1] These findings suggest syphilis-related nephropathy is caused by immune-complex deposition. This hypothesis is supported by the identification of elutable anti-treponemal antibodies or a treponemal antigen in the glomeruli of patients with syphilis-related kidney disease.[3]

Diagnosis of a syphilis-related nephropathy may be difficult and requires thorough questioning and physical examination. Diagnostic criteria include a recent infection, co-existence of kidney disease with a late primary or secondary stage, positive serological tests, remission after initiating penicillin therapy and exclusion of other potential causes such as other underlying diseases or co-medication.[4]

Table 1. *Serology tests of patient with rectal ulceration and nephrotic syndrome*

	At time of diagnosis	After 2 weeks	After 6 months
TPPA	1250	2500	320
FTA-abs Ig	Positive	Positive	Weakly positive
VDRL	16	16	<1
Anti-HIV-Ig	Negative		Negative
Chlamydia IgM	800	400	
Chlamydia IgG	400	200	

TPPA = Treponema pallidum antibodies; FTA-abs Ig = fluorescent treponemal antibody-absorption immunoglobulin; VDRL = Venereal Disease Research Laboratory.

CONCLUSION

Sexually transmitted diseases, including syphilis, remain an important cause of secondary forms of the nephrotic syndrome. Recognition of syphilis-related nephropathy is important, as complete recovery can simply be achieved by initiating antibiotic therapy.

REFERENCES

1. Hunte W, al-Ghraoui F, Cohen RJ. Secondary syphilis and the nephrotic syndrome. J Am Soc Nephrol. 1993;3:1351-5.
2. Chen YC, Lee N, Chang CT, Wu MS. Salt loss and hyponatraemia in a patient with syphilitic nephritis. Nephrol Dial Transplant. 2005;20:1248-50.
3. Walker PD, Deeves EC, Sahba G, Wallin JD, O'Neill WM Jr. Rapidly progressive glomerulonephritis in a patient with syphilis. Identification of antitreponemal antibody and treponemal antigen in renal tissue. Am J Med. 1984;76:1106-12.
4. Hruby Z, Kuzniar J, Rabczynski J, Bogucki J, Steciwko A, Weyde W. The variety of clinical and histopathologic presentations of glomerulonephritis associated with latent syphilis. Int Urol Nephrol. 1992;24:541-7.

LETTER TO THE EDITOR

Giant atria in a patient with systemic lupus erythematosus

B. Weijs[1]*, P. van Paassen[2], S.C.A.M. Bekkers[1]

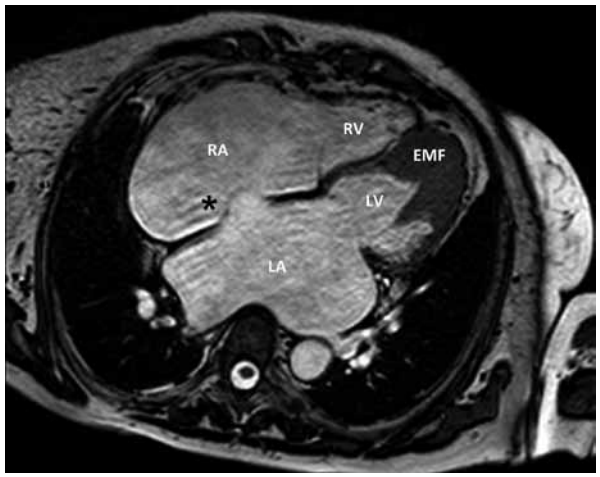
Departments of [1]Cardiology, [2]Clinical and Experimental Immunology, Department of Internal Medicine, CARIM, Maastricht University Medical Centre, the Netherlands, *corresponding author: tel.: +31 (0)43-387 51 19, fax: +31 (0)43-387 51 04, e-mail: b.weijs@mumc.nl

Cardiac disease is common among patients with systemic lupus erythematosus (SLE) and includes endomyocardial fibrosis (EMF).[1] However, the far-reaching consequences due to diastolic dysfunction are relatively unknown. Early cardiac evaluation and subsequent treatment in SLE patients may improve outcome.[2-4]

A 56-year-old Caucasian woman with SLE presented with atrial fibrillation (AF) and symptoms of progressive heart failure. Her chest X-ray revealed severe cardiomegaly and an obtuse carinal angle. The electrocardiogram showed AF and inverted T waves in the precordial leads. Cardiovascular magnetic resonance imaging (*figure 1*) revealed massive biatrial enlargement with intra-atrial septal aneurysm, obliteration of the apex, decreased LV ejection fraction (0.37) and severe tricuspid regurgitation. The late gadolinium enhanced images were non-diagnostic due to arrhythmia artifacts.

