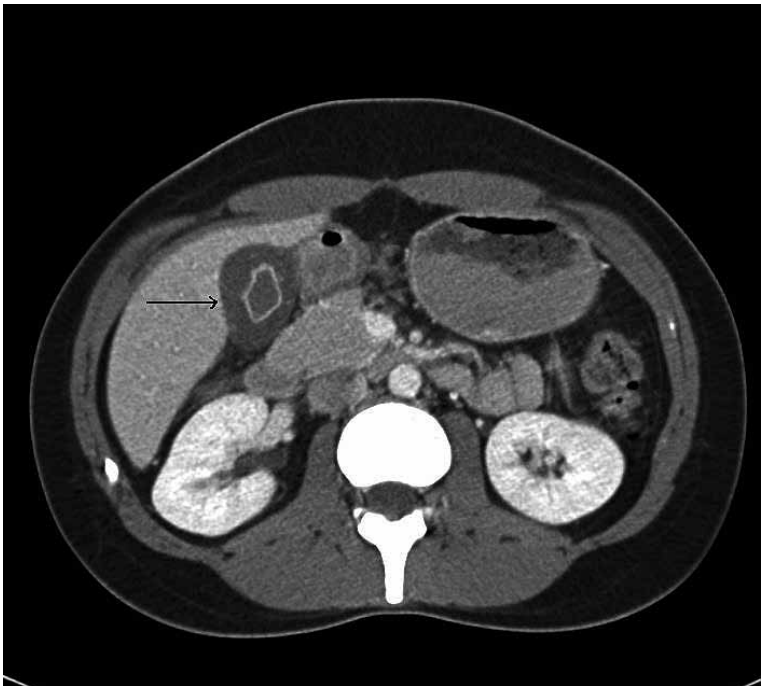
Netherlands
The Journal of Medicine
PUBLISHED IN COLLABORATION WITH THE NETHERLANDS ASSOCIATION OF INTERNAL MEDICINE



Acute abdomen and liver enzyme abnormalities: what is your diagnosis?

June 2010, vol. 68. No. 6, 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

Netherlands
The Journal of Medicine

EDITORIAL

Abundance of research talent in internal medicine

M. Levi
Department of Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, m.m.levi@amc.uva.nl

Internal Medicine is a broad medical speciality at the centre of clinical medicine and traditionally has strong ties with both fundamental and clinical research. Indeed, many of the current great advances in biomedical research, including molecular genetics and imaging technology, are applicable to Internal Medicine and the step from bench to bedside and vice versa is made ever faster. A recent survey of published research in Internal Medicine in the Netherlands showed the relatively strong position of the field as compared with other disciplines in medicine and confirmed the solid tradition to connect medicine to science in Internal Medicine.[1] To be able to maintain this situation, it is of utmost importance that the next generation of internists is equally attracted to research and successful alike. Although many of us have the impression that many young specialists in Internal Medicine or residents in training for internist are indeed eager to combine specialised medicine with research, there is no formal inventory to check the number of young individuals in Internal Medicine that are successful in pursuing a research career and a relative comparison with other research areas within and outside medicine is not available. One way of looking at this is to analyse the number of granted fellowships within the Incentives Scheme for Innovational Research ('Vernieuwingsimpuls') that was started about a decade ago by the Netherlands Organisation for Scientific Research (NWO), in collaboration with the Royal Netherlands Academy for Science (KNAW) and the Dutch Ministry of Education and Science. The aim of this programme is to '...promote innovation in the field of academic research. The scheme is directed at providing encouragement for individual postdoctoral researchers at various stages of their careers. Since it is vital to the universities that talented researchers should enter the profession and gain promotion within it, two main purposes of the Innovational Research Incentives Scheme are to provide the scope for adventurous, talented and pioneering researchers to conduct creative research of their own choice and to encourage them to make a permanent career of academic research.'[2] The fellowships within this scheme enable the selected young researchers to develop their own innovative lines of research and the programme encompasses all research areas at large. The scheme consists of three types of fellowships, each directed at a different stage in researchers' academic careers. Veni grants (€ 250,000 for three years) are for excellent researchers who have recently obtained their doctorates and who have already demonstrated an outstanding talent for academic research. Vidi grants (€ 800,000 for five years) are for excellent researchers who have already been active in postdoctoral research for some years, thereby demonstrating the ability to generate original ideas and translate these hypotheses into research successes. Vici grants are for excellent senior researchers who have shown that they have the ability to successfully develop their own innovative lines of research and to act as coaches for young researchers. A Vici grant is typically € 1,500,000 for a period of five years. A recent evaluation of the programme has underscored its success in maintaining and fostering research talent in the Netherlands.[3]

An analysis of successful applications for Veni, Vidi and Vici grants in the last four years is shown in *table 1*. About 20 to 25% of the total number of granted fellowships are awarded within the discipline of biomedical science. Remarkably, more than half of these fellowships are granted in the area of Internal Medicine. Thereby, Internal Medicine wins about 10% of the total number of fellowships for talented researchers across all research areas in the Netherlands. Interestingly, this distribution is true for all three types of grants. *Figure 1* shows the subdisciplines of Internal Medicine in which successful young researchers can be found. Cardiovascular research and oncology are traditionally strong research fields in the Netherlands but also infectious diseases and immunology harbour relatively large numbers of fellowship awardees. *Table 2* shows that despite measures to increase the number of female researchers, the majority of Veni grant winners are still male. This is even more salient in Internal Medicine, which is remarkable since for many years the numbers of women and men in training for the specialism

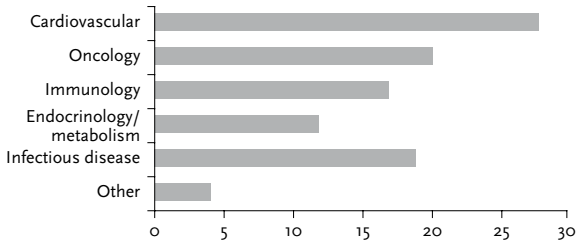
Table 1. *Grants in the Innovational Research Programme ('Vernieuwingsimpuls') by the Netherlands Organisation for Scientific Research 2005-2009*

	Veni programme	Vidi programme	Vici programme
Total number of fellowships	439	336	121
Fellowships in biomedical sciences	90	80	29
Fellowships in Internal Medicine	51	35	14

Table 2. *Characteristics of granted fellowships in the Innovational Research Programme ('Vernieuwingsimpuls') by the Netherlands Organisation for Scientific Research 2005-2009*

	Total Veni grants	Internal Medicine Veni grants
% Grants to females	38%	29%
Mean age of the fellowship winners (± SD)	29.7 (4.1)	32.3 (6.8)
Grants per university medical centre (range)	6-15	1-13

Figure 1. *Grants in the various subdisciplines of Internal Medicine in the Innovational Research Programme ('Vernieuwingsimpuls') by the Netherlands Organisation for Scientific Research 2005-2009*



are equal. Apparently, there are other factors that relatively prohibit women from pursuing a research career in Internal Medicine, which is also reflected by the fact that the proportion of women who are granted the more advanced Vidi and Vici fellowships is even progressively smaller (data not shown). Interestingly, despite the fact that many of the Veni fellowships in Internal Medicine are granted after completion of the six-year residency programme, the mean age of the Veni winner in Internal Medicine is only slightly higher compared with Veni fellows in other disciplines of science. Apparently, talented young individuals have the ability to somehow carry on with their research during their residency, rendering the postdoctoral period a highly efficient combination of medical specialisation and continuing research. Another interesting finding is that the number of Veni laureates within the area of Internal Medicine is not equally distributed amongst university medical centres (*table 2*), although relatively small numbers may have affected this outcome.

Taken together, there seems to be an abundance of research talent in Internal Medicine in the Netherlands, holding a great promise for the future and indicating that the discipline is likely to maintain its strong position at the crossroad of science and medicine. The Innovational Research Scheme indeed provides a useful instrument for young talented researchers in Internal Medicine to build on their research careers and thereby is an important supportive instrument for developing a new generation of academic medical specialists.

REFERENCES

1. Levi M. How academic is internal medicine in the Netherlands? A bibliometric analysis. Neth J Med. 2009;67:318-9.
2. Netherlands Organization for Scientific Research. Innovational Research Incentives Scheme Programme brochure round 2010 (Vernieuwingsimpuls Veni – Vidi – Vici). www. nwo. nl.
3. Technopolis. Evaluatie Vernieuwingsimpuls 2000 – 2006. www. nwo. nl.

Netherlands
The Journal of Medicine

REVIEW

Seronegative polyarthritis as severe systemic disease

A.P. Rozin[1]*, T. Hasin[2], K. Toledano[1], L. Guralnik[3], A. Balbir-Gurman[1]

Departments of [1]Rheumatology, [2]Intensive Cardiac Care Unit, [3]Medical Imaging, Rambam Health Care Campus and Technion, Haifa, Israel, *corresponding author: tel.: 972-4-8542268, fax: 972-4-8542985, e-mail: a_rozin@rambam.health.gov.il

ABSTRACT

Background: Severe extra-articular disease is associated with high levels of rheumatoid factor (RF) in patients with seropositive rheumatoid arthritis (RA) and a poor prognosis. It is said that patients with seronegative rheumatoid arthritis have a more benign course and less destructive disease. We observed several patients with seronegative non-rheumatoid polyarthritis, with aggressive extra-articular systemic disease.

Objectives: Review of seronegative systemic polyarthritis with clinical presentation of typical cases.

Methods: Medline search for systemic manifestations of seronegative polyarthritis. Clinical presentations: 1. A 56-year-old woman was admitted to the cardiac intensive care unit with stabbing presternal chest pain aggravated by breathing and progressive dyspnoea, which gradually developed over a period of two weeks with one episode of fever at 38.0 °C. She had suffered chronic pain in her buttocks for three years with polyarthralgia and evanescent palmar-plantar rash. Imaging showed bilateral sacroiliitis (HLA B27 negative) and a large pericardial effusion. Extra-articular manifestations of SAPHO syndrome were proposed and she was successfully treated with combined therapy: pulse methylprednisolone, azathioprine, colchicine and prednisone. 2. A 47-year-old woman with psoriatic arthropathy developed high fever with leucocytosis and thrombocytosis and lung infiltrates during exacerbation of her joint disease. She was treated with pulse methylprednisolone followed by corticosteroid tapering, anti-TNF (infliximab) and methotrexate with complete resolution. 3. A 19-year-old man with inflammatory bowel disease developed acute pericarditis with response to 6-mercaptopurine, salazopyrine and prednisone.

Results: We discuss a range of seronegative arthritis diseases with possible systemic manifestations including the main procedures for early diagnosis. Infection, malignancy, hypersensitivity, granulomatous disease and other collagen diseases such as systemic lupus erythematosus should be excluded, but investigations for an underlying disease should not delay early corticosteroid and immunosuppressive therapy.

Conclusion: A high level of suspicion of extra-articular disease should always be maintained when treating active seronegative polyarthritis.

KEYWORDS

Seronegative polyarthritis, sacroiliitis, pleuro-pericarditis, pneumonitis, SAPHO syndrome

RHEUMATOID FACTOR AND ITS BIOLOGICAL ROLE

Rheumatoid factor (RF), as a marker of seropositive rheumatoid arthritis, is an autoantibody (usually IgM) against the Fc portion of IgG. By forming immune complexes, RF initiates the universal mechanism of immunoglobulin elimination by the reticuloendothelial system. That is why RF is present in small titres in all people, being in higher titres (above 20 IU/ml) in less than 5% of the population. The incidence of high titres of rheumatoid factor increases with age and more than 20% of people over the age of 65 years have an elevated rheumatoid factor. High levels of RF are associated with severe destructive joint disease, extra-articular involvement (lung, vasculitis, subcutaneous nodules) and poor prognosis. As a much more specific addition to RF detection, anti-cyclic citrullinated peptide antibodies (anti-CCP) has recently become an important diagnostic tool for confirming RA seropositivity.

SERONEGATIVITY

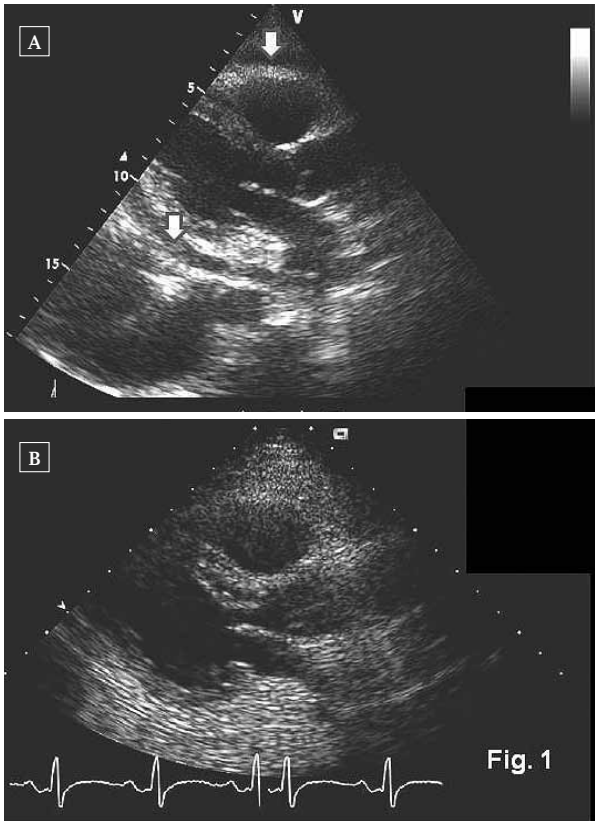
Patients who do not have detectable RF are said to be 'seronegative' (SNA). Patients with seronegative rheumatoid arthritis are thought to have a more benign course and less destructive disease. Spondyloarthropathies are diseases not associated with increased RF. In this article seronegative polyarthritis is considered to be not only spondyloarthropathy disease but all arthritides with negative IgM RF. We observed several patients with seronegative non-rheumatoid polyarthritis (SNA), with aggressive extra-articular systemic disease, requiring prompt diagnostic and therapeutic decisions.

CLINICAL PRESENTATIONS OF SERONEGATIVE SYSTEMIC POLYARTHRITIS

Case 1

Our first case concerns a 56-year-old woman who was admitted to the cardiac intensive care unit with stabbing presternal chest pain aggravated by breathing and progressive dyspnoea. Her symptoms developed gradually over a period of two weeks with one episode of fever at 38.0 °C. Her preceding disease comprised chronic pain in the buttocks for three years with polyarthralgia and evanescent palmar-plantar rash. The pain in the buttocks had increased before admission. Her other medical problems were acute gastroenteritis one month ago, subacute thyroiditis one year ago with further normal thyroid function, achalasia with severe oesophageal distension for 30 years, pleural effusion of unknown origin nine years ago and hypersensitivity to penicillin. She had not received any medications before admission. Vital signs on admission were as follows: blood pressure 120/90 mmHg, pulse 110 beats/min, temperature 37.0 °C, respiratory rate 32/min. Physical examination revealed congested neck veins, left subscapular dullness, decreased respiratory sounds, muffled cardiac sounds, no abdominal organ enlargement, and tenderness above the sacroiliac joints. Chest X-Ray showed cardiomegaly and bilateral pleural effusion. CT angiogram of the chest excluded pulmonary embolism and showed large pericardial effusion, bilateral small pleural effusion, multiple lung infiltrates, and achalasia. ECG demonstrated low voltage. Pelvis CT displayed stage II bilateral sacroiliitis. Echocardiography confirmed large pericardial effusion with fibrin deposits (*figure 1A*), preserved ventricular function and no valve disease. Laboratory investigation showed microcytic anaemia (haemoglobin 10.1 g/dl), leucocytosis 16,900 per µl (polymorphonuclear neutrophils (PMN) 78%), thrombocytosis 6 x 10^5 per µl, albumin 2.5 g/dl, normal troponin, erythrocyte sedimentation rate (ESR) 50 mm/h, C-reactive protein 21.1 mg/dl (n<0.5), mildly elevated alkaline phosphatase and borderline increased ferritin, normal renal function, and no proteinuria. Blood and urinary cultures were negative. Pericardiocenthesis yielded 0.5 litre of serosanguinous exudate containing 72% PMN and 28% mononuclear cells, a high lactate dehydrogenase concentration (620 IU/l) and normal glucose level. Gram staining, culture, pericardial fluid cytology and Ziehl-Neelsen staining were all negative. Blood and pericardial fluid virology tests, also including EBV, Coxsackie viruses and hepatitis, were negative. The diagnosis of polyserositis and pneumonitis in the presence of sterile blood and pericardial exudate and high level of acute phase response (ESR, CRP, low albumin) led us to a possible autoimmune disease, probably related to sacroiliitis and palmar-plantar rash (SAPHO syndrome). The low level of ferritin did not support the diagnosis of Still's disease. Hypersensitivity was unlikely because of the lack of an offending drug and exposure. Malignancy had not been found. Immunological profile: RF, anti-CCP, ANA, antibodies to extractable nuclear antigens (SSA, SSB, SM, RNP), ANCA, anticardiolipin antibodies, and

Figure 1. *A. Echocardiography on admission (parasternal long axis) demonstrating pericardial effusion (arrows). B. Echocardiography two months after admission (parasternal view) demonstrating resolution of pericardial effusion*



cryoglobulins were all negative. Protein electrophoresis revealed polyclonal hypergammaglobulinaemia. Systemic lupus erythematosus and collagen vascular disease, despite negative immunological tests, were doubtful. Methylprednisolone intravenous pulses of 500 mg per day for three days with further prednisone therapy (1 mg/kg) brought about a rapid improvement. The white blood count (WBC) count increased to 40,000 per µl after corticosteroid (CS) administration and decreased to 10,600 per µl after starting azathioprine (AZA) therapy as steroid sparing agent. AZA was considered after relapse of the chest pain after CS tapering to a dose below 20 mg/day. Pleural and pericardial effusion were completely resolved (*figure 1B*).

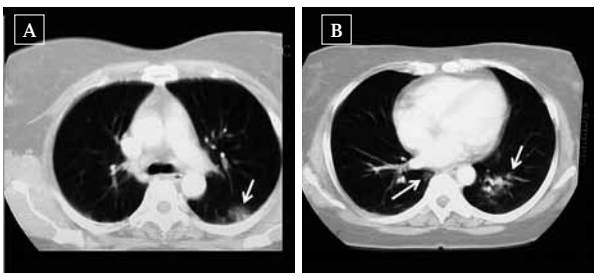
Case 2

Our second case concerns a 47-year-old woman with a two-year history of psoriatic arthropathy and fever of 39.5 °C, asymmetric joint and spine pain, finger swelling and stiffness on admission without respiratory complaints. The chest CT revealed two small infiltrates in lower lobe of the left lung and one small basal right lower lobe infiltrate with mediastinal lymphadenopathy (*figure 2*). Cell blood count showed leucocytosis (13,000) and thrombocytosis (7 x 10^6). Tuberculosis, bacterial and viral pneumonia, granulomatous disease and malignancy were excluded, trial of antibiotic therapy failed and the patient responded to methylprednisolone therapy. Further activity of inflammatory disease was suppressed by CS, methotrexate and anti-TNF (infliximab) therapy with complete resolution of lung abnormalities, active polyarthritis and fever.

Case 3

A 19-year-old man was admitted with abdominal pain and bloody diarrhoea and chest pain, aggravated by deep breathing. Abdominal CT and colonoscopy revealed ulcerative colitis and sacroiliitis and chest CT showed moderate pericardial effusion. The patient responded to intravenous corticosteroid pulse therapy, and combined therapy including 6-mercaptopurine, salazopyrine, and prednisone with complete resolution of active colitis and pericardial effusion. Chest pain and pericardial effusion was an unusual systemic presentation of ulcerative colitis!

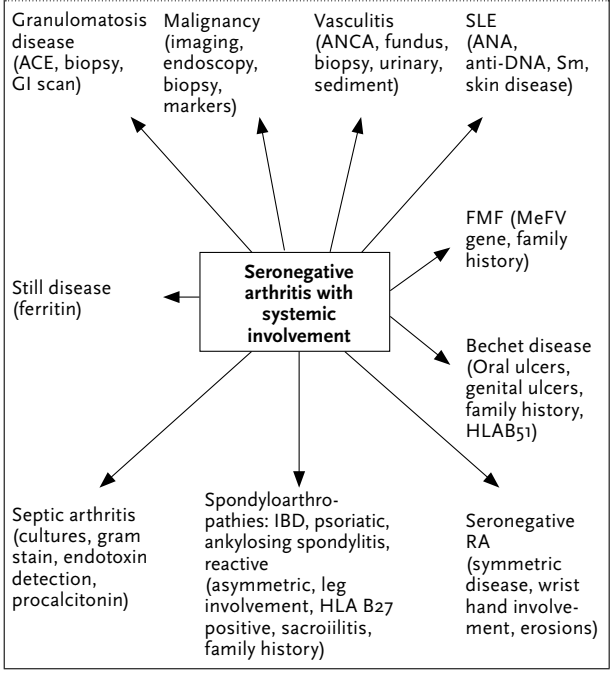
Figure 2. *A. CT scan of the chest at the level of carina, lung window. Small patchy ground glass subpleural opacity in the apical segment of the left lower lobe is seen. B. CT scan of the chest at the level of right inferior pulmonary vein, lung window. Vague ground glass opacity around pulmonary vessels of the left lower lobe and small patchy opacity in the right lower lobe are seen.*



DIAGNOSTIC ALGORITHM FOR SERONEGATIVE SYSTEMIC POLYARTHRITIS

We discuss a range of seronegative arthritis diseases including the main procedures for early diagnosis (*figure 3*).

Figure 3. *Diagnostic algorithm of seronegative polyarthritis as severe systemic disease*



PREDICTORS OF SYSTEMIC MANIFESTATIONS

In the multivariate analysis, extra-articular RA (ExRA) manifestations were predicted by the presence of a positive RF (RR 1.56), ANA test (RR 1.58), smoking (RR 1.52) and severe disability (Steinbrocker Class III-IV at diagnosis) (RR 1.42) but not by age and sex.[1] In a subgroup of 12.8% with severe ExRA (Malmo criteria) the main predictors were smoking (RR 2.94), early disability (RR 2.45). RF was weakly associated with ExRA Malmo (severe extra-articular disease) compared with seronegative RA, but smoking, early disability and old age were stronger predictors than

RF for severe ExRA.[1] Such data estimating extra-articular disease and its severity for seronegative non-RA arthritis are unknown, probably because its lower incidence and prevalence.

SYSTEMIC INFLAMMATION AND MORTALITY

The systemic inflammatory process is a major predictor of mortality in patients with RA. Extra-articular disease confers a mortality risk ratio five times that of patients without such manifestations.[2] Seronegative non-RA arthritis patients with systemic involvement have an unclear lifespan compared with those without systemic disease.

NOT ONLY IGM RFS ARE INVOLVED IN SYSTEMIC DISEASE

Not only IgM RFs, but also IgG, IgA and IgE RF variants are proposed in the pathogenesis of severe RA and non-RA polyarthritis with extra-articular involvement.[3-6] Seronegative arthritis may be seropositive for IgG, IgA and IgE RFs.

EXTRA-ARTICULAR MANIFESTATIONS

Extra-articular manifestations of ankylosing spondylitis are common and well defined: microscopic ileal and coecal inflammation (50% of patients),[7,8] anterior uveitis (40%),[9] aortic regurgitation and conduction abnormalities (9%),[10] pericarditis (1%),[11] upper lobe lung fibrosis with reticulo-nodular opacities and cysts, bronchial and pleural thickening (1-2%),[12] renal amyloidosis (4-9%),[13] and IgA nephropathy.[14] Extra-articular disease of SAPHO syndrome (pleural effusion[15-17]) and psoriatic arthropathy (diastolic left ventricular dysfunction,[18] cryptogenic organising pneumonia,[19] idiopathic interstitial pneumonia with IgA nephropathy[20]) is only presented in occasional reports. Nevertheless, this disease may be aggressive and requires a rapid differential work-up and appropriate management. Investigations for an underlying disease should not delay early corticosteroid and immunosuppressive therapy.

MECHANISMS OF SYSTEMIC DISEASE AND EXTRA-ARTICULAR MANIFESTATIONS

Systemic spread of extra-articular RFs, infectious antigens or new intrinsic or extrinsic non-infectious antigens may serve as a nidus for development of granulation inflammatory tissue (pannus) destroying adjacent tissue. However, immune complexes containing exogenous antigens have never been detected in RA and spondyloarthropathies. Although preceding infections are common, germ-free state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats[21] and elevated levels of IgM and IgA antibodies to *Proteus mirabilis* and IgM antibodies to *Escherichia coli* are associated with early seropositive RA.[22]

Systemic involvement may implicate even late onset of inflammatory spondyloarthropathy presenting as undifferentiated arthritis, fever, loss of weight and large oedema, probably the most original presentation of arthritis specific to old males.[23]

Pleuropericarditis associated with inflammatory bowel disease (IBD) is a rare extraintestinal complication.[24-29] Cardiac involvement may also present as myopericarditis, conduction defects, and be complicated with disseminated intravascular coagulation.[25] Pericardial effusion may be asymptomatic with cardiac tamponade.[24] At least 29 cases of pleuropericarditis associated with IBD have been reported with good response to NSAIDs as well as corticosteroids.[25] Of note, this complication can also develop during remission, its diagnosis can be very difficult and a high level of suspicion should be maintained.[27,28]

Further findings show common lung involvement in patients with SNA and RA. Bronchoalveolar lavage (BAL) was performed on 13 asymptomatic patients with SNA; (6 with peripheral psoriatic arthritis, 2 with axial psoriatic arthritis, 3 with ankylosing spondylitis, 2 with sacroiliitis).[30] BAL revealed a significant decrease of neutrophilic granulocytes and an increase in B lymphocytes in patients with SNA in comparison with 64 patients with rheumatoid arthritis (RA; 24 seronegative, 39 seropositive) and 15 healthy controls. Patients with SNA and RA had a significant increase of lymphocytes, especially T, T-helper and activated cells. In addition patients with RA had a significant increase of natural killer cells and lower percentage of alveolar macrophages and T-supressor cells. Transbronchial biopsy was performed on nine patients with SNA and on 59 patients with RA. Abnormal histological features of lung tissue were observed in four out of nine patients with SNA (two with fibrosis, one with follicular lymphoid hyperplasia and one with desquamative interstinal pneumonitis). The abnormal lung histology in RA patients was more pronounced; however, the differences between SNA and RA were not significant. The data from BAL and histology suggest that the pulmonary involvement in SNA and RA is caused by an unspecified immunological process.[30] Pulmonary lymphocyte alveolitis in spondyloarthropathy has been reported as a subclinical disease using BAL with distal airspace cytology.[31]

The assessment of cytokines and their soluble receptors in the synovial fluid (SF) of inflammatory arthropathies may be useful in studying pathogenetic and immunoregulatory mechanisms underlying different diseases.[32] The two immune arthropathies, RA and reactive arthritis (ReA), were characterised by increased SF levels of IL-12, sCD25 and of the sTNF-RII/sTNF-RI ratio compared with controls. ReA differed, however, from RA by showing lower IL-8 and IL-4 levels, higher IFN-gamma levels and a higher IL-12/IL-10 ratio, suggesting a more prevalent Th1 profile in ReA SF. The data indicate that the measurement of SF cytokines and soluble receptors may discriminate between each inflammatory arthropathy and might be useful in clinical practice.[32] Cytokine 'storm' may be involved in highly active inflammation in our patients with severe systemic disease.[33] Cytokine storm or hypercytokinaemia is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells, with highly elevated levels of various cytokines.[34] The primary symptoms are high fever, swelling and redness, extreme fatigue and nausea. In some cases the immune reaction may be fatal.

T helper (Th) 17 cells have been implicated in the pathogenesis of psoriasis and psoriatic arthropathy (two of our patients). Interleukin (IL)-23 stimulates survival and proliferation of Th17 cells, and thus serves as a key master cytokine regulator for these diseases. In psoriasis, IL-23 is overproduced by dendritic cells and keratinocytes, and this cytokine stimulates Th17 cells within the dermis, joints or other target organs to make IL-17A and IL-22. IL-22 drives keratinocyte and inflammatory cell proliferation.[35] Targeting of these key cytokines is likely to lead to dramatic clinical improvement and prevention of severe systemic disease.

Most of our patients were febrile with high leucocytosis. The conventional view of the steps that lead to fever production is that they begin with the biosynthesis of pyrogenic cytokines by stimulated (pathogens or immune conflict) mononuclear phagocytes, their release into the circulation and transport to the thermoregulatory centre in the preoptic area (POA) of the anterior hypothalamus, and their induction there of cyclo-oxygenase (COX)-2-dependent prostaglandin (PG) E_2, the putative final mediator of the febrile response. A new unified model postulates that the steps in the production of lipopolysaccharide (LPS or Il-1) fever occur in the following sequence: the immediate activation by LPS of the complement (C) cascade, the stimulation by the anaphylatoxic C component C5a of Kupffer cells, their consequent, virtually instantaneous release of PGE_2, its excitation of hepatic vagal afferents, their transmission of the induced signals to the POA via the ventral noradrenergic bundle, and the activation by the thus, locally released norepinephrine (NE) of neural α_1- and glial α_2-adrenoceptors. The activation of the first causes an immediate, PGE_2-independent rise in core temperature (T_c) (the early phase of fever; an antioxidant-sensitive PGE_2 rise, however, accompanies this first phase), and of the second a delayed, PGE_2-dependent T_c rise (the late phase of fever). Meanwhile-generated pyrogenic cytokines and their consequent upregulation of blood-brain barrier cells COX-2 also contribute to the latter rise.[36,37] Many patients with psoriatic arthritis have neutrophilic and eosinophilic leucocytosis.[38] Isolated polymorphonuclear leucocytes (PMN) from psoriatic patients have normal concentrations of proteolytic enzymes and they have β-adrenergic receptors of normal density and affinity. PMN from psoriatic patients responded normally to the synthetic chenotactic peptide, f-Met-Leu-Phe. The chemotactic activities of sera from psoriatic patients were similar to those of normal sera. Sera from psoriatic patients enhanced chemokinesis of PMN more than normal control sera at a final concentration of 1%; no difference in chemokinetic response between psoriatic and normal sera was found at serum concentrations greater than 2.5%. This study suggests that the peripheral PMN from psoriatic patients are normal, but the sera of psoriatic patients has more chemokinetic activity for PMN than does normal serum.[38] Fever and leucocytosis are the first lines of innate immunity implicated in severe autoimmune disease of our patients.

CONCLUSION

SNA may be a severe disease with multiple system involvement, organ and life threatening. Non-IgM RFs, innate immune system, cytokines, cell and antibody mediated immunity, gram-negative pathogens and nonmicrobial antigens may be implicated in pathogenesis of systemic disease. High level of suspicion of extra-articular disease should always be maintained. After excluding infection early, aggressive, combined immunosuppressive therapy should be initiated for systemic manifestations of SNA.

REFERENCES

1. Turesson C, O'Fallon W, Crowson C, Gabriel S, Matteson E. Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years. Ann Rheum Dis. 2003;62:722-7.
2. Turesson C, O'Fallon WM, Crowson CS, Gabriel SE, Matteson EL. Occurrence of extra-articular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis. J Rheumatol. 2002;29:62-7.
3. Scott DG, Bacon PA, Allen C, Elson CJ, Wallington T. IgG rheumatoid factor, complement and immune complexes in rheumatoid synovitis and vasculitis: Comparative and serial studies during cytotoxic therapy. Clin Exp Immunol. 1981;43:54-63.

4. Dunne JV, Carson DA, Spiegelberg HL, Alspaugh MA, Vaughan JH. IgA rheumatoid factor in the sera and saliva of patients with rheumatoid arthritis and Sjogren's syndrome. Ann Rheum Dis. 1979;38:161-5.

5. Pai S, Pai L, Birkenfeldt R. Correlation of serum IgA rheumatoid factor levels with disease severity in rheumatoid arthritis. Scand J Rheumatol. 1998;27:252-6.

6. Gioud-Paquet M, Auvinet M, Raffin T, et al. IgM rheumatoid factor (RF), IgA RF, IgE RF, and IgG RF detected by ELISA in rheumatoid arthritis. Ann Rheum Dis. 1987;46:65-71.

7. De Keyser F, Baeten D, Van de Bosch F, et al. Gut inflammation and spondyloarthropathies. Curr Rheumatol Rep. 2002;4:525-32.

8. Leirisalo-Repo M, Turunen U, Stenman S, Helenius P, Seppala K. High frequency of silent inflammatory bowel disease in spondyloarthropathy. Arthritis Rheum. 1994;37:23-31.

9. Rosenboum JT. Characterization of uveitis associated with spondyloarthritis. J Rheumatol. 1989;16:792-6.

10. Crowly JJ, Donnely SM, Tobin M, et al. Doppler echocardiographic evidence of left ventricular diastolic dysfunction in ankylosing spondylitis. Am J Cardiol. 1993;71:1337-40.

11. Yildirir A, Aksayek S, Calguneri M, Oto A, Kes S. Echocardiographic evidence of cardiac involvement in ankylosing spondylitis. Clin Rheumatol. 2002;21:129-34.

12. Souza AS, Muller NL, Marchiori E, Soarez-Souza LV, de Souza Rocha M. Pulmonary abnormalities in ankylosing spondylitis: inspiratory and expiratory high-resolution CT findings in 17 patients. J Thorac Imag. 2004;19:259-63.

13. Nabokov AV, Shabunin MA, Smirnov AV. Renal involvement in ankylosing spondylitis (Bechterew's disease). Nephrol Dial Transplant. 1996;11:1172-5.

14. Vilar MJ, Cury SE, Ferraz MB, Sesso R, Atra E. Renal abnormalities in ankylosing spondylitis. Scand J Rheumatol. 1997;26:19-23.

15. Fernandez-Campillo J, Garcia-Pachon E. Pleural effusion associated with the SAPHO syndrome. Chest. 2001;120;1752.

16. Vaile JH, Langlands DR, Prichard MG. SAPHO syndrome: a new pulmonary manifestation? J Rheumatoid. 1995; 22:2190-1.

17. Ravelli A, Martini A. SAPHO syndrome and pulmonary disease. J Rheumatol. 1996; 23:1482-3.

18. Saricaoglu H, Gullulu S, Bulbul Baskan E, Cordan J, Tunali S. Echocardiographic findings in subjects with psoriatic arthropathy. J Eur Acad Dermatol Venereol. 2003;17:414-7.

19. Webber NK, Elston CM, O'Toole EA. Generalized pustular psoriasis and cryptogenic organizing pneumonia. Br J Dermatol. 2008;158:853-4.

20. Hiki Y, Kokubo T, Horii A, et al. A case of severe IgA nephropathy associated with psoriatic arthritis and idiopathic interstitial pneumonia. Acta Pathol Jpn. 1993;43:522-8.

21. Taurog JD, Richardson JA, Croft JT, et al. The germ-free state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. J Exp Med. 1994;180:2359-64.

22. Newkirk MM, Goldbach-Mansky R, Senior BW, Klippel J, Schumacher HR Jr, El-Gabalawy HS. Elevated levels of IgM and IgA antibodies to Proteus mirabilis and IgM antibodies to Escherichia coli are associated with early rheumatoid factor (RF)-positive rheumatoid arthritis. Rheumatology. 2005; 44:1433-41.

23. Le Parc JM. Inflammatory arthritis of the elderly. Rev Prat. 2005;55:2115-20.

24. Bansal D, Chahoud G, Ison K, et al. Pleuropericarditis and pericardial tamponade associated with inflammatory bowel disease. J Ark Med Soc. 2005;102:16-9.

25. Orii S, Chiba T, Nakade I, et al. J Clin Gastroenterol. 2001;32:251-4.

26. Iaquinto G, Sorrentini I, Ptillo FE, Berardesca G. Pleuropericarditis in a patient with ulcerative colitis in longstanding 5-aminosalicylic acid therapy. Ital J Gastroenterol. 1994;26:145-7.

27. de Wazieres B, Fahd P, Fest T, Dupond JL, Vuitton D. Pleuropericarditis and Pyoderma gangrenosum during remission of a hemorrhagic rectocolitis. Rev Mal Respir. 1991;8:595-7.

28. Gonzales Martin T, Dapena Vielba F, Ergueta Martin P, Bermejo Garcia J, Gonzalez Sarmiento E, Maranon Cabello A. Acute pleuropericarditis and cardiac tamponade as extraintestinal complications of ulcerative colitis. An Med Interna. 1990;7:581-4.

29. Patwardhan RV, Heilpern RJ, Brewster AC, Darrah JJ. Pleuropericarditis: an extraintestinal complication of inflammatory bowel disease. Report of three cases and review of literature. Arch Intern Med. 1983;143:94-6.

30. Scherak O, Kolarz G, Popp W, Wottawa A, Ritschaka L, Braun O. Lung involvement in rheumatoid factor-negative arthritis. Scand J Rheumatol. 1993:22:225-8.

31. Jeandel P, Bonnet D, Chouc PY, Molinier S, Raphenon G, Marter G, et al. Demonstration of subclinical pulmonary alveolitis in spondyloarthropathies. Rev Rhum Ed Fr. 1994;61:301-9.

32. Ribbens C, Andre B, Kaye O, Kaiser MJ, Bonnet V, de Groote D, Franchimont N, Malaise MG. Increased synovial fluid levels of interleukin-12, sCD25 and sTNF-RII/sTNF-RI ratio delineate a cytokine pattern characteristic of immune arthropathies. Eur Cytokine Netw. 2000;11:669-76.

33. Ferrara JL, Abhyankar DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transplant Proc. 1993;2:1216-7.

34. Osterholm MT. Preparing for the next pandemic. NEJM. 2005;352:1839-42.

35. Fitch E, Harper E, Skorcheva I, Kurtz SE, Blauvelt A. Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. Curr Rheumatol Rep. 2007;9:461-7.

36. Blatteis CM, Li S, Li Z, Feleder C, Perlik V. Cytokines, PGE2 and endotoxic fever: a reassessment. Prostaglandines & Other Lipid Mediators. 2005;76:1-18.

37. Blatteis CM. The onset of fever: new insights into its mechanism. Prog Brain Res. 2007;162:3-14.

38. Fraki JE, Jakoi L, Davies AO, Lefkowitz RJ, Snyderman R, Lazarus GS. Polymorphonuclear leukocyte function in psoriasis: chmotaxis, chemokinesis, beta-adrenergic receptors, and proteolytic enzymes of polymorphonuclear leukocytes in the peripheral blood from psoriatic patients. J Invest Dermatol. 1983;81:254-7.

Netherlands
The Journal of Medicine

REVIEW

Platelet-vessel wall interaction in health and disease

E.C. Löwenberg[1,2*], J.C.M. Meijers[1,2], M. Levi[1,3]

Departments of [1]Vascular Medicine, [2]Experimental Vascular Medicine, [3]Internal Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)20-566 87 91, fax: +31 (0)20-566 93 43, e-mail: e.c.lowenberg@amc.uva.nl

ABSTRACT

Upon vessel wall injury platelets rapidly adhere to the exposed subendothelial matrix which is mediated by several cellular receptors present on platelets or endothelial cells and various adhesive proteins such as von Willebrand factor, collagen and fibrinogen. Subsequent platelet activation results in the recruitment of additional platelets and the generation of platelet aggregates forming a stable platelet plug. In addition, activated platelets form a strong link between primary and secondary haemostasis as they provide the phospholipid surface that is necessary for the assembly of activated coagulation factor complexes required for thrombin generation.

Other than the physiological function acting as a first line of defence against bleeding, platelets may also contribute to pathological thrombus formation. Platelets play an important role in thromboembolic diseases and may contribute to the formation of occlusive thrombi which can lead to severe complications such as stroke or myocardial infarction.

Improved understanding of the respective roles of the various cellular receptors, adhesive proteins and regulatory proteins involved in platelet-vessel wall interaction and subsequent thrombus formation, both under physiological and pathological conditions, has led to the development and investigation of a broad range of antiplatelet drugs. This review provides an overview of the current knowledge on the mechanisms involved in the interaction between platelets and vascular endothelium and discusses recent advancements in the development of drugs interfering with platelet-vessel wall interaction at various stages of thrombus formation.

KEYWORDS

Adhesion molecules, antithrombotic agents, platelets, (sub) endothelium, thrombus formation, von Willebrand factor

INTRODUCTION

Platelets are circulating blood cells that do not interact with the intact vessel wall under normal circumstances. However, when the vessel wall is injured and the endothelium is disrupted, a rapid and complex interaction between circulating platelets and exposed (sub) endothelial structures occurs.[1] This interaction, mediated by various cellular receptors on the surface of platelets or endothelial cells and adhesive proteins such as von Willebrand factor (vWF) and fibrinogen, ultimately results in the adhesion of platelets to the vessel wall followed by aggregation of platelets to each other. In addition to this physiological function as a first line of defence against bleeding, platelets may also contribute to pathological thrombus formation. In systemic inflammatory syndromes such as sepsis, disseminated intravascular platelet activation may lead to microvascular thrombosis as well as an amplification of the inflammatory response through the release of inflammatory cytokines and growth factors. Furthermore, activated platelets form a strong link between the processes of primary and secondary haemostasis as they provide the phospholipid layer necessary for the assembly of activated coagulation factor complexes which in turn is required for thrombin generation and subsequent conversion of fibrinogen into fibrin.

This review provides a comprehensive overview of what is currently known about the mechanisms involved in platelet-vessel wall interaction, both under physiological and pathological conditions. The respective roles of various important cellular receptors, adhesive proteins and regulatory proteins, as well as the development of drugs interfering with platelet-vessel wall interaction at different levels of thrombus formation will be discussed.

ROLE OF PLATELETS IN NORMAL HAEMOSTASIS

Upon vessel wall injury, rapid and complex interactions between circulating platelets and exposed (sub)endothelial structures occur[1] resulting in platelet adhesion to the damaged endothelium. The mechanism by which platelets adhere to the vascular wall to achieve haemostasis is fairly well understood, with vWF-mediated platelet adhesion being the most important route, particularly in situations of high shear stress such as small arteries, arterioles and stenosed vessels. VWF is an adhesive glycoprotein synthesised by megakaryocytes and endothelial cells and is either constitutively secreted or targeted to storage organelles in the endothelium, called Weibel-Palade bodies, or to α-granules which are present in platelets or megakaryocytes.[2] The mature vWF molecule consists of disulphide-linked multimers with a molecular weight of 20,000,000 or more.[3] Under normal conditions vWF circulates in plasma in its inactive form whereas the largest and most active forms, high molecular weight (HMW) or ultra-large vWF multimers, are present in storage organelles and not found in circulating blood. However, upon endothelial cell activation vWF is acutely released from endothelial storage sites and can be detected transiently as ultra-large vWF in the circulation.[4,5] Subsequent binding of vWF to the exposed sub-endothelial structures, such as collagen, induces a conformational shift in the vWF molecule from its latent to its active form, thereby exposing the binding site for platelet glycoprotein (GP) receptor Ib. Through the simultaneous binding of collagen and platelets vWF can serve as a molecular bridge between platelets and the sub-endothelial matrix mediating platelet adhesion to the vessel wall.[6]

In addition to its important role in platelet adhesion, vWF may also be a ligand for the major platelet integrin αIIbβ3 (GPIIb/IIIa) thereby facilitating platelet aggregation. When platelets bind to vWF they become activated and undergo a conformational change. This shape change induces several effects, including the acute release of the contents of storage organelles, such as fibrinogen and ADP, further enhancing platelet activation.[7] Second, the conformational change induces the expression of active GPIIb/IIIa on the surface of activated platelets through which platelets can bind to either vWF or fibrinogen forming platelet aggregates.[8] Finally, the platelet membrane turns into a phospholipid surface upon activation and shape change. Since this negatively charged surface is necessary for the assembly of activated coagulation factor complexes which in turn is required for thrombin generation, platelets form a strong link between the processes of primary and secondary haemostasis. Secondary haemostasis ultimately results in the formation of a fibrin network stabilising the growing platelet thrombus.

Another adhesive protein crucially involved in platelet-vessel wall interaction is collagen. Collagen types I and IV may directly bind to platelet glycoprotein receptor Ia/IIa (integrin α2β1).[9] The importance of this GPIa/IIa mediated collagen-platelet binding seems limited to low shear rate conditions. In addition GPIa/IIa binding to collagen may also facilitate the interaction between collagen and another platelet glycoprotein receptor, GPVI. GPVI belongs to the immunoglobin superfamily, which forms an important group of cellular adhesive receptors. Although GPVI may be directly involved in platelet adhesion to collagen, it seems likely to predominantly act as an activator of the GPIa/IIa receptor via intracellular signalling.[10]

Other adhesive proteins present in the extracellular matrix that are involved in the interaction between platelets and the (sub) endothelium include fibronectin, thrombospondin, laminin and vitronectin. Fibronectin is produced by megakaryocytes. It is stored in platelet α-granules and secreted upon thrombin-mediated platelet activation. Through binding to platelet receptor GPIIb/IIIa fibronectin can mediate platelet-platelet interaction.

Another protein that is stored in α-granules and secreted upon platelet activation is thrombospondin, which binds to the platelet membrane where it can interact with fibrinogen, fibrin, fibronectin, collagen and other platelets. The physiological function of its release during platelet activation might lie in its capability to overcome the antithrombotic activity of physiological nitric oxide, thereby providing a positive feedback for efficient platelet adhesion and aggregation.[11] Binding of thrombospondin to platelets is mediated by platelet GPIV receptor. Other receptors that are possibly involved in the thrombospondin-platelet interaction include integrin α5β3 and GPIb.[12]

A third adhesive protein located in the extracellular matrix that is involved in the interaction between platelets and endothelial structures is the large glycoprotein laminin. Similar to collagen laminin can amplify platelet activation through GPVI binding. Recent data suggest that laminin may also interact with vWF and the GPIb/IX/V complex thereby supporting platelet adhesion under high shear stress conditions.[13] The extracellular adhesive protein vitronectin can bind to platelet receptor GPIIb/IIIa or the integrin αvβ3 and appears to be functionally similar to fibronectin.[14]

The superfamily of selectins, including L-selectin (expressed on leucocytes), E-selectin (expressed on endothelial cells) and P-selectin (expressed on both platelets and endothelial cells), forms an important group of cellular adhesive receptors. P-selectins are of particular importance for the interaction between platelets, leucocytes and the blood vessel wall. Platelet and endothelial cell activation induces the release of P-selectins from platelet α-granules and endothelial Weibel-Palade bodies[15] which

then become integrated into the cell membrane and mediate both platelet and leucocyte adhesion. Via the induction of tissue factor expression on circulating monocytes, P-selectin may promote fibrin formation as tissue factor is the primary initiator of thrombin generation which ultimately results in the conversion of fibrinogen to fibrin.[16] Increased levels of soluble P-selectin have been demonstrated during acute coronary syndromes as well as systemic inflammation.[16]

ROLE OF PLATELETS IN THROMBOTIC DISEASE

In addition to the physiological role as a first line of defence against bleeding in response to vascular injury, platelets may also interact with the intact endothelium and contribute to pathological thrombus formation. Although key events leading to thrombus formation in normal haemostasis may be similar in pathological thrombus formation in diseases like stroke or myocardial infarction, factors such as altered shear rate and local dysfunction of endothelial cells, possibly in association with inflammatory mechanisms, appear to play important contributory roles.[17] In case of arterial occlusion, increased shear rate can induce circulating vWF to unfold, bind to platelets through GPIb/IX/V interaction and initiate GPIIb/IIIa-mediated platelet aggregation, which ultimately may result in complete occlusion of the respective artery causing heart attack or stroke.[18-20] Another example of platelet-vessel wall interaction contributing to disease is the development of atherosclerosis in which platelets appear to be crucial.[21,22] In apoE deficient mice platelets were demonstrated to adhere to the vascular wall of the carotid artery even before the invasion of leucocytes and the development of manifest atherosclerotic lesions.[21] Furthermore, platelet receptors GPIbα and IIb/IIIa were found to be the main mediators of platelet adhesion[21] and prolonged antibody-mediated blockade of the platelet GPIbα receptor was demonstrated to inhibit the accumulation of leucocytes in the vascular endothelium as well as atherosclerotic lesion formation. The latter was further supported in different mouse models showing that interruption of platelet-vessel wall interaction, through either antibody-mediated inhibition or by knocking out various platelet receptors including GPIIb, GPIbα and P-selectin, substantially reduces atherosclerotic lesion formation.[22-24] Subsequently, atherosclerotic plaques may rupture thereby exposing the underlying subendothelial structures which could lead to pathological thrombus formation through vWF-GPIb/IX/V and/or collagen-GPVI interactions.

Inflammation is yet another pathological condition in which platelets are important.[25] Platelet activation is common in inflammatory states, including cardiovascular diseases such as unstable angina and acute myocardial infarction,[26,27] and systemic inflammatory syndromes like sepsis where intravascular platelet activation may lead to microvascular thrombosis and contribute to organ failure, morbidity and mortality.[28] The development of severe cardiovascular complications and the outcome of cardiovascular interventions (e.g. percutaneous interventions or bypass surgery) can be actively initiated and influenced by platelets and can therefore also be reduced by effective platelet inhibition.[29,30] Activated platelets can interact with various cell types at the vascular wall, including endothelial cells, neutrophils, monocytes and endothelial progenitor cells. Under normal circumstances platelets will not interact with the intact endothelium; however, inflamed endothelial cells develop properties that make them adhesive for platelets. Various *in vitro* studies performed with human umbilical vein endothelial cells have shown that platelets adhere to activated human endothelial cells and that this interaction is mediated by platelet receptor GPIIb/IIIa, involving platelet-bound fibrinogen, fibronectin and vWF, as well as endothelial receptors, such as intercellular adhesion molecule-1 (ICAM-1), αvβ3 integrin and GPIb.[31-33] Furthermore, platelet activation induces a local release of platelet granule contents containing various potent inflammatory substances which further enhance the inflammatory response and alter chemotactic, adhesive and proteolytic properties of endothelial cells.[34,35] Under inflammatory conditions platelets within the blood stream can interact with circulating leucocytes and once recruited to the vascular endothelium attract these cells to the vascular wall. Leucocyte infiltration into the vessel wall requires multiple steps, involving adhesive and signalling events. These steps include selectin-mediated adhesion and rolling of leucocytes over the endothelium, integrin-mediated firm adhesion of leucocytes, leucocyte activation, and finally the actual infiltration of inflammatory cells into the endothelium.[36] The interaction between platelets, and both leucocytes and the vascular wall can occur in various sequences. First, platelets can form aggregates with leucocytes thereby promoting leucocyte recruitment, either by activating leucocyte adhesion receptors or by directly serving as a bridging molecule between leucocytes and the endothelium. When adhered to the vessel wall, platelets can attract leucocytes by releasing chemoattractants and providing an adhesive surface for leucocyte adhesion. During these complex interactions platelets, leucocytes and endothelial cells all become activated in a cascade-like fashion. An important role is reserved for P-selectin (briefly discussed above) and its main ligand, P-selectin glycoprotein ligand-1 (PSGL-1). These adhesion molecules were originally characterised to be important for initial rolling interactions between leucocytes and the vessel wall, which are required for subsequent leucocyte recruitment

to sites of inflammation or infection.[37,38] However, various animal studies using different models of thrombosis have revealed an important role for P-selectin and PSGL-1 in the process of thrombosis as well. Mice deficient in either of these molecules were demonstrated to form thrombi which were markedly reduced in mass, fibrin level and tissue factor accumulation. Furthermore, compared with wild type mice, P-selectin or PSGL-1 deficient mice were shown to have reduced numbers of infiltrating inflammatory cells in the affected vessel walls.[39,40] The observed differences in tissue factor expression and fibrin generation were already observed in the first 20 seconds after injury, prior to leucocyte recruitment. Similar results were obtained in wild-type mice treated with anti-P-selectin blocking antibodies prior to thrombosis induction and further support a crucial role for P-selectin and PSGL-1 in the recruitment of tissue factor bearing microparticles in early thrombi and subsequent fibrin formation.[41]

PROTEINS REGULATING PLATELET-VESSEL WALL INTERACTION

Although much information is available on mechanisms involved in platelet activation, little is known about the signalling pathways that negatively regulate platelet function. Here we will discuss two proteins importantly involved in the regulation of platelet-vessel wall interaction, platelet-endothelial-cell adhesion molecule-1 (PECAM-1) and von Willebrand factor cleaving protease ADAMTS13.

The immunoglobulin (Ig) gene superfamily consists for a large part of molecules that are involved in the recognition of adhering cells, including intercellular adhesion molecule (ICAM) 1 to 3, vascular adhesion molecule (VCAM) and platelet-endothelial cell adhesion molecule-1 (PECAM-1). In contrast to the cellular adhesion receptors ICAM and VCAM, which play important roles in the interaction between leucocytes and endothelial cells, PECAM-1 is not so much involved in mediating platelet-endothelial cell interaction, but rather acts as a negative regulator of platelet activation through the down modulation of platelet receptor GPIb/IX/V and GPVI. As discussed before, GPIb/IX/V and GPVI bind to vWF and collagen, respectively, and are involved in the initial interaction between circulating platelets and the vessel wall, either under physiological or pathological circumstances. Binding of vWF to the GPIb/IX/V complex appears to simultaneously activate PECAM-1, thereby forming a negative feedback loop. Various regulatory functions of PECAM-1 have been identified in murine platelets.[42] For one, PECAM-1 was shown to negatively regulate early GPIb-initiated platelet signalling responses. Second, PECAM-1 appeared to control the rate and extent of GPIb-mediated activation of platelet receptor GPIIb/IIIa. Furthermore, PECAM-1 was demonstrated to limit the size and rate of platelet thrombus formation under conditions of physiological flow. Via the down modulation of both GPIb/IX/V complexes and GPVI receptors, PECAM-1 can prevent unnecessary platelet activation under high shear and acts as a negative regulator of both platelet activation and aggregation.

VWF-mediated platelet adhesion forms the most important route for platelet-vessel wall interaction under conditions of high shear stress. VWF multimer size, and thus also vWF activity, is primarily regulated by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13), whereas recently other factors have also been identified to cleave vWF, such as plasmin and leucocyte proteases.[43-45]

ADAMTS13, sometimes also referred to as vWF cleaving protease, rapidly cleaves acutely released large vWF multimers into smaller fragments, thereby reducing its propensity to facilitate platelet adhesion and aggregation.[46] ADAMTS13 is synthesised in hepatic stellate cells, endothelial cells, megakaryocytes or platelets, and is enzymatically active in circulating blood.[47-50] However, under static conditions the specific cleavage site located in the vWF A2 domain (Tyr1605-Met1606) is buried within the vWF molecule which normally circulates in plasma in a globular form and thus cannot be recognised by its cleaving protease. Various circumstances can induce vWF unfolding, thereby exposing the ADAMTS13 cleavage site and increasing the proteolytic degradation of vWF by ADAMTS13. These include high shear conditions,[51] denaturing agents such as urea[52] and mutations as seen in the bleeding disorder von Willebrand disease type 2A.[53] In contrast, reduced ADAMTS13 activity may lead to insufficient vWF processing causing a prothrombotic state as is often observed in thrombotic thrombocytopenic purpura (TTP). TTP is a rare and life-threatening condition classified in the group of thrombotic microangiopathies in which a systemic microvascular aggregation of platelets can cause ischaemia in the brain, kidney and other organs, thrombocytopenia, and mechanical injury to erythrocytes.[54] TTP is strongly associated with a severe deficiency of ADAMTS13. The majority of cases are caused by a congenital or acquired (autoantibody-mediated) deficiency resulting in the presence of endothelial cell-attached ultra-large vWF multimers which readily bind to platelet receptor GPIb and promote platelet adhesion and aggregation.[55,56] Other factors that have been proposed to possibly inhibit ADAMTS13 activity include free haemoglobin, inflammatory cytokine IL-6, leucocyte elastase, thrombin, activated coagulation factor X and plasmin.[44,57,58] However, most data pointing to an inhibitory function of these proteins on ADAMTS13 activity are obtained *in vitro* and for free haemoglobin and IL-6 supraphysiological concentrations were used.

Recently, ADAMTS13 deficiencies have also been reported in non-TTP diseases that are accompanied by extreme endothelial activation, including severe sepsis, various inflammatory states and severe malaria.[59-61] However, the clinical relevance of these observations remains unsure.

DEVELOPMENT OF DRUGS INTERFERING WITH PLATELET-VESSEL WALL INTERACTION

Improved understanding of the roles of various platelet receptors and adhesive proteins involved in vascular cell adhesion has contributed to the development and evaluation of many antithrombotic agents interfering with either of these processes. In this section the various drugs targeting different pathways involved in platelet-vessel wall interaction will be discussed.

Drugs targeting vWF- GPIb/IX/V interaction

As was previously discussed, the initial adhesion of platelets to the damaged vessel wall depends, in particular under high shear stress conditions, on the binding of the platelet GPIb/IX/V complex to subendothelial bound vWF. Through platelet GPIb/IX/V-vWF interaction platelets start rolling over the stretched vWF molecules that line the damaged endothelium, which is followed by firm platelet adhesion and further thrombus formation at the site of vascular damage. One of the strategies pursued to inhibit thrombus formation is to hamper this initial step of platelet adhesion to thrombogenic surfaces by interfering with this vWF-GPIb/IX/V axis.

Clinical evidence for the role of vWF-GPIb/IX/V interaction in thrombus formation was found in patients affected with von Willebrand disease or Bernard Soulier syndrome, caused by abnormalities in the vWF molecule and the GPIb/IX/V complex respectively.[62] VWF plays a highly important role in normal haemostasis. As discussed, it promotes platelet adhesion and aggregation. In addition it serves as a stabilising carrier molecule for coagulation factor VIII.[2] Von Willebrand disease is the most common inherited bleeding disorder. Either qualitative defects (type 2) or quantitative defects (type 1 and type 3) in the vWF molecule lead to impaired vWF-GPIb/IX/V interaction, causing mucocutaneous bleeding and reduced circulating FVIII levels resulting in (spontaneous) bleeding tendencies as seen in haemophilia.[63] The autosomal recessive bleeding disorder Bernard Soulier syndrome is characterised by giant platelets, low platelet count and prolonged bleeding time. Affected patients clinically present with bleeding problems such as epistaxis, mucocutaneous, gingival or trauma-induced bleeding. Various mutations affecting different parts of the GPIb/IX/V complex have been indentified, but most mutations are found in the GPIb region resulting in decreased expression of platelet receptor GPIbα on the platelet surface.[64]

The clinical relevance of von Willebrand disease and Bernard Soulier syndrome emphasises the importance of vWF-GPIb/IX/V interaction in normal thrombus formation. Inhibition at this level appears to be an attractive approach for antithrombotic treatment as targeting the initial interaction between platelets and the vascular wall can be expected to give strong effects. Furthermore, the particular importance of vWF-GPIb/IX/V interaction for platelet adhesion under high shear stress conditions implies that drug-mediated interference at this axis will be more specific for arterial systems where shear stress is generally high. In contrast venous systems with lower shear rates can be expected to be less affected, resulting in fewer bleeding complications. Finally, the important roles for platelets (adhesion and activation) and thrombus formation in restenosis after angioplasty, which remains a significant clinical problem despite technical improvements, make the vWF-GPIb/IX/V axis an appealing target for antithrombotic therapy.[65] In particular since interference at this level will, in addition to platelet adhesion inhibition, also reduce subsequent platelet activation. This will result in decreased platelet granule content release, previously shown to affect thrombin generation as well as smooth muscle cell migration and proliferation, thereby leading to decreased fibrin deposition and neointima formation.[66]

Molecules that have been tested for inhibition of the vWF-GPIb/IX/V interaction *in vivo* include snake venoms, such as crotalin and agkistin, peptides derived from vWF (e.g. AR545C, RG12986 and VCL), and various monoclonal antibodies. The clinical applicability of the first two groups seems limited, as snake venoms have been shown to induce significant bleeding and vWF-derived peptides appear to have a short duration of action after administration.[67-72] In contrast, monoclonal antibodies (moAbs) interfering with vWF-GPIb/IX/V interactions appear to have an effective and long-term antithrombotic effect without affecting bleeding and thus seem most promising. MoAbs directed against either the vWF A1 domain or the GPIb/IX/V complex have been tested *in vivo* and have been reviewed elsewhere.[73]

Various moAbs directed against the vWF A1 domain involved in GPIb/IX/V binding have been identified, however, only a few have been reported to have *in vivo* antithrombotic efficacy. This is mainly due to the low cross-reactivity of the antibodies between different species and the common occurrence of pronounced bleedings. Nevertheless one monoclonal antibody, named AJvW2, which was first isolated from a murine moAb blocking the vWF A1 domain in 1997, generated successful results in several *in vivo* animal studies.[74-76] In line with these animal

data the first human clinical trial, testing the humanised antibody in healthy volunteers, demonstrated effective vWF-GPIb/IX/V inhibition without an effect on bleeding time or significant clinical adverse events.[77] Although these initial results are encouraging, additional clinical studies are needed to further investigate whether the antibody can serve as an effective and safe agent for antithrombotic treatment in humans.

Also several moAbs directed against GPIb have been developed and characterised *in vitro,* but again only little *in vivo* data exists. Again this is partly due to low cross-reactivity of the anti-GPIb monoclonal antibodies with GPIb of different species. Furthermore, severe thrombocytopenia and increased bleeding times were often observed (e.g. with AP-1, PG-1, PP4-3C, 6B4 IgG) hampering further use of these anti-GPIb moAbs *in vivo*.[78-80] However, the moAb 6B4 Fab was shown to have a strong antithrombotic effect in various *in vitro, in vivo* and *ex vivo* models in baboons without affecting platelet count or bleeding time.[80,81] Further studies with humanised 6B4 Fab are needed to confirm these results in humans and to determine the potential use of this compound for antithrombotic treatment in humans.

Drugs targeting collagen-vWF interaction

Collagen is one of the most thrombogenic subendothelial compounds triggering thrombus formation when it becomes exposed upon endothelial damage. Under low shear stress conditions platelets can directly bind to collagen, whereas under high shear rates binding of vWF to collagen is a prerequisite for platelet adhesion. The physiological importance of collagen-vWF interaction is emphasised by the clinical bleeding tendency observed in a family with impaired binding of vWF to collagen.[82] Due to the particular importance of vWF-collagen binding for platelet adhesion under high shear conditions, one can hypothesise that inhibition of vWF-collagen binding specifically decreases thrombus formation in situations of high shear stress, such as stenosed vessels, whereas haemostasis in healthy vessels will be less affected. Various compounds interfering with the binding of vWF to collagen have been developed and characterised, but only three have been evaluated for their effects *in vivo*. Although *in vitro* promising results were obtained for leech antiplatelet protein (LAPP), no effect was observed on thrombus formation in an *in vivo* baboon model.[83] In contrast, another leech protein named saratin was shown to reduce thrombus formation and stenosis without affecting platelet count or bleeding time in a rat model, and was demonstrated to reduce platelet deposition on human atherosclerotic plaques in a pig model.[84,85] *In vivo* testing of a monoclonal antibody inhibiting vWF-collagen binding (82D6A3) in baboons demonstrated a strong antithrombotic effect without significantly affecting bleeding time, platelet count or coagulation parameters.[86] Although these results seem encouraging, further studies with a humanised form of the antibody should be performed to confirm these data in humans.

Drugs targeting collagen-GPVI and collagen-GPIa/IIa interaction

The major collagen receptor GPVI is solely expressed on megakaryocytes and platelets. It plays important roles in both platelet adhesion and subsequent activation of integrins. Since GPVI deficiency, causing impaired platelet-collagen binding, leads to only mild bleeding problems,[87,88] the GPIV-collagen interaction might serve as an attractive and safe target for antiplatelet therapy. Several drugs have been developed of which some have also been evaluated *in vivo*. Both antimurine and antihuman GPVI monoclonal antibodies have been shown to inhibit thrombosis without significantly prolonging bleeding times in mice and monkeys, respectively.[89,90] The dimeric form of soluble GPVI was expected to serve as a competitive inhibitor *in vivo,* but appeared to have no antithrombotic effect when tested in a comparative study using soluble GPVI and anti-GPVI antibodies.[91] Surprisingly the peptide EXP3179, which is an active metabolite of the angiotensin II type 1 receptor antagonist losartan, was demonstrated to specifically inhibit GPVI-mediated platelet adhesion after acute vessel injury in mice.[92] Drug-mediated interference with collagen-GPVI interaction has clearly been shown to have antithrombotic potential; however, further research including clinical studies are needed to validate this approach.

Another important collagen receptor that is present on platelets and capable of binding different types of collagen is integrin $\alpha 2\beta 1$, or glycoprotein Ia/IIa. When platelets become activated, membrane bound GPIa/IIa receptors shift from a low affinity state to a conformation with high affinity for collagen binding. The clinical relevance of GPIa/IIa mediated platelet-collagen interaction emerged from the mild bleeding tendencies observed in GPIa/IIa deficient patients. Furthermore, *in vitro* experiments studying the effect of various GPIa/IIa antagonists on platelet adhesion and aggregation, demonstrated a contributory role for GPIa/IIa in thrombus formation.[93] However, opposite results were obtained in mice, with the observation of normal platelet adhesion and absence of bleeding problems in $\alpha 2$ or $\beta 1$ deficient mice.[94] Further studies, including in a non-human primate model, should be performed to determine whether the observed differences in bleeding in mice and man are due to species differences, and next, to confirm whether drugs targeting the GPIa/IIa-collagen interaction could have antithrombotic potential in humans.

Drugs targeting GPIIb/IIIa-fibrinogen or -vWF interactions

In contrast to the previously discussed compounds that inhibit collagen-vWF-GPIb/IX/V interactions

and thus hamper the initial step of platelet adhesion to thrombogenic surfaces, GPIIb/IIIa blocking agents interfere with the final step of platelet aggregation which is comprised of fibrinogen binding to platelet receptor GPIIb/IIIa. GPIIb/IIIa is the receptor that is most abundantly present on the platelet surface.[95] Upon platelet activation GPIIb/IIIa shifts from its latent to its active confirmation which enables the activated platelet receptor to interact with various adhesive proteins present in the plasma, including fibrinogen, vWF, vitronectin and fibronectin. Binding of platelets to other platelets however, is exclusively mediated by fibrinogen and vWF. Clinical evidence for the essential role of GPIIb/IIIa in platelet aggregation and thrombus formation in humans is provided by patients affected with the bleeding disorder, Glanzmann's thrombasthenia. In Glanzmann's thrombasthenia specific gene mutations cause an absence or non-functionality of the GPIIb/IIIa receptor resulting in impaired platelet aggregation which clinically presents with a life-long severe bleeding tendency. The potential antithrombotic effect of GPIIb/IIIa inhibition was demonstrated in both GPIIb/IIIa deficient mice (demonstrating a bleeding tendency similar to that seen in patients with Glanzmann's thrombasthenia) and mice treated with monoclonal anti-GPIIb/IIIa antibodies, which were shown to be protected from thrombosis in several thrombosis models using various thrombosis inducing stimuli.[96,97] A more detailed overview of the development of moAbs antagonising GPIIb/IIIa starting from the early 1980s is provided by De Meyer *et al.*[73] Although the first reports on the use of anti-GPIIb/IIIa moAbs were somewhat disappointing since side effects such as thrombocytopenia and significant bleeding were common,[98-101] more recent work has pointed to the beneficial effects of competitive GPIIb/IIIa-binding compounds in the prevention of platelet aggregation and thrombus formation. Although the GPIIb/IIIa antagonists abciximab, eptifibatide and tirofiban are currently approved by the FDA and used to manage myocardial infarction and coronary syndromes in humans, limitations remain, of which the risk of bleeding complications is the most important. One monoclonal antibody that might serve as an effective but safer alternative is the humanised antibody YM337 which recognises platelet glycoprotein IIb/IIIa specifically, whereas abciximab also binds other integrins such as $\alpha v\beta 3$ and $\alpha M\beta 2$. In both, animal and human studies, YM337 has been shown to effectively inhibit thrombus formation without affecting bleeding times.[102-104] However, in contrast to *ex vivo* antiplatelet experiments performed in rhesus monkeys, where a significantly smaller effect on template bleeding times was observed for YM337 compared to abciximab treatment,[105] no difference in bleeding time was observed in healthy human individuals treated with either YM377 or abciximab.[104] Further studies should be performed to confirm the potential use of YM337 as an antithrombotic drug in humans.

CONCLUSION

Platelet-vessel wall interactions play important roles in thrombus formation, either under physiological circumstances serving as a first line of defence against bleeding, or under pathological circumstances such as atherosclerosis or inflammation, where complex interactions between circulating platelets and the vascular wall can result in platelet adhesion, aggregation and finally pathological thrombus formation contributing to disease. Interactions between platelets and the vascular endothelium are mediated by various cellular receptors present on the surface of platelets and endothelial cells, adhesive proteins such as von Willebrand factor and fibrinogen, and important regulatory proteins including PECAM-1 and ADAMTS-13. The improved understanding of the different proteins involved in platelet-vessel wall interaction has led to the development of effective antithrombotic drugs targeting these processes at various levels. In the recent past several drugs targeting the collagen-vWF-GPIb/IX/V axis (interfering with collagen-GPVI or collagen-GPIa/IIa binding, or antagonising interactions between receptor platelet GPIIb/IIIa IPV and fibrinogen or vWF) have been developed and tested *in vivo*. Although encouraging results have been obtained and some drugs, including the GPIIb/IIIa-antagonists abciximab, eptifibatide and tirofiban, are currently approved by the FDA and used for the management of myocardial infarction and coronary syndromes in humans, some major limitations remain to be overcome of which the risk of (major) bleeding is the most important. Further studies should confirm whether the various developed compounds can actually serve as effective and safe alternatives for the treatment and prevention of thrombosis in humans.

REFERENCES

1. Hawiger JJ. Adhesive interactions of blood cells and vascular wall in hemostasis and thrombosis. In: Colman RW, Hirsch J, Marder V, Salzman E, editors. Hemostasis and Thrombosis. Basic Principles and Clinical Practice. 3 ed. Philadelphia: J.B. Lippincott; 1994. p. 639-53.
2. Ruggeri ZM. Structure and function of von Willebrand factor. Thromb Haemost. 1999;82(2):576-84.
3. Wagner DD. Cell biology of von Willebrand factor. Annu Rev Cell Biol. 1990;6:217-46.
4. Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD. Involvement of large plasma von Willebrand factor (vWF) multimers and unusually large vWF forms derived from endothelial cells in shear stress-induced platelet aggregation. J Clin Invest. 1986;78(6):1456-61.

5. Ruggeri ZM. Von Willebrand factor. Curr Opin Hematol. 2003;10(2):142-9.

6. Ware JA, Heistad DD. Seminars in medicine of the Beth Israel Hospital, Boston. Platelet-endothelium interactions. N Engl J Med. 1993;328(9):628-35.

7. Nurden AT, Nurden P. Advantages of fast-acting ADP receptor blockade in ischemic heart disease. Arterioscler Thromb Vasc Biol. 2003;23(2):158-9.

8. Andrews RK, Lopez JA, Berndt MC. Molecular mechanisms of platelet adhesion and activation. Int J Biochem Cell Biol. 1997;29(1):91-105.

9. Jung SM, Moroi M. Activation of the platelet collagen receptor integrin alpha(2)beta(1): its mechanism and participation in the physiological functions of platelets. Trends Cardiovasc Med. 2000;10(7):285-92.

10. Clemetson KJ, Clemetson JM. Platelet collagen receptors. Thromb Haemost. 2001;86(1):189-97.

11. Isenberg JS, Romeo MJ, Yu C, Yu CK, Nghiem K, Monsale J, et al. Thrombospondin-1 stimulates platelet aggregation by blocking the antithrombotic activity of nitric oxide/cGMP signaling. Blood. 2008;111(2):613-23.

12. Jurk K, Clemetson KJ, de Groot PG, Brodde MF, Steiner M, Savion N, et al. Thrombospondin-1 mediates platelet adhesion at high shear via glycoprotein Ib (GPIb): an alternative/backup mechanism to von Willebrand factor. FASEB J. 2003;17(11):1490-2.

13. Inoue O, Suzuki-Inoue K, Ozaki Y. Redundant mechanism of platelet adhesion to laminin and collagen under flow: involvement of von Willebrand factor and glycoprotein Ib-IX-V. J Biol Chem. 2008;283(24):16279-82.

14. Thiagarajan P, Kelly KL. Exposure of binding sites for vitronectin on platelets following stimulation. J Biol Chem. 1988;263(6):3035-8.

15. Furie B, Furie BC, Flaumenhaft R. A journey with platelet P-selectin: the molecular basis of granule secretion, signalling and cell adhesion. Thromb Haemost. 2001;86(1):214-21.

16. Shebuski RJ, Kilgore KS. Role of inflammatory mediators in thrombogenesis. J Pharmacol Exp Ther. 2002;300(3):729-35.

17. Ruggeri ZM. Platelets in atherothrombosis. Nat Med. 2002;8(11):1227-34.

18. Kroll MH, Hellums JD, McIntire LV, Schafer AI, Moake JL. Platelets and shear stress. Blood 1996;88(5):1525-41.

19. Gawaz M. Role of platelets in coronary thrombosis and reperfusion of ischemic myocardium. Cardiovasc Res. 2004;61(3):498-511.

20. Bhatt DL, Topol EJ. Scientific and therapeutic advances in antiplatelet therapy. Nat Rev Drug Discov. 2003;2(1):15-28.

21. Massberg S, Brand K, Gruner S, Page S, Muller E, Muller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med. 2002;196(7):887-96.

22. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. Nat Med. 2003;9(1):61-7.

23. Massberg S, Schurzinger K, Lorenz M, Konrad I, Schulz C, Plesnila N, et al. Platelet adhesion via glycoprotein IIb integrin is critical for atheroprogression and focal cerebral ischemia: an in vivo study in mice lacking glycoprotein IIb. Circulation. 2005;112(8):1180-8.

24. Burger PC, Wagner DD. Platelet P-selectin facilitates atherosclerotic lesion development. Blood. 2003;101(7):2661-6.

25. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest. 2005;115(12):3378-84.

26. Ott I, Neumann FJ, Gawaz M, Schmitt M, Schomig A. Increased neutrophil-platelet adhesion in patients with unstable angina. Circulation. 1996;94(6):1239-46.

27. Furman MI, Barnard MR, Krueger LA, Fox ML, Shilale EA, Lessard DM, et al. Circulating monocyte-platelet aggregates are an early marker of acute myocardial infarction. J Am Coll Cardiol. 2001;38(4):1002-6.

28. Levi M. Platelets in sepsis. Hematology. 2005;10(Suppl 1):129-31.

29. Topol EJ, Moliterno DJ, Herrmann HC, Powers ER, Grines CL, Cohen DJ, et al. Comparison of two platelet glycoprotein IIb/IIIa inhibitors, tirofiban and abciximab, for the prevention of ischemic events with percutaneous coronary revascularization. N Engl J Med. 2001;344(25):1888-94.

30. Kastrati A, Mehilli J, Neumann FJ, Dotzer F, ten BJ, Bollwein H, et al. Abciximab in patients with acute coronary syndromes undergoing percutaneous coronary intervention after clopidogrel pretreatment: the ISAR-REACT 2 randomized trial. JAMA. 2006;295(13):1531-8.

31. Gawaz M, Neumann FJ, Dickfeld T, Reininger A, Adelsberger H, Gebhardt A, et al. Vitronectin receptor (alpha(v)beta3) mediates platelet adhesion to the luminal aspect of endothelial cells: implications for reperfusion in acute myocardial infarction. Circulation. 1997;96(6):1809-18.

32. Bombeli T, Schwartz BR, Harlan JM. Adhesion of activated platelets to endothelial cells: evidence for a GPIIbIIIa-dependent bridging mechanism and novel roles for endothelial intercellular adhesion molecule 1 (ICAM-1), alphavbeta3 integrin, and GPIbalpha. J Exp Med. 1998;187(3):329-39.

33. Etingin OR, Silverstein RL, Hajjar DP. von Willebrand factor mediates platelet adhesion to virally infected endothelial cells. Proc Natl Acad Sci USA. 1993;90(11):5153-6.

34. May AE, Kalsch T, Massberg S, Herouy Y, Schmidt R, Gawaz M. Engagement of glycoprotein IIb/IIIa (alpha(IIb)beta3) on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. Circulation. 2002;106(16):2111-7.

35. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature. 1998;391(6667):591-4.

36. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76(2):301-14.

37. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD. Leukocyte rolling and extravasation are severely compromised in P selectin-deficient mice. Cell. 1993;74(3):541-54.

38. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO, Wagner DD. Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J Exp Med. 1995;181(6):2277-82.

39. Myers D, Jr., Farris D, Hawley A, Wrobleski S, Chapman A, Stoolman L, et al. Selectins influence thrombosis in a mouse model of experimental deep venous thrombosis. J Surg Res. 2002;108(2):212-21.

40. Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. Nat Med. 2002;8(10):1175-81.

41. Falati S, Liu Q, Gross P, Merrill-Skoloff G, Chou J, Vandendries E, et al. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. J Exp Med. 2003;197(11):1585-98.

42. Rathore V, Stapleton MA, Hillery CA, Montgomery RR, Nichols TC, Merricks EP, et al. PECAM-1 negatively regulates GPIb/V/IX signaling in murine platelets. Blood. 2003;102(10):3658-64.

43. Bonnefoy A, Legrand C. Proteolysis of subendothelial adhesive glycoproteins (fibronectin, thrombospondin, and von Willebrand factor) by plasmin, leukocyte cathepsin G, and elastase. Thromb Res. 2000;98(4):323-32.

44. Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. Blood. 2004;104(1):100-6.

45. Raife TJ, Cao W, Atkinson BS, Bedell B, Montgomery RR, Lentz SR, et al. Leukocyte proteases cleave von Willebrand factor at or near the ADAMTS13 cleavage site. Blood. 2009;114(8):1666-74.

46. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood. 2002;100(12):4033-9.

47. Zhou W, Inada M, Lee TP, Benten D, Lyubsky S, Bouhassira EE, et al. ADAMTS13 is expressed in hepatic stellate cells. Lab Invest. 2005;85(6):780-8.

48. Turner N, Nolasco L, Tao Z, Dong JF, Moake J. Human endothelial cells synthesize and release ADAMTS-13. J Thromb Haemost. 2006;4(6):1396-404.

49. Suzuki M, Murata M, Matsubara Y, Uchida T, Ishihara H, Shibano T, et al. Detection of von Willebrand factor-cleaving protease (ADAMTS-13) in human platelets. Biochem Biophys Res Commun. 2004;313(1):212-6.

50. Majerus EM, Zheng X, Tuley EA, Sadler JE. Cleavage of the ADAMTS13 propeptide is not required for protease activity. J Biol Chem. 2003;278(47):46643-8.

51. Tsai HM, Sussman II, Nagel RL. Shear stress enhances the proteolysis of von Willebrand factor in normal plasma. Blood. 1994;83(8):2171-9.

52. Furlan M, Robles R, Lammle B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood. 1996;87(10):4223-34.

53. Tsai HM, Sussman II, Ginsburg D, Lankhof H, Sixma JJ, Nagel RL. Proteolytic cleavage of recombinant type 2A von Willebrand factor mutants R834W and R834Q: inhibition by doxycycline and by monoclonal antibody VP-1. Blood. 1997;89(6):1954-62.

54. Tsai HM. Advances in the pathogenesis, diagnosis, and treatment of thrombotic thrombocytopenic purpura. J Am Soc Nephrol. 2003;14(4):1072-81.

55. Moake JL. Thrombotic microangiopathies. N Engl J Med. 2002;347(8):589-600.

56. Levy GG, Nichols WC, Lian EC, Foroud T, McClintick JN, McGee BM, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413(6855):488-94.

57. Studt JD, Hovinga JA, Antoine G, Hermann M, Rieger M, Scheiflinger F, et al. Fatal congenital thrombotic thrombocytopenic purpura with apparent ADAMTS13 inhibitor: in vitro inhibition of ADAMTS13 activity by hemoglobin. Blood. 2005;105(2):542-4.

58. Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. Blood. 2005;105(3):1085-93.

59. Ono T, Mimuro J, Madoiwa S, Soejima K, Kashiwakura Y, Ishiwata A, et al. Severe secondary deficiency of von Willebrand factor-cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: its correlation with development of renal failure. Blood. 2006;107(2):528-34.

60. Franchini M, Montagnana M, Targher G, Lippi G. Reduced von Willebrand factor-cleaving protease levels in secondary thrombotic microangiopathies and other diseases. Semin Thromb Hemost. 2007;33(8):787-97.

61. Lowenberg EC, Charunwatthana P, Cohen S, van den Born BJ, Meijers JC, Yunus EB, et al. Severe malaria is associated with a deficiency of von Willebrand factor cleaving protease, ADAMTS13. Thromb Haemost. 2010;103(1):181-7.

62. Nurden AT, Caen JP. Specific roles for platelet surface glycoproteins in platelet function. Nature. 1975;255(5511):720-2.

63. Sadler JE. New concepts in von Willebrand disease. Annu Rev Med. 2005;56:173-91.

64. Ramasamy I. Inherited bleeding disorders: disorders of platelet adhesion and aggregation. Crit Rev Oncol Hematol. 2004;49(1):1-35.

65. Harker LA. Role of platelets and thrombosis in mechanisms of acute occlusion and restenosis after angioplasty. Am J Cardiol. 1987;60(3):20-8B.

66. Rekhter MD, O'Brien E, Shah N, Schwartz SM, Simpson JB, Gordon D. The importance of thrombus organization and stellate cell phenotype in collagen I gene expression in human, coronary atherosclerotic and restenotic lesions. Cardiovasc Res. 1996;32(3):496-502.

67. Chang MC, Lin HK, Peng HC, Huang TF. Antithrombotic effect of crotalin, a platelet membrane glycoprotein Ib antagonist from venom of Crotalus atrox. Blood. 1998;91(5):1582-9.

68. Yeh CH, Chang MC, Peng HC, Huang TF. Pharmacological characterization and antithrombotic effect of agkistin, a platelet glycoprotein Ib antagonist. Br J Pharmacol. 2001;132(4):843-50.

69. Inbal A, Gurevitz O, Tamarin I, Eskaraev R, Chetrit A, Novicov I, et al. Unique antiplatelet effects of a novel S-nitrosoderivative of a recombinant fragment of von Willebrand factor, AR545C: in vitro and ex vivo inhibition of platelet function. Blood. 1999;94(5):1693-700.

70. McGhie AI, McNatt J, Ezov N, Cui K, Mower LK, Hagay Y, et al. Abolition of cyclic flow variations in stenosed, endothelium-injured coronary arteries in nonhuman primates with a peptide fragment (VCL) derived from human plasma von Willebrand factor-glycoprotein Ib binding domain. Circulation. 1994;90(6):2976-81.

71. Wells DS, Hensel R, Loullis C, Brophy P, Mullin ME, Murray E, et al. Disposition of a novel recombinant antithrombotic agent, RG 12986, in cynomolgus monkeys. Drug Metab Dispos. 1996;24(10):1102-6.

72. Azzam K, Garfinkel LI, Bal dit SC, Cisse TM, Drouet L. Antithrombotic effect of a recombinant von Willebrand factor, VCL, on nitrogen laser-induced thrombus formation in guinea pig mesenteric arteries. Thromb Haemost. 1995;73(2):318-23.

73. De Meyer SF, Vanhoorelbeke K, Ulrichts H, Staelens S, Feys HB, Salles I, et al. Development of monoclonal antibodies that inhibit platelet adhesion or aggregation as potential anti-thrombotic drugs. Cardiovasc Hematol Disord Drug Targets. 2006;6(3):191-207.

74. Kageyama S, Yamamoto H, Nagano M, Arisaka H, Kayahara T, Yoshimoto R. Anti-thrombotic effects and bleeding risk of AJvW-2, a monoclonal antibody against human von Willebrand factor. Br J Pharmacol. 1997;122(1):165-71.

75. Yamamoto H, Vreys I, Stassen JM, Yoshimoto R, Vermylen J, Hoylaerts MF. Antagonism of vWF inhibits both injury induced arterial and venous thrombosis in the hamster. Thromb Haemost. 1998;79(1):202-10.

76. Kageyama S, Yamamoto H, Nakazawa H, Yoshimoto R. Anti-human vWF monoclonal antibody, AJvW-2 Fab, inhibits repetitive coronary artery thrombosis without bleeding time prolongation in dogs. Thromb Res. 2001;101(5):395-404.

77. Machin SJ, Clarke C, Ikemura O, Kageyama S, Mackie IJ, Talbot JA, et al. A humanized monoclonal antibody against vWF A1 domain inhibits vWF:RiCof activity and platelet adhesion in human volunteers. J Thromb Haemost. 2003;1(Suppl 1):328.

78. Cadroy Y, Hanson SR, Kelly AB, Marzec UM, Evatt BL, Kunicki TJ, et al. Relative antithrombotic effects of monoclonal antibodies targeting different platelet glycoprotein-adhesive molecule interactions in nonhuman primates. Blood. 1994;83(11):3218-24.

79. Becker BH, Miller JL. Effects of an antiplatelet glycoprotein Ib antibody on hemostatic function in the guinea pig. Blood. 1989;74(2):690-4.

80. Cauwenberghs N, Meiring M, Vauterin S, van Wyck V, Lamprecht S, Roodt JP, et al. Antithrombotic effect of platelet glycoprotein Ib-blocking monoclonal antibody Fab fragments in nonhuman primates. Arterioscler Thromb Vasc Biol. 2000;20(5):1347-53.

81. Wu D, Meiring M, Kotze HF, Deckmyn H, Cauwenberghs N. Inhibition of platelet glycoprotein Ib, glycoprotein IIb/IIIa, or both by monoclonal antibodies prevents arterial thrombosis in baboons. Arterioscler Thromb Vasc Biol. 2002;22(2):323-8.

82. Ribba AS, Loisel I, Lavergne JM, Juhan-Vague I, Obert B, Cherel G, et al. Ser968Thr mutation within the A3 domain of von Willebrand factor (VWF) in two related patients leads to a defective binding of VWF to collagen. Thromb Haemost. 2001;86(3):848-54.

83. Schaffer LW, Davidson JT, Siegl PK, Gould RJ, Nutt RF, Brady SF, et al. Recombinant leech antiplatelet protein prevents collagen-mediated platelet aggregation but not collagen graft thrombosis in baboons. Arterioscler Thromb. 1993;13(11):1593-601.

84. Cruz CP, Eidt J, Drouilhet J, Brown AT, Wang Y, Barnes CS, et al. Saratin, an inhibitor of von Willebrand factor-dependent platelet adhesion, decreases platelet aggregation and intimal hyperplasia in a rat carotid endarterectomy model. J Vasc Surg. 2001;34(4):724-9.

85. Vilahur G, Duran X, Juan-Babot O, Casani L, Badimon L. Antithrombotic effects of saratin on human atherosclerotic plaques. Thromb Haemost. 2004;92(1):191-200.

86. Wu D, Vanhoorelbeke K, Cauwenberghs N, Meiring M, Depraetere H, Kotze HF, et al. Inhibition of the von Willebrand (VWF)-collagen interaction by an antihuman VWF monoclonal antibody results in abolition of in vivo arterial platelet thrombus formation in baboons. Blood. 2002;99(10):3623-8.

87. Moroi M, Jung SM, Shinmyozu K, Tomiyama Y, Ordinas A, Diaz-Ricart M. Analysis of platelet adhesion to a collagen-coated surface under flow conditions: the involvement of glycoprotein VI in the platelet adhesion. Blood. 1996;88(6):2081-92.

88. Sugiyama T, Okuma M, Ushikubi F, Sensaki S, Kanaji K, Uchino H. A novel platelet aggregating factor found in a patient with defective collagen-induced platelet aggregation and autoimmune thrombocytopenia. Blood. 1987;69(6):1712-20.

89. Nieswandt B, Schulte V, Bergmeier W, Mokhtari-Nejad R, Rackebrandt K, Cazenave JP, et al. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. J Exp Med. 2001;193(4):459-69.

90. Matsumoto Y, Takizawa H, Nakama K, et al. Ex vivo evaluation of anti-GPVI antibody in cynomolgus monkeys: dissociation between anti-platelet aggregatory effect and bleeding time. Thromb Haemost. 2006;96(2):167-75.

91. Gruner S, Prostredna M, Koch M, et al. Relative antithrombotic effect of soluble GPVI dimer compared with anti-GPVI antibodies in mice. Blood. 2005;105(4):1492-9.

92. Grothusen C, Umbreen S, Konrad I, et al. EXP3179 inhibits collagen-dependent platelet activation via glycoprotein receptor-VI independent of AT1-receptor antagonism: potential impact on atherothrombosis. Arterioscler Thromb Vasc Biol. 2007;27(5):1184-90.

93. Vanhoorelbeke K, Ulrichts H, Schoolmeester A, Deckmyn H. Inhibition of platelet adhesion to collagen as a new target for antithrombotic drugs. Curr Drug Targets Cardiovasc Haematol Disord. 2003;3(2):125-40.

94. Nieswandt B, Brakebusch C, Bergmeier W, et al. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. EMBO J. 2001;20(9):2120-30.

95. Wagner CL, Mascelli MA, Neblock DS, Weisman HF, Coller BS, Jordan RE. Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets. Blood. 1996;88(3):907-14.

96. Hodivala-Dilke KM, McHugh KP, Tsakiris DA, et al. Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest. 1999;103(2):229-38.

97. Smyth SS, Reis ED, Vaananen H, Zhang W, Coller BS. Variable protection of beta 3-integrin--deficient mice from thrombosis initiated by different mechanisms. Blood. 2001;98(4):1055-62.

98. Hanson SR, Pareti FI, Ruggeri ZM, et al. Effects of monoclonal antibodies against the platelet glycoprotein IIb/IIIa complex on thrombosis and hemostasis in the baboon. J Clin Invest. 1988;81(1):149-58.

99. Torem S, Schneider PA, Hanson SR. Monoclonal antibody-induced inhibition of platelet function: effects on hemostasis and vascular graft thrombosis in baboons. J Vasc Surg. 1988;7(1):172-80.

100. Krupski WC, Bass A, Kelly AB, Ruggeri ZM, Harker LA, Hanson SR. Interruption of vascular thrombosis by bolus anti-platelet glycoprotein IIb/IIIa monoclonal antibodies in baboons. J Vasc Surg. 1993;17(2):294-303.

101. Kotze HF, Badenhorst PN, Lamprecht S, et al. Prolonged inhibition of acute arterial thrombosis by high dosing of a monoclonal anti-platelet glycoprotein IIb/IIIa antibody in a baboon model. Thromb Haemost. 1995;74(2):751-7.

102. Kaku S, Kawasaki T, Hisamichi N, et al. Antiplatelet and antithrombotic effects of YM337, the Fab fragment of a humanized anti-GPIIb/IIIa monoclonal antibody in monkeys. Thromb Haemost. 1996;75(4):679-84.

103. Kaku S, Umemura K, Mizuno A, et al. Evaluation of a GPIIb/IIIa antagonist YM337 in a primate model of middle cerebral artery thrombosis. Eur J Pharmacol. 1998;345(2):185-92.

104. Harder S, Kirchmaier CM, Krzywanek HJ, Westrup D, Bae JW, Breddin HK. Pharmacokinetics and pharmacodynamic effects of a new antibody glycoprotein IIb/IIIa inhibitor (YM337) in healthy subjects. Circulation. 1999;100(11):1175-81.

105. Suzuki K, Sakai Y, Hisamichi N, et al. Comparison of the antiplatelet effect of YM337 and abciximab in rhesus monkeys. Eur J Pharmacol. 1997;336(2-3):169-76.

ERRATUM

Unfortunately in the photo quiz 'An 86-year-old man with a unilateral pectoral swelling' by Hunag-Bin Tasai[1,3], Chin-Chi Kuo[2,4], Jeng-Wen Huang[5*], Kuan-Yu Hung[5], which was published in Neth J Med. 2010;68(4):183, an error was made in the authors' names. The correct authors' names should have been:

Hunag-Bin Tsai[1,3], Chih-Wei Lin[2], Chin-Chi Kuo[4], Jeng-Wen Huang[5], Kuan-Yu Hung[5*]

[1]Departments of Internal Medicine and [2]Medical Imaging, Buddhist Dalin Tzu Chi General Hospital, Chiayi County, Taiwan, [3]Tzu Chi University School of Medicine, Hualien, Taiwan, [4]Departments of Internal Medicine, National Taiwan University Hospital Yun-Lin Branch, Yun-Lin, Taiwan, [5]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, *corresponding author: tel.: +886-2-23562082, fax: +886-2-239341176, e-mail: kyhung@ntu.edu.tw

We apologise for any inconvenience.

Netherlands
The Journal of Medicine

REVIEW

Hepatic veno-occlusive disease associated with toxicity of pyrrolizidine alkaloids in herbal preparations

Zhe Chen, Ji-Rong Huo*

Department of Digestive Disease, The Second Xiang-Ya Hospital of Central South University, Changsha, Hunan Province, China, *corresponding author: tel.: +86-731-85295035, e-mail: HJR198@hotmail.com

ABSTRACT

Hepatic veno-occlusive disease (VOD) is frequently linked to stem cell transplantation (SCT), mainly related to the conditioning regime, and contributes to considerable morbidity and mortality. However, pyrrolizidine alkaloid (PA)-induced VOD has long been overlooked. The pathogenesis of VOD remains poorly understood; studies suggest that endothelial cell injury, cytokines and haemostatic derangement are all involved in the pathogenesis of VOD. Until recently, treatment options have been limited and no uniformly effective therapy has been established. Thus, treatment is largely supportive and symptomatic. Ongoing work, including development of new animal models and clinical studies, is needed to help us fully understand the pathogenesis of VOD and enable us to devise effective solutions. Furthermore, it is strongly advised that supervisory measures be taken to standardise the use of herbal medication.

KEYWORDS

Hepatic veno-occlusive disease; pyrrolizidine alkaloids; coagulation parameters

INTRODUCTION

Hepatic veno-occlusive disease (VOD), first described in 1920 by Willmot and Robertson,[1] is a clinical syndrome characterised by hepatomegaly, ascites, weight gain and jaundice.[2-4] It is due to several factors. In Western countries, VOD is now recognised as a complication most commonly associated with high-dose chemotherapy and stem cell transplantation (SCT).[5,6] The incidence of VOD following SCT ranges from 5 to 70% in different reports,[7-9] depending on the variations in patients' characteristics, diverse criteria for diagnosis, sample size, variable distribution of risk factors, differences in conditioning therapy used and the capacity to diagnose early and mild VOD.[5,10] VOD has also been reported after solid organ transplantation, especially kidney transplantation, mainly related to the toxicity of azathioprine.[11,12] Many cases of VOD after kidney transplantation had been reported, and in one report, VOD occurred in up to five of the 200 patients who underwent kidney transplantation.[13-15] In addition, VOD has been described in association with other agents, such as oral contraceptives, chemotherapeutic drugs (actinomycin D, mithramycin, dacarbazine, cytosine arabinoside, 6-thioguanines, cyclophosphamide, gemtuzumab ozogamicin),[5] alcohol and radiation injury.

While hepatic impairment resulting from conventional pharmaceutical drugs is widely acknowledged, the potential hepatotoxicity of herbal preparations and other botanicals has been underestimated due to public misconception that they are harmless. They are commonly used for self-medication without supervision. Several species of pyrrolizidine alkaloid (PA)-containing plants can cause VOD and have been associated with epidemics in developing countries such as Jamaica, India, Egypt, Iraq and South Africa.[9,16,17] Most of them are results of food contamination or when PA-containing plants are misused for medical purposes. Nowadays, there is growing concern even in the developed countries over the use of PA-containing herbal remedies in the treatment of arthritis, thrombophlebitis, gout and diarrhoea. This review outlines the pathogenesis of PA-associated VOD, with an emphasis on endothelial cell injury and coagulation parameters. The current status and future directions of treatment are also discussed.

PYRROLIZIDINE ALKALOIDS

Pyrrolizidine alkaloids are a group of more than 350 natural toxins sharing a basic structure derived from esters of three necine bases: platynecine, retronecine or otonecine. They are named for their inclusion of a pyrrolizidine nucleus (a pair of linked pyrrole rings). Each pyrrole can be diagrammed as a five-sided structure with four carbons and one nitrogen forming the ring, and pyrroles are incorporated into the chlorophyll molecule.[18] It is assumed that more than 6000 plant species belonging to the families of *Compositae*, *Boraginaceae* and *Leguminosae* contain PAs at different levels and in different patterns. In turn it has been estimated that about 3% of the world's flowering plants contain one or more of the toxic PAs.[19] Among them, the *Senecio, Crotalaria, Cynoglossum, Heliotropeum, Echium* and *Symphytum* species are of particular importance due to their toxicity to livestock and humans (*table 1*).[19-21] Acute intoxications caused by PAs are characterised by hepatotoxicity and haemorrhagic liver necrosis. Long-term exposure caused hepatic megalocytosis, veno-occlusive disease in liver and to a lesser extent in the lungs, proliferation of the biliary tract epithelium, fatty liver degeneration and liver cirrhosis. Moreover, many PAs are genotoxic and carcinogenic in rodents. In humans, PAs cause primarily hepatic veno-occlusive disease.[22]

Although PAs can be found in all plant organs, they are usually concentrated more in roots than in leaves.[23,24] Research done by Couet *et al.*[23] revealed that the roots of comfrey had a range of 1400 to 8300 ppm PAs content while the leaves had only 15 to 55 ppm. It is also suggested that small, younger leaves contain more PAs than large, older leaves.[25] A necine base is the main structure of PAs.[26] A pyrrolizidine alkaloid should contain at least one 1,2 unsaturated necine base, which is usually esterified to necic acid for the induction of hepatoxicity. Different structural characteristics of PAs determine different degrees of toxicity. Its toxicity will increase if the hydroxyl groups are esterified in positions 7 and 9, if the necic acids have branched chains or are unsaturated, or if the necic acid forms a cyclic diester ring, as observed in senecionine and monocrotaline (*figure 1*).[22]

The PAs, which have minimal toxicity in their original form, are metabolised in the liver through a CYP (P450 cytochrome)

Figure 1. *Structure of two representative PAs, senecionine and monocrotaline*

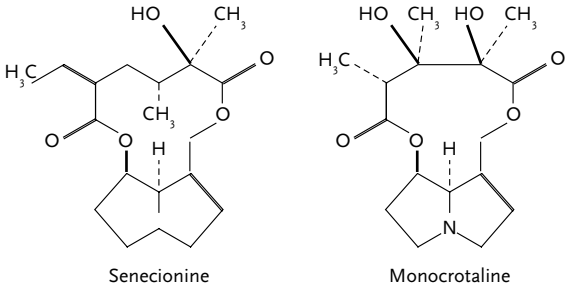


Table 1. *Common plants containing PAs*

Family	Species	Usage, constituents and toxicity
***Boraginaceae* family**	*Anchusa officinalis*	Used as an expectorant and a diuretic, also used to treat skin diseases. Total alkaloid content is about 0.12%
	Borago officinalis	It is used in Western herbs to treat inflammatory diseases and cough, also used as a diuretic. Total alkaloid content is less than 0.001%
	Cynoglossum officinale	Used to treat diarrhoea and topically for bruises. Total alkaloid content is 0.7 to 1.5%
	Helitropium arborescens	It is commonly used in Africa as medicinal herb. Contains heliotrine and indicine. Total alkaloid content is about 0.01%
	Lithospermum officinale	Used to treat gout, kidney stones, diarrhoea, and also as for contraceptive purposes. Contains lithosenine. Total alkaloid content is about 0.003%
	Myosotis scorpioides	Used as a sedative and externally as an eye wash. Contains myoscorpine, scropioidine, and symphytine. Total alkaloid content is 0.08%
	Symphytum officinale	Commonly called comfrey. It is widely used as an 'alternative'. Contains intermedine, lycopsamine, symphytine, echimidine and symglandine. Total content of PAs is about 0.5%
***Asteracea* family**	*Emilia sonchifolia*	Used to treat influenza, cough and bronchitis. Contains senkirkine and doronine. Total alkaloid content is 0.2%
	Eupatorium cannabinum	Used as an antithermic, also a diuretic. Contains supinine, rinderine, echinatine and lycopsamine
	Petasites hybridus	It is used in Europe for numerous diseases, especially abdominal pain. Contains, senecionine, integerrimine, retrosine, seneciphylline, jacobine, et cetera. Total content is about 0.01%
	Senecio aureus	Used as a treatment for injuries, also a diaphoretic and diuretic. Contains senecionine, riddelline, retrorsine, monocrotaline and otosenine
	Tussilago farfara	It is widely used in Europe to treat lung disorders and gastrointestinal disorders. Contains senkirkine and senecionine
***Fabacaea* family**	*Cortalaria spp.*	Contaminated cereal crops blamed for human poisoning. Contains crotananine, monocrotaline and cronaburmine

3A-mediated transformation to N-oxides and conjugated dienic pyrroles. Pyrroles are alkylating compounds that are highly reactive with proteins and nucleic acids. The complex of pyrroles with proteins and nucleic acids may persist in tissues and generate chronic injury, whereas N-oxides may be transformed into epoxides and toxic necines.[23,27] CYP3A inducers could enhance PAs' toxicity, while CYP3A inhibitors could decrease it. PAs can decrease glutathione (GSH) in sinusoidal endothelial cells and oxidative stress plays an important role in PA-induced VOD. It has been demonstrated that intraportal infusion of GSH can prevent VOD in monocrotaline-induced rat models; a possible reason is that GSH could conjugate with dehydromonocrotaline to form GSDHP, a compound of much lower toxicity that is released in high concentration into bile.[28,29] The enhanced oxidative stress can also affect collagen α1 transcription directly and/or through the activation of hepatic stellate cells, thus, ultimately leading to VOD.[30] Moreover, PAs can inhibit the proliferation of hepatocytes; decrease the levels of the antiapoptotic protein Bcl-x, while increasing the expression of the proapoptotic protein Bax. The latter leads to the release of cytochrome C from mitochondria and activates the intrinsic apoptotic pathway.[22]

Not everyone taking PAs-containing food or plants shows signs of VOD, and a strict dose-dependency may be absent. As we know, the most important drug-metabolising enzyme system regarding the biotransformation of exogenous compounds is cytochrome P450s, and more than 150 different cytochrome P450s have been detected. Thus, differences in enzyme activities lead to individual variabilities in handling PAs.[31] Besides, the external use of PAs is safer than oral or systemic administration given that the hepatic bioavailability is minimal. The systemic bioavailability after external use is about 20- to 50-fold lower than that after oral ingestion,[22] but the absorption of PAs will increase when inflammation or lesions are present on the skin.

HISTOPATHOLOGY AND PATHOGENESIS

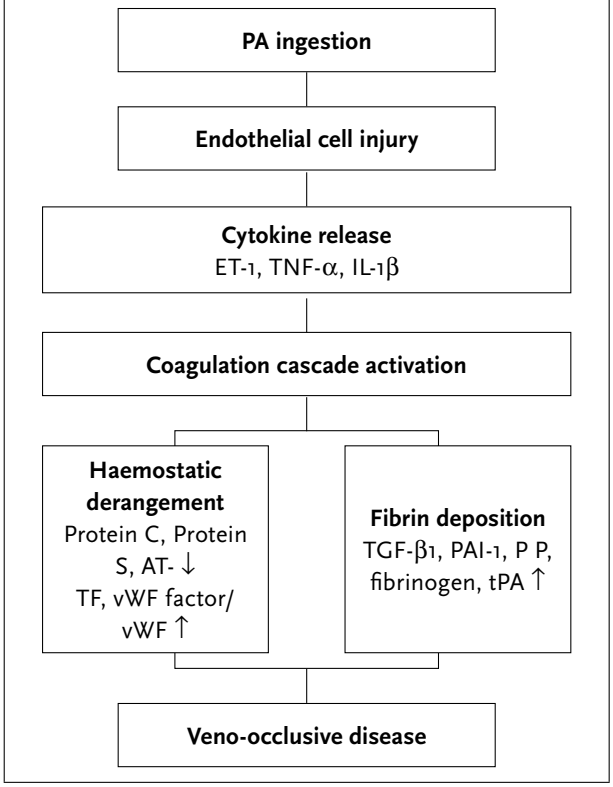
Injury to sinusoidal endothelial cells and hepatocytes in zone 3 of the liver acinus is considered to be the initial event in the development of VOD.[32-34] The reason may be that zone 3 is rich in cytochrome P450 and GST enzymes, but contains lower levels of GSH than other zones.[35-37] In fact, sinusoidal endothelial cells are more sensitive to damage than hepatocytes. This is supported by the following evidence.[37-39] First, the concentration of GSH in sinusoidal endothelial cells, which is required for the detoxification of PAs, is less than half that in hepatocytes. Second, when precursor amino acids are added to culture media, hepatocytes can synthesise GSH while sinusoidal endothelial cells cannot. Third, portal hypertension is a presenting feature of VOD rather than a late event secondary to progressive parenchymal failure. Fourth, sinusoidal endothelial cells possess the CD14 surface antigen, a receptor for lipopolysaccharide (LPS), and this makes them the first cells to be exposed to toxins and bacteria in portal blood.

Many animal models have been established to study the histological features of VOD.[40-43] Injury to the hepatic venules, which is characterised by subendothelial oedema, red cell exudation, deposition of fibrin and factor α/ von-Willebrand factor (VWF) within venular walls,[32,44] is believed to be the first histological change. In the early stage of VOD, damage to the endothelium of central venule (CV), subendothelium and sinusoidal haemorrhage, varying degrees of coagulative necrosis in the centrilobular and concentric narrowing of terminal hepatic venules and sinusoids are present, but fibrosis is mild or absent. Late VOD is characterised by subendothelial and adventitial fibrosis, while damage to CV and haemorrhage persist throughout. All these lesions distribute unevenly in the liver, and cirrhosis may ultimately appear.[9,10,42]

COAGULATION PATHWAYS

The role of coagulation pathways in the pathogenesis of VOD is still debatable (*figure 2*). Although VOD is considered a nonthrombotic vascular disease, sufficient

Figure 2. *The potential role of coagulation pathways in the pathogenesis of VOD*



evidence suggests that haemostatic derangement may be relevant to the occurrence of VOD.[5,45,46] The endothelial injury caused by PAs triggers the coagulation cascade and induces a hypercoagulable state.[10,33,34] In animal models, monocytes are recruited to the lobule and venous endothelium at an early stage.[42] VWF, thrombomodulin and several cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), endothelin-1 (ET-1), P-selection and E-selection, are released by monocytes and (or) endothelial cells[32,37,47,48] as responses to the toxicity of PAs. Both TNF-α and IL-1β are procoagulant. The expression of several coagulation factors, such as tissue factor (TF)[49,50] and plasminogen activation inhibitor-1 (PAI-1), is increased by the stimulation of TNF-α and IL-1β.[51,52] Furthermore, platelets are activated, and the expression of fibrosis markers, such as transforming growth factor beta-1 (TGFβ1)[53,54] and N-terminal propeptide for type III procollagen (PIIIP),[37,55] is increased, and these eventually lead to fibrous obliteration of the affected venules.

Decrease of natural anticoagulants, as well as changes in fibrinolytic activity, have also been found in many clinical cases of VOD. A study about changes of coagulation parameters in 44 consecutive patients who underwent allogeneic SCT suggested that PIIIP, tissue plasminogen activator (t-PA), and protein C were predictive markers for VOD ($p<0.0001$).[56] David *et al.*[57] reported that reduction of antithrombin, protein C, protein S and elevation of D-dimer, prothrombin fragment F1+2 could be detected in patients with VOD, and the elevation of PAI-1 and VWF were characteristic markers of VOD. In addition, Park *et al.*[58] suggested that increase in t-PA, PAI-1 and decrease in antithrombin-III (ATIII) might be useful markers for VOD. A consecutive study revealed that ADAMTS 13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13), a metalloproteinase produced specifically in hepatic stellate cells that cleaves unusually large VWF multimers (UL-VWFMS),[59] was involved in the pathogenesis of VOD. Activity of ADAMTS 13 was significantly reduced in VOD patients, and could be a predictor for the occurrence of VOD.[60,61]

Among all the coagulation parameters, PAI-1 and protein C are thought to be of great importance. PAI-1 is considered to be not only an independent predictor of VOD, but also associated with the severity of disease, clinical course and response to treatment.[62-64] Protein C is a sensitive and early marker of liver dysfunction due to its short plasma half-life, and in a multivariate analysis, it was the only variable that could distinguish VOD from non-VOD patients independently.[65-67]

In summary, coagulation pathways play an important part in VOD. It is, however, unclear whether these changes are involved in the pathogenesis or are merely consequences of the disease.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of VOD is based on the classical triad of weight gain, painful hepatomegaly and jaundice. Symptoms usually appear after one to two months of continuous exposure to PAs.[42] Clinically, the diagnosis is usually made in accordance with the criteria put forth by the Seattle[1] or Baltimore[68] group (*table 2*). The specificity of these two criteria is about 92%, but the sensitivity is rather low.[32] None of the signs are specific for the diagnosis. Moreover, a history of ingestion is usually difficult to obtain because patients are reluctant to divulge the use of herbal preparations, even on repeated questioning, either assuming their safety or fearing not being taken seriously for using herbs.

Histological biopsy of liver is the gold standard for the diagnosis of VOD. This procedure is usually delayed because of thrombocytopenia, clotting abnormalities and extensive ascites, which are contraindications to liver biopsy.[37,64] As an alternative, a catheter-based percutaneous transjugular approach is being used. This approach allows the measurement of hepatic venous pressure gradient (HVPG) and a liver biopsy at the same time. Studies show that an HVPG greater than 10 mmHg is correlated with VOD, the specificity, positive predictive value and sensitivity are 91%, 86% and 52%, respectively.[32,70]

Ultrasonography may also be helpful for the diagnosis by showing ascites, hepatomegaly, attenuated hepatic flow, hepatic vein dilation and thickening of gallbladder wall. These non-specific findings can be used to exclude other diseases that mimic VOD, such as extrahepatic biliary obstruction and malignant infiltration of the liver. Pulse Doppler ultrasound usually shows a decreased or

Table 2. *Diagnostic criteria for veno-occlusive disease (VOD)*

1) **Seattle criteria**[1]
Presence of at least 2 of the following 3 clinical features within 30 days after transplantation:
   1. Jaundice
   2. Hepatomegaly with right upper quadrant pain
   3. Ascites and /or unexplained weight gain

2) **Baltimore criteria**[64]
Presence of hyperbilirubinaemia (total serum bilirubin >2 mg/dl) within 21 days after transplantation and at least 2 of the following features:
   1. Hepatomegaly (usually painful)
   2. Weight gain >5% from baseline
   3. Ascites

3) **Modified Seattle criteria**[7]
Presence of 2 of the following features within 20 days after transplantation:
   1. Hyperbilirubinaemia (total serum bilirubin >2 mg/dl)
   2. Hepatomegaly or right upper quadrant pain of liver origin
   3. Unexplained weight gain (>2% of baseline body weight) because of fluid accumulation

inverted portal blood flow, which is a relatively late finding in patients with VOD.[71] Thus, it may be of prognostic value.[72] Magnetic resonance imaging findings, include hepatomegaly, hepatic vein narrowing, periportal cuffing, gallbladder wall thickening, ascites, and reduced portal venous flow velocity,[73] can be used as a method for differential diagnosis.

PROGNOSIS

The reported mortality of VOD varies from 20 to 50%. While there is a gradual resolution of symptoms in mild and moderate patients, the mortality of severe patients approaches 100%, often involving multiple organ failure (MOF). Patients can be classified into three groups[5,9,10] (mild, moderate and severe) according to the severity of disease and the outcome (*table 3*). Unfortunately, this classification is a retrospective assessment of the disease and is not useful for its management. A regression model proposed by Bearman *et al.*[74] suggests that patients who develop hyperbilirubinaemia and significant fluid retention earlier and worsen faster are at high risk of severe VOD. Research also finds that the severity of clinical features is associated with the number of histological changes rather than the occlusive of small hepatic venules.[75] Moreover, an HVPG greater than 20 mmHg indicates a poor prognosis.[74]

Table 3. *Classification of veno-occlusive disease (VOD) according to its severity*

Mild VOD
1. No adverse effects of liver disease are present
2. No treatment of VOD is needed
3. The illness is self-limited

Moderate VOD
1. Presence of an adverse effect of liver disease
2. Treatment of VOD is needed (such as diuretics for fluid retention or medication to relieve pain from hepatomegaly)

Severe VOD
1. Signs and symptoms of VOD do not resolve by day 100
2. Patients die of complications of VOD

PREVENTION

Human exposure originates from PA-containing herbs, teas and dietary supplements. Possible routes of human dietary exposure are accidental or intentional ingestion of PA-containing plants, consumption of PA-contaminated honey, and exposure through products of animal origin such as milk and eggs. Exposure may also result from the intentional consumption of herbal medicinal products. A study showed that 60% of people using herbal remedies took them along with conventional medicines and the remedies are often made by simple processes with no brand name and neither written recommendations nor warnings.[76] In most cases of PA-associated VOD, the daily intake ranges from several milligrams to hundreds of milligrams.[18] Thus, supervision and instruction of PA content in food and herb medicine are crucial.

European Food Safety Authority (EFSA) has recommended to obtain more data on carry over of PAs into milk as infants may have high exposure via this pathway. Also highlighted was the need for quantitative assessment of the contribution of honey to human exposure, as honey is found to contain residual amounts of PA metabolites.[77] Some countries and organisations have set standards for the use of PA-containing herb preparations. In the USA and many European countries, herbal supplements must be approved by national public health institutions before sale. Many herbs known to contain toxic PAs and to be potentially damaging, such as Senecio, are not allowed as ingredients of herbal supplements. Regulatory efforts have been taken by many countries to prevent these injuries: the German government has set a limit for daily exposure to PAs of no more than 0.1 µg for less than six weeks per year;[19] in Belgium the limit for PAs in herbs is 1 ppm (1 µg per gram of herb); the American Herb Products Association (AHPA) recommends that all products with botanical ingredients which contain toxic pyrrolizidine alkaloids bear the following cautionary statement on the label: For external use only. Do not apply to broken or abraded skin. Do not use when nursing.[18] In addition, extract manufactures are seeking methods of removing the PAs from their finished products. In China, the government should support timely risk management actions as real and potential risks to the health of consumers occur. The Chinese traditional herbal medicine (*table 4*) should meet specific and appropriate standards of safety and quality and the herbal products should be accompanied by necessary information for safe use.

TREATMENT

The success of treatment for VOD depends on early diagnosis and early intervention. Various analytical techniques, particularly chromatography methods in conjunction with mass spectrometry, can be used to detect PAs in plants or plant-derived products. It is of great importance to avoid further contact with the suspicious toxin as soon as possible once the diagnosis is confirmed or symptoms appear. So far, there is no uniformly effective treatment for VOD, and supportive care in established VOD is the cornerstone of management. The purpose of supportive care is to maintain intravascular volume and renal perfusion without causing extravascular fluid accumulation.[2] General measures include restriction of water and sodium supply together with the usage of

Table 4. *Commonly used Chinese herbs that are currently known to contain pyrrolizidine alkaloid*

Chinese herbs	Plant species	Effectiveness and constituents
Herba crotalariae asssamicae	*Crotalaria asamica Benth.*	Mainly used to treat cough, swelling and toothache. Contains monocrotaline. Alkaloid content is 2-3% in stems and leaves
Herba crotalariae sessiliflorae	*Crotalaria sessiliflora L.*	Used in the treatment of bruises, also as an anticancer agent. Contains monocrotaline, reteonecine and platynecic acid
Radix cynoglossi officinalis	*Cynoglossum officinale L.*	Used to clear heat and expectoration, also useful for the treatment of cough, aphonia and rhinorrhagia. Contains heliosupine, lasiocarpine, heliotrine and platyphylline. The concentration is less than 1%
Gynura root	*Gynura segetum (lour.) Merr.*	Used extensively in Chinese folk medicine to promote microcirculation, relieve pain and cure injury. It contains at least five kinds of PAs, such as senecionine, seneciphylline and integerrimine. Many cases of Gynura root-related VOD have been reported
Senecio scandens	*Senecio scandens Buch.-Ham.*	It can clear away the heat-evil and expel superficial evils. In traditional Chinese medicine, it is widely used to treat acute inflammatory diseases, such as bacterial diarrhoea, enteritis. It contains nine hepatotoxic PA (senecionine, seneciphylline, et al.) with a content of 6.95–7.19 mg/g
Flos farfarae	*Tussilago farfara L.*	Used to relieve cough and sputum. Contains tussilagine, isotussilagine, senkirkine and senecionine
Herba tephroseritis kirilowii	*Senecio kirilowii Turcz. ex.*	Used as an antipyretic-detoxicate drug, also a diuretic. Contains senecionine, seneciphylline and integerrimine
Liparis japonica	*DC.* *Liparis japonicus (Miq.) Maxim.*	Used to promote blood circulation to restore menstrual flow, also as a cardiac tonic and a sedative

loop diuretics and spironolactone. Avoidance of other hepatotoxic drugs is as important as the application of drugs that have protective effects on the liver. The role of albumin or other colloids is debatable: it can help maintain the intravascular volume in patients with severe hypoalbuminaemia, but will eventually accumulate in extravascular spaces. Low-dose dopamine is recommended if renal dysfunction appears. Paracentesis can be used to reduce the ascites and relieve symptoms such as abdominal distention and dyspnoea. When fluid overload or renal failure is present, haemodialysis is needed. Mechanical ventilation may be helpful to relieve respiratory failure.[5,9,32,78,79] Transjugular intrahepatic portosystemic shunt (TIPS) has been used to relieve refractory ascites and portal hypertension, but the outcome is poor in many reports.[80,81] The recent review on clinical practice guidelines for TIPS did not recommend TIPS for VOD.[82] Finally, if hepatic failure is imminent, orthotopic liver transplantation is necessary,[83] but a suitable liver donor is seldom readily available.

Since cytokine activation may be involved in the pathogenesis of VOD, inhibitors of cytokines may be useful. As a potent inhibitor of cytokine production, the effect of methylprednisolone is promising. In a report, Khoury *et al.*[49] used high-dose methylprednisolone (a dose of 500 mg/m^2 intravenously every 12 hours for a total of six doses) to treat 20 patients with VOD, where the response rate was 60%. In another report, 48 patients with VOD were treated with methylprednisolone (a dose of 0.5 mg/kg *iv* every 12 hours for a total of 14 doses and then discontinued without taper), 30 patients (63%) responded with a reduction in bilirubin of 50% or more.[84] The regimen was well tolerated with minimal side effects. Despite these encouraging results, randomised controlled trials are required to determine the exact effect of methylprednisolone for the treatment of VOD. Pentoxifylline, a modulator of TNF-α which inhibits the transcription of TNF messenger RNA, was reported to have a protective effect against VOD with no significant adverse effects.[85] Further studies are needed to prove the therapeutic effect of pentoxifylline.

Based upon the histological findings of fibrin deposition and intense factor VIII/VWF staining in VOD, as well as the coagulation abnormalities observed, thrombolytic therapy with or without anticoagulation has been developed. Several reports about the use of t-PA have been published, but most of them are case reports or research studies including no more than ten patients.[86-88] In the largest study so far, 42 cases of VOD were treated with the combination of recombinant human tissue plasminogen activator (rh-tPA) and heparin. The response rate was 29%. Major bleeding occurred in 24% of the patients and 7% of the patients died of it.[89] The data suggested that the therapeutic effect of t-PA was better with earlier application, but once multiorgan dysfunction developed, it was unlikely to be successful. The efficacy of ATIII is controversial: it failed to be effective in a number of studies,[32] while in a trial of 48 patients being treated with ATIII, the overall mortality was decreased with no overt bleeding or thrombosis complications.[90]

One of the most promising agents being used is defibrotide (DF). DF is a large, single-stranded polydeoxyribonucleotide derived from porcine mucosa. It is identified to have antithrombotic, anti-ischaemic, anti-inflammation

and thrombolytic properties without significant systemic anticoagulant effects.[91] The efficacy of DF may due to its ability to increase levels of endogenous prostaglandins (PGI_2 and E_2), stimulate the expression of thrombomodulin, and increase the function of t-PA while decreasing the activity of PAI-1.[92-95] It is well tolerated: adverse events, such as flushing, nausea, and gastrointestinal disturbances, are slight (incidence ranging from 1 to 9%). Chopra *et al.*[96] reported a European multicentre programme of using DF to treat 40 patients with VOD. Overall, 17 out of 40 patients showed complete response to DF (42.5%) with no significant toxicity. Treatment of severe VOD with DF is also encouraging. In a report, 19 patients with severe VOD were treated with DF. The dose ranged from 5 to 60 mg/kg/day, and the improvement of symptoms was seen in eight patients (42%). Six of the eight responders survived 100 days after transplantation, contrasted with the 2% survival reported in comparable patients.[97] A multicentre, phase II trial was conducted to confirm the effect and safety of DF. In the trial, 150 patients with severe VOD and MOF were randomised to 25 mg/kg/day or 40 mg/kg/day of DF. A complete response rate of 46% was observed with 41% survived at day 100 after transplantation and 25 mg/kg/day was the preferred dose.[98] Although many therapeutic modalities with intriguing results have been reported, further multicentre randomised-controlled trials either as individual therapy or in combination with others are needed.

So far, literature about PA-associated VOD is only in the form of case reports, and the available reports do not provide sufficiently reliable data to be used in establishing a health-based guidance. The majority of the large-scale clinical trials on treatment are about VOD after SCT. Nevertheless, similar pathogenesis and limited case reports suggest that these methods may be equally useful for VOD caused by PAs.

CONCLUSIONS

Hepatoxicity of PAs has perplexed human beings for more than 80 years, since senecio poisoning was first described in South Africa.[4] Many studies on animal models and patients with VOD have been done. The use of biological markers may allow an earlier diagnosis while the exact pathogenesis is still unknown. Recent studies suggest that coagulation pathways may play an important role in the pathogenesis of VOD. Clinically, thrombolytic therapy, with or without anticoagulant agents, has been tried with unfavourable results. The bleeding complication is sometimes fatal. DF is a promising therapy, but further studies are needed to determine who to treat, when to treat them and how much to treat them with. As no uniformly effective treatment is present, prevention of this formidable toxicity of PAs is of great importance. And a better understanding of the pathogenesis of VOD is essential if we are to improve the survival rate.

REFERENCES

1. Willmot FC, Robertson GW. Senecio disease, or cirrhosis of the liver due to Senecio poisoning. Lancet. 1920;2:848-9.
2. McDonald GB, Sharma P, Matthews DE, Shulman HM, Thomas ED. Venoocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. Hepatology. 1984;4:116-22.
3. Bayraktar UD, Seren S, Bayraktar Y. Hepatic venous outflow obstruction: three similar syndrome. World J Gastroenterol. 2007;13:1912-7.
4. Richardson PG, Guinan EC. The pathology, diagnosis and treatment of hepatic veno-occlusive disease: current status and novel approaches. Br J Haematol. 1999;107:485-93.
5. Kumar S, Deleve LD, Kamath PS, Tefferi A. Hepatic veno-occlusive disease (Sinusoidal Obstruction Syndrome) after hematopoietic stem cell transplantation. Mayo Clin Proc. 2003;78:589-98.
6. Gozdzik J, Krasowska-kwiecien A, Wedrychowicz A. Sinusoidal obstruction disease (SOS), previous hepatic venoocclusive disease (VOD)—still serious complication after hematopoietic stem cell transplantation. Przegl Lek. 2008;65: 203-8.
7. Shulman HM, Hinterberger W. Hepatic veno-occlusive disease—liver toxicity syndrome after bone marrow transplantation. Bone Marrow Transplant. 1992;10: 197-214.
8. Carreras E, Bertz H, Arcese W, et al. Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: a prospective cohort study of the European group for blood and marrow transplantation. Blood. 1998;92: 3599-604.
9. Helmy A. Review article: updates in the pathogenesis and therapy of hepatic sinusoidal obstruction syndrome. Aliment Pharmacol Ther. 2006;23:11-25.
10. Baron F, Deprez M, Beguin Y. The veno-occlusive disease of the liver. Haematologica. 1997;82:718-25.
11. Azoulay D, Castaing D, Lemoine A, et al. Successful treatment of severe azathioprine-induced hepatic veno-occlusive disease in a kidney-transplanted patient with transjugular intrahepatic portosystemic shunt. Clin Nephrol. 1998;50:118-22.
12. Hola K, Brahm J, Alvo M, et al. Hepatic veno-occlusive disease associated to the use of azathioprine in a renal transplant recipient. Rev Med Chil. 1995;124:1489-91.
13. Jeffries MA, McDonnell WM, Tworek JA, Merion RM, Moseley RH. Venoocclusive disease of the liver following renal transplantation. Dig Dis Sci. 1998;43:229-34.
14. Liano F, Moreno A, Matesanz R, et al. Veno-occlusive hepatic disease of the liver in renal transplantation: is azathioprine the cause? Nephron. 1989;51:509-16.
15. Adler M, Delhaye M, Deprez C, et al. Hepatic vascular disease after kidney transplantation: report of two cases and review of literature. Nephrol Dial Transplant. 1987;2:183-8.
16. Tandon BN, Tandon HD, Tandon RK, Narndranathan M, Joshi YK. An epidemic of hepatic veno-occlusive disease in central India. Lancet. 1976;2:271-2.
17. Moayad YA, Muntaha H. An outbreak of veno-occlusive disease of the liver in northern Iraq. Eastern Mediterr Health J. 1998;4:142-8.
18. Dharmananda S. Safety issues affecting herbs: pyrrolizidine alkaloids. Available from URL: http://www.itmonline.org/arts/pas.htm 2001.
19. Stegelmeier BL, Edgar JA, Colegate SM, et al. Pyrrolizidine alkaloid plants, metabolism and toxicity. J Nat Toxins. 1999;8:95-116.
20. International programme on chemical safety. Geneva: World Health Organization; 1998; pp 1-232.

21. Huxtable RJ. Human health implication of pyrrolizidine alkaloids and herbs containing them. In: Cheeke PR, editor. Toxicants of plant origins. Boca Raton, FL: CRC Press Inc. 2001;p.41-79.

22. Chojkier M. Hepatic sinusoidal-obstruction syndrome: toxicity of pyrrolizidine alkaloids. J Hepatol. 2003;39:437-66.

23. Couet CE, Crews C, Hanley AB. Analysis, separation, and bioassay of pyrrolizidine alkaloids from comfrey (symphytum officinale). Nat Toxin. 1996;4:163-7.

24. Hartmann T, Ehmke A, Eilert U, et al. Sites of synthesis, translocation and accumulation of pyrrolizidine alkaloid N-oxides in Senecio vulgaris L. Planta. 1989;177:98-107.

25. Mattocks AR. Toxic pyrrolizidine alkaloids in comfrey. Lancet. 1980;2:1136-7.

26. Mattocks AR. Toxicity of pyrrolizidine alkaloids. Nature. 1968;217:723-8.

27. Yang YC, Yan J, Churchwell M, et al. Development of a ^{32}P-postlabeling/HPLC method for detection of dehydroretronecine-derived DNA adducts in vivo and in vitro. Chem Res Toxicol. 2001;14:91-100.

28. Wang XD, Kanel GC, Deleve LD. Support of sinusoidal endothelial cell glutathione prevents hepatic veno-occlusive disease in the rat. Hepatology. 2000;31:428-34.

29. Yan CC, Huxtable RJ. Effects of monocrotaline, a pyrrolizidine alkaloid, on glutathione metabolism in the rat. Biochem Phamacol. 1996;51:375-9.

30. Chojkier M, Houglum K, Lee KS, et al. Long and short term D-alpha-tocopherol supplementation inhibits liver collagen alpha (1) gene expression. Am J Physiol. 1998;275:1480-5.

31. Stickel F, Egerer G, Seitz HK. Hepatotoxicity of botanicals. Public Health Nutrition. 2000;3:113-24.

32. Wadleigh M, Ho V, Momtaz P, Richardson P. Hepatic veno-occlusive disease: pathogenesis, diagnosis and treatment. Curr Opin Hematol. 2003;10:451-62.

33. Lee JH, Lee KH, Kim S, et al. Relevance of protein C and S, antithrombin III, von Willebrand factor, and factor VIII for the development of hepatic veno-occlusive disease in patients undergoing allogeneic bone marrow transplantation: a prospective study. Bone Marrow Transplant. 1998;22:883-8.

34. Peres E, Kintzel P, Dansey R, et al. Early intervention with antithrombin III therapy to prevent progression of hepatic venoocclusive disease. Blood Coagul Fibrinolysis. 2008;19:203-7.

35. el Mouelhi M, Kauffman FC. Sublobular distribution of transferases and hydrolases associated with glucuronide, sulfate and glutathione conjugation in human liver. Hepatology. 1986;6:450-6.

36. Kera Y, Penttila KE, Lindros KO. Glutathione replenishment capacity is lower in isolated perivenous than in periportal hepatocytes. Biochem J. 1988;254:411-7.

37. Coppell JA, Brown SA, Perry DJ. Veno-occlusive disease: cytokines, genetics, and haemostasis. Blood Rev. 2003;17:63-70.

38. Deleve LD. Dacarbazine toxicity in murine liver cells: a model of hepatic endothelial injury and glutathione defense. J Pharmacol Exp Ther. 1994;268:1261-70.

39. Deleve LD. Glutathione defense in non-parenchymal cells. Semin Liver Dis. 1998;18:403-13.

40. Chen MY, Cai JT, Du Q, Wang LJ, Chen JM, Shao LM. Reliable experimental model of hepatic veno-occlusive disease caused by monocrataline. Hepatobiliary Pancreat Dis Int. 2008 ;7:395-400.

41. Yeong ML, Clark SP, Waring JM, Wilson RD, Wakefield SJ. The effects of comfrey derived pyrrolizidine alkaloids on rat liver. Pathology. 1991;23:35-8.

42. Deleve LD, McCuskey RS, Wang XD, et al. Characterization of a reproducible rat model of hepatic veno-oclcusive disease. Hepatology. 1999;29:1779-91.

43. Gao XSh, Xiao ShSh, He JF. Analysis of alkaloids in Sedum aizoon and establishment of hepatic veno-occlusive model in mice. Chin J Integr Trad West Med Dig. 2006;14:311-3.

44. Shulman HM, Gown AM, Nugent DJ. Hepatic veno-occlusive disease after bone marrow transplantation. Immunohistochemical identification of the material within occluded central venules. Am J Pathol. 1987;127:549-58.

45. Scrobohaci ML, Drouet L, Monem-Mansi A, et al. Liver veno-occlusive disease after bone marrow transplantation changes in coagulation parameters and endothelial markers. Thromb Res. 1991;63:509-19.

46. Faioni EM, Mannucci PM. Venoocclusive disease of the liver after bone marrow transplantation: the role of hemostasis. Leuk Lymphoma. 1997;25:233-45.

47. Castenskiold EC, Kelsey SM, Collins PW, et al. Functional hyperactivity of monocytes after bone marrow transplantation: possible relevance for the development of post-transplant complications or relapse. Bone Marrow Transplant. 1995;15:879-84.

48. Collins PW, Gutteridge CN, O'Driscoll A, et al. von Willebrand factor as a marker of endothelial cell activation following BMT. Bone Marrow Transplant. 1992;10:499-506.

49. Khoury H, Adkins D, Brown R, et al. Dose early treatment with high-dose methylprednisolone after the course of hepatic regimen-related toxicity? Bone Marrow Transplant. 2000;25:737-43.

50. Ho VT, Revta C, Richardson PG. Hepatic veno-occlusive disease after hematopoietic stem cell transplantation: update on defibrotide and other current investigational therapies. Bone Marrow Transplant. 2008;41:229-37.

51. Fearns C, Loskutoff DJ. Induction of plasminogen activator inhibitor 1 gene expression in murine liver by lipopolysaccharide. Cellular localization and role of endogenous tumor necrosis factor-alpha. Am J Pathol. 1997;150:579-90.

52. Speiser W, Kapiotis S, Kopp CW, et al. Effect of intradermal tumor necrosis factor-α-induced inflammation on coagulation factors in dermal vessel endothelium. Thromb Haemost. 2001;85:362-7.

53. Pihusch V, Pihusch W, Penovici M, Kolb HJ, Hiller E, Pihusch R. Transforming growth factor beta-1 released from platelets contributes to hypercoagulability in veno-occlusive disease following hematopoetic stem cell transplantation. Thromb Res. 2005;116:233-40.

54. Anscher MS, Peters WP, Reisenbichler H, Petros WP, Jirtle RL. Transforming growth factor beta as a predictor of liver and lung fibrosis after autologous bone marrow transplantation for advanced breast cancer. N Engl J Med. 1993;328:1592-8.

55. Eltumi M, Trivedi P, Hobbs J, et al. Monitoring of veno-occlusive disease after bone marrow transplantation by serum aminopropepide of type III procollagen. Lancet. 1993;342:518-21.

56. Tanikawa S, Mori S, Ohhashi K, et al. Predictive markers for hepatic veno-occlusive disease after hematopoietic stem cell transplantation in adults: a prospective single center study. Bone Marrow Transplant. 2000;26:881-6.

57. David M, Toth O, Nagy A, et al. Pathogenesis of hepatic veno-occlusive disease in patients undergoing hematopoietic stem cell transplantation. 35th Hemophilia Symposium Hamburg 2004.

58. Park YD, Yasui M, Yoshimoto T, et al. Changes in hemostatic parameters in hepatic veno-occlusive disease following bone marrow transplantation. Bone Marrow Transplant. 1997;19:915-20.

59. Uemura M, Tatsumi K, Matsumoto M, et al. Localization of ADAMTS 13 to the stellate cells of human liver. Blood. 2005;106:922-4.

60. Park YD, Yoshioka A, Kawa K, et al. Impaired activity of plasma von Willebrand factor-cleaving protease may predict the occurrence of hepatic veno-occlusive disease after stem cell transplantation. Bone Marrow Transplant. 2002;29:789-94.

61. Matsumoto M, Kawa K, Uemura M, et al. Prophylactic fresh frozen plasma may prevent development of hepatic VOD after stem cell transplantation via ADAMTS 13-mediated restoration of von Willebrand factor plasma levels. Bone Marrow Transplant. 2007;40:251-9.

62. Kaleelrahman M, Eaton JD, Leeming D, et al. Role of plasminogen activator inhibitor-1 (PAI-1) levels in the diagnosis of BMT-associated hepatic veno-occlusive disease and monitoring of subsequent therapy with defibrotide (DF). Hematology. 2003;8:91-5.

63. Pihusch M, Wegner H, Goehring P, et al. Diagnosis of hepatic veno-occlusive disease by plasminogen activator inhibitor-1 plasma antigen levels: a prospective analysis in 350 allogeneic hematopoietic stem cell recipients. Transplantation. 2005;80:1376-82.

64. Sartori MT, Spiezia L, Cesaro S, et al. Role of fibrinolytic and clotting parameters in the diagnosis of liver veno-occlusive disease after

hematopoietic stem cell transplantation in a pediatric population. Thromb Haemost. 2005;93:682-9.

65. Faioni EM, Krachmalnicoff A, Bearman SI, et al. Naturally occurring anticoagulants and bone marrow transplantation: plasma protein C predicts the development of venocclusive disease of the liver. Blood. 1993;81:3458-62.

66. Scrobohaci ML, Drouet L, Monem-Mansi A, et al. Liver veno-occlusive disease after bone marrow transplantation changes in coagulation parameters and endothelial markers. Thromb Res. 1991;63:509-19.

67. Yoshimoto K, Ono N, Okamura T, Sata M. Recent progress in the diagnosis and therapy for veno-occlusive disease of the liver. Leuk Lymphoma. 2003;44:229-34.

68. Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44:778-83.

69. Steenkamp V, Stewart MJ, Zuckerman M. Clinical and analytical aspects of pyrrolizidine poisoning caused by South African traditional medicines. Ther Drug Monit. 2000;22:302-6.

70. Shulman HM, Gooley T, Dudley MD, et al. Utility of transvenous liver biopsies and wedged hepatic venous pressure measurement in sixty marrow transplant recipients. Transplantation. 1995;59:1015-22.

71. Hommeyer SC, Teefey SA, Jacobson AF, et al. Venocclusive disease of the liver: prospective study of US evaluation. Radiology. 1992;184:683-6.

72. Lassau N, Auperin A, Leclere J, Bennaceur A, Valteau-Couanet D, Hartmann O. Prognostic value of doppler-ultrasonography in hepatic veno-occlusive disease. Transplantation. 2002;74:60-6.

73. van den Bosch MA, van Hoe L. MR imaging findings in two patients with hepatic veno-occlusive disease following bone marrow transplantation. Eur Radiol. 2000;10:1290-3.

74. Bearman SI, Anderson GL, Mori M, Hinds MS, Shulman HM, McDonald GB. Venoocclusive disease of liver: development of a model for predicting fatal outcome after marrow transplantation. J Clin Oncol. 1993;11:1729-36.

75. Shulman HM, Fisher LB, Schoch HG, Henne KW, McDonald GB. Veno-occlusive disease of the liver after marrow transplantation: histological correlates of clinical signs and symptoms. Hepatology. 1994;19:1171-81.

76. Firenzuoli F, Gori L, Ippolito FM. Symposium on pharmacovigilance of herbal medicines, London, March 28, 2006. Evid Based Complement Alternat Med. 2007.

77. Scientific Panel Members. Opinion of the scientific panel on contaminants in the food chain on a request from the European Commission related to pyrrolizidine alkaloids as undesirable substances in animal feed. The EFSA J. 2007;447:1-50.

78. Rubenfeld GD, Crawford SW. Withdrawing life support from mechanically ventilated recipients of bone marrow transplants: A case for evidence-based guidelines. Ann Intern Med. 1996;125:625-33.

79. Leroy B, Le Franc P, Thomas P, Colombel F, Scherpereel P. Hepatic veno-occlusive disease caused by Deticene: a cause of acute hypovolemic shock. Ann Fr Anesth Reanim. 1990;9:550-2.

80. Azoulay D, Castaing D, Lemoine A, Hargreaves GM, Bismuth H. Transjugular intrahepatic portosystemic shunt (TIPS) for severe veno-occlusive disease of the liver following bone marrow transplantation. Bone Marrow Transplant. 2000;25:987-92.

81. Rajvanshi P, McDonald GB. Expanding the use of transjugular intrahepatic portosystemic shunts for veno-occlusive disease. Liver Transplant. 2001;7:154-9.

82. Boyer TD, Haskal ZJ. The role of transjugular intrahepatic portosystemic shunt in the management of portal hepertension. Hepatology. 2005;41:386-400.

83. Membreno FE, Ortiz J, Foster PF, et al. Liver transplantation for sinusoidal obstructive syndrome (veno-occlusive disease): case report with review of the literature and the UNOS database. Clin Transplant. 2008;22:397-404.

84. Al Beihany A, Al Omar H, Sahovic E, et al. Successful treatment of hepatic veno-occlusive disease after myeloablative allogeneic hematopoietic stem cell transplantation by early administration of a short course of methylprednisolone. Bone Marrow Transplant. 2008;41:287-91.

85. Bianco JA, Appelbaum FR, Nemunaitis J, et al. Phase I-II trial of pentoxifylline for the prevention of transplant-related toxicities following bone marrow transplantation. Blood. 1991;78:1205-11.

86. Bearman SI, Shuhart MC, Hinds MS, McDonald GB. Recombinant human tissue plasminogen activator for the treatment of established severe venocclusive disease of the liver after bone marrow transplantation. Blood. 1992;80:2458.

87. Rosti G, Bandini G, Belardinelli A, et al. Alteplase for hepatic veno-occlusive disease after bone-marrow transplantation. Lancet. 1992;339:1481.

88. Hagglund H, Ringden O, Ljungman P, Winiarski J, Ericzon B, Tyden G. No beneficial effects, but severe side effects caused by recombinant human tissue plasminogen activator for treatment of hepatic veno-occlusive disease after allogeneic bone marrow transplantation. Transplant Proc. 1995;27:3535.

89. Bearman SI, Lee LJ, Baron AE, McDonald GB. Treatment of hepatic venocclusive disease with recombinant human tissue plasminogen activator and heparin in 42 marrow transplant patients. Blood. 1997;89:1501-6.

90. Edward Peres, Polly Kintzel, Roger Dansey, et al. Early intervention with antithrombin III therapy to prevent progression of hepatic venoocclusive disease. Blood Coagul Fibrinolysis. 2008;19:203-7.

91. Morabito F, Gentile M, Gay F, et al. Insights into defibrotide: an update review. Expert Opin Boil Ther. 2009;9:763-72.

92. Berti F, Rossoni G, Biasi G, Buschi A, Mandelli V, Tondo C. Defibrotide, by enhancing prostacyclin generation, prevents endothelin-1 induced contraction in human saphenous veins. Prostaglandins. 1990;40:337-50.

93. Zhou Q, Chu X, Ruan C. Defibrotide stimulates expression of thrombomodulin in human endothelial cells. Thromb Haemost. 1994;71:507-10.

94. Paul W, Gresele P, Momi S, Bianchi G, Page CP. The effect of defibrotide on thromboembolism in the pulmonary vasculature of mice and rabbits and in the cerebral vasculature of rabbits. Br J Pharmacol. 1993;110:1565-71.

95. Echart CL, Graziadio B, Somaini S, et al. The fibrinolytic mechanism of defibrotide: effect of defibrotide on plasmin activity. Blood Coagul Fibrinolysis. 2009;20:627-34.

96. Chopra R, Eaton JD, Grassi A, et al. Defibrotide for the treatment of hepatic veno-occlusive disease: results of the European compassionate-use study. Br J Haematol. 2000;111:1122-9.

97. Richardson PG, Elias AD, Krishnan A, et al. Treatment of severe veno-occlusive disease with defibrotide: compassionate use results in response without significant toxicity in a high-risk population. Blood. 1998;92:737-44.

98. Richardson P, Soiffer RJ, Antin JH, et al. Defibrotide (DF) for the treatment of severe veno-occlusive disease (sVOD) and multi-organ failure (MOF) post SCT: final results of a multi-center, randomized, dose-finding trial. Blood. 2006;108:178.

Netherlands
The Journal of Medicine

CASE REPORT

Intravascular lymphoma as an unusual cause of multifocal cerebral infarctions discovered on FDG-PET/CT

K. Boslooper, D. Dijkhuizen, A.W.G. van der Velden, M. Dal, J.F. Meilof, K. Hoogenberg*

Department of Internal Medicine, Martini Hospital, Groningen, the Netherlands,
*corresponding author: tel.: +31 (0)50-524 58 70, fax: +31 (0)50-524 58 89, e-mail: k.hoogenberg@mzh.nl

ABSTRACT

Intravascular large B-cell lymphoma (IVLBCL) is a rare and aggressive variant of diffuse large B-cell lymphoma with frequent involvement of the central nervous system. Its atypical presentation often delays the diagnosis and due to its aggressive behaviour, the diagnosis is made post-mortem in half of the cases. We report a case of a 67-year-old male patient presenting with speech difficulties and balance disturbances in whom a magnetic resonance imaging (MRI) scan showed multiple lesions of the white matter, denoted as embolic infarctions. He was treated for a suspected endocarditis with antibiotics, but deteriorated neurologically with persistent fever. A consecutive FDG-PET/CT revealed an increased uptake in the adrenals, of which a biopsy showed IVLBCL. The patient was successfully treated with systemic R-CHOP with intrathecal methotrexate and achieved complete remission after six cycles of chemotherapy. The potential role of FDG-PET/CT is illustrated by this case leading to an exceptional diagnosis of IVLBCL.

KEYWORDS

Intravascular diffuse large B-cell lymphoma, cerebral infarction, FDG-PET/CT

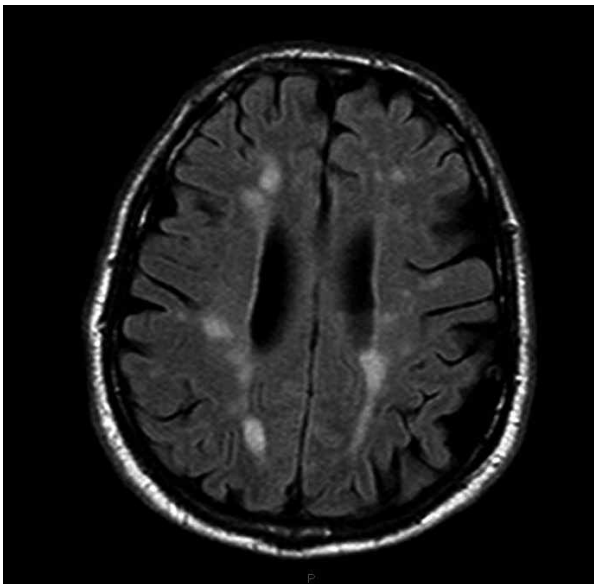
INTRODUCTION

Cerebrovascular accidents (CVA) are vascular events with a high prevalence, currently estimated to be the second cause of death in the Western world.[1] They are either ischaemic or haemorrhagic in origin, of which approximately 80% are due to ischaemia. Ischaemic CVA is usually caused by atherosclerosis, carotid artery stenosis or cardiac emboli. However, in a small minority of patients cerebral infarction may be cryptogenic of origin.[1,2] In the present report, we describe a patient with such cryptogenic multifocal cerebral infarctions and symptoms of systemic illness. A FDG-PET/CT guided towards a rare haematological disorder causing the unexplained neurological symptoms.

CASE REPORT

A 67-year-old man, with a history of hypertension and diabetes mellitus type 2, was referred to the department of neurology because of progressive speech difficulties and disturbances in balance. At physical examination he had a broad-based gait and he was remarkably slow of speech. CT scanning showed multiple small subcortical infarctions highly suspicious for ischaemic CVA. The patient was admitted to hospital and treated according to our stroke protocol. However, he slowly deteriorated and a consecutive magnetic resonance imaging (MRI) showed widespread lesions of the white matter, suspect for multiple emboli (*figure 1*). The combination of a continuous subfebrile temperature and a possible vegetation on a biscuspid aortic valve at transthoracic echocardiography raised the possibility of an endocarditis causing cerebral emboli. While blood cultures remained sterile, pragmatic treatment with intravenous antibiotics appeared to improve the condition of the patient with partial neurological recovery. However, several weeks later he was readmitted because of recurrence of neurological deficits and a relapse of the fever. The erythrocyte sedimentation rate (ESR) was 44 mm/hour (normal range (N) <15), the C-reactive protein (CRP) was 34 mg/l (N <10) and lactate dehydrogenase (LDH) was 728 IU/l (N <500). A repeated MRI disclosed new subcortical lesions. Blood cultures were negative

Figure 1. *T2-weighted axial magnetic resonance image (MRI) showing widespread subcortical lesions of the white matter and diffuse cerebral atrophy*



again and cardiological re-evaluation did not show any signs of aortic valve vegetations at this time. Virus and autoimmune serology were negative and lumbar aspiration was not diagnostic. Because of the persisting subfebrile temperature with elevated inflammation markers, a FDG-PET/CT was performed. This showed an increased FDG uptake in both adrenals (*figures 2* and *3*). A biopsy of the left adrenal gland demonstrated an intravascular, CD20-positive, diffuse large B-cell lymphoma (IVLBCL, *figures 4* and *5*). An additional bone marrow biopsy did not show lymphoma location. The patient further

Figure 2. *Coronal image of whole body FDG-PET with increased uptake in both adrenals*

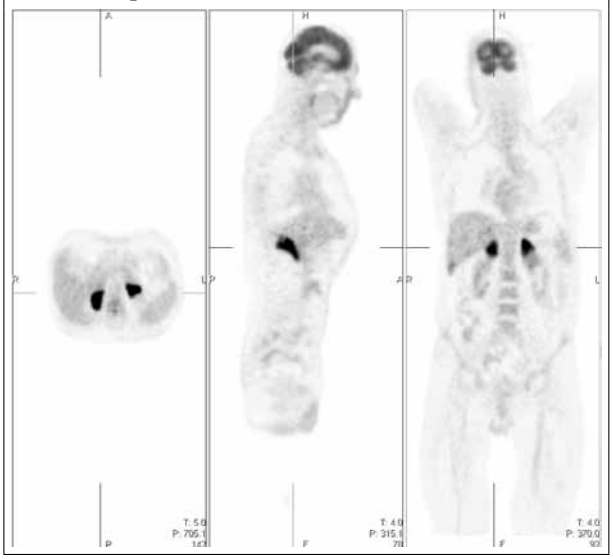


Figure 3. *Coronal image of fused PET/CT with increased uptake in both adrenals*

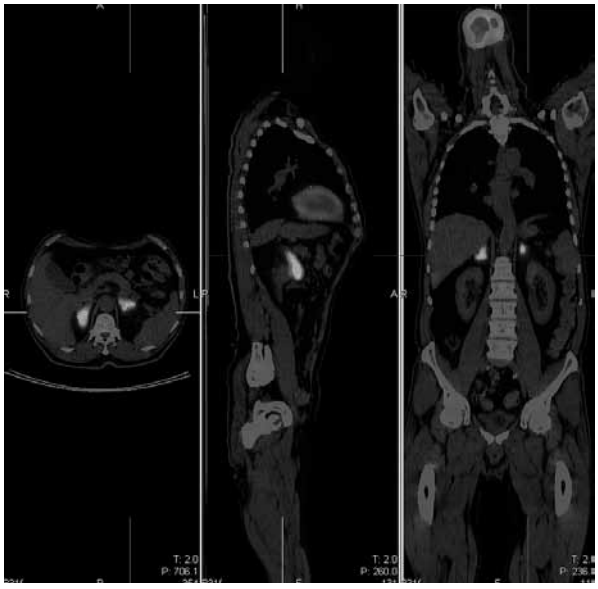


Figure 4. *Biopsy of left adrenal: extensive infiltration of malignant lymphoid cells, located in sinusoids (haematoxylin-eosin staining, original magnification x50)*

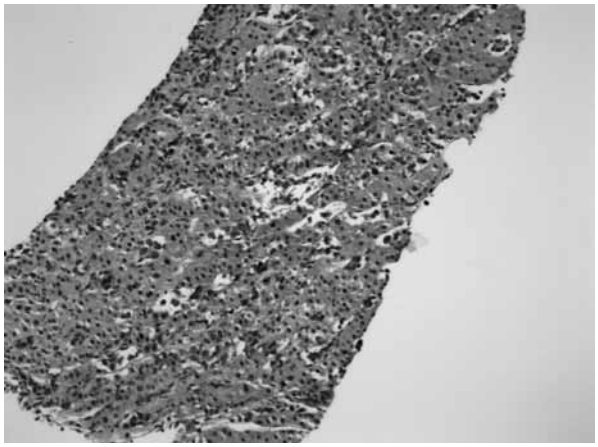
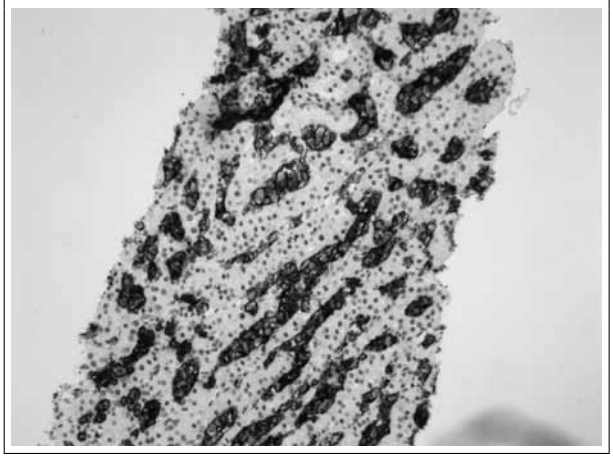


Figure 5. *CD20 staining showing the intravascular location of lymphoid cells (original magnification x50)*



deteriorated, being subconscious at the time of diagnosis with development of a right hemiparesis. Chemotherapy with systemic R-CHOP and intrathecal methotrexate was instituted. While his condition initially worsened, due to a cytokine release syndrome, he recovered well during treatment and the neurological symptoms faded. After six cycles of chemotherapy complete remission was established on a repeated FDG-PET/CT scan. Until now, seven months after finishing chemotherapy, the patient is still in remission and in good condition.

DISCUSSION

Intravascular diffuse large B-cell lymphoma

Intravascular diffuse large B-cell lymphoma (IVLBCL) is a rare and aggressive form of non-Hodgkin's lymphoma. This lymphoma variant was first reported by Pleger and Tappeiner in 1959 and is considered to be endothelial in origin.[3] The current World Health Organisation classification defines IVLBCL as an extranodal diffuse large B-cell lymphoma characterised by the presence of neoplastic lymphocytes only in the lumina of small vessels,[4] leading to occlusion of the vessel and consequently tissue infarction.[5]

Clinical manifestations

Clinically two distinct forms are recognised. The 'classical' or Western variant frequently involves the central nerve system (CNS) and skin, while in Asian countries the disease predominately presents with a haemophagocytic syndrome.[6]

The Western