Long-term treatment included conventional heart failure therapy, anticoagulation and adequate rate control for AF. Because of refractory congestive heart failure symptoms, she was operated on. EMF was confirmed and a tricuspid valvuloplasty, atrial reduction and manual dissection of the obliterated ventricle was performed. Afterwards her cardiac condition stabilised for three years. Unexpectedly, she recently died because of a complicated infection as a consequence of long-term immunosuppressive therapy.

Figure 1. *MRI scan*



LA = left atrium; RA = right atrium; LV = left ventricle; RV = right ventricle; EMF = endomyocardial fibrosis; (*) = intra-atrial septal aneurysm.

REFERENCES

1. Doria A, Laccarino L, Sarzi-Puttini P, Atzeni F, Turriel M, Petri M. Cardiac involvement in systemic lupus erythematosus. Lupus. 2005;14(9):683-6.
2. van der Laan-Baalbergen NE, Mollema SA, Kritikos H, Schoe A, Huizinga TW, Bax JJ, et al. Heart failure as presenting manifestation of cardiac involvement in systemic lupus erythematosus. Neth J Med. 2009;67(9):295-301.
3. Buss SJ, Wolf D, Korosoglou G, et al. Myocardial left ventricular dysfunction in patients with systemic lupus erythematosus: new insights from tissue Doppler and strain imaging. J Rheumatol. 2010;37(1):79-86.
4. Sherer Y, Levi Y, Schoenfeld Y. Marked improvement of severe cardiac dysfunction after one course of intravenous immunoglobulin in a patient with systemic lupus erythematosus. Clin Rheumatol. 1999;18:238-40.

INFORMATION FOR AUTHORS

Aims and scope

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

Manuscripts

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

Language

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

Submission

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

Preparation of manuscripts

Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process, number the lines in the margin and the pages.

Subheadings should not exceed 55 characters, including spaces.

Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for generic names of substances and materials.

A *Covering letter* should accompany the manuscript, identifying the corresponding person (with the address, telephone number, fax number and e-mail address). Conflicts of interest, commercial affiliations, consultations, stock or equity interests should be specified. In the letter one to three sentences should be dedicated to what this study adds. The letter should make it clear that the final manuscript has been seen and approved by all authors. All authors should sign the letter. The letter should either be submitted through http://mc.manuscriptcentral.com/nethjmed or faxed to the editorial office (+31 (0)20-691 96 58).

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

The *Title page* should include authors' names, degrees, academic addresses, correspondence address, including telephone number, fax number, e-mail address and grant support. Also the contribution of each author should be specified.

The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

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

Keywords: Include three to five keywords in alphabetical order.

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

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

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

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

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

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

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

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med. 2001;59:184-95.
2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

Please note that all authors should be listed when six or less; when seven or more, list only the first three and add et al. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against the reference list after your manuscript has been revised.
The use of bibliographic software programmes that are designed to generate reference lists such as Reference Manager© or Endnote© is highly encouraged. Authors can use the predefined output 'Vancouver' style from these programmes.

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

Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors. *Legends for figures* should be typed, with double spacing, on a separate page.

Case reports
Case reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in *the Netherlands Journal of Medicine*. Neth J Med. 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references. In addition, we require that authors of case reports answer the following two questions (Neth J Med. 2008;66(7):289-90): 1) What was known on this topic? and 2) What does this add? The answers will appear in a separate box in the text.

Mini reviews
Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material which has already been published in a previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The text should not exceed 2500 words.

Letters to the editor (correspondence)
Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please use SI units for measurements and provide the references conform the Vancouver style (N Engl J Med. 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the Journal, the referred article should be quoted in the list of references.

Photo quiz
A photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

Book reviews
The editorial board will consider articles reviewing books.

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

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

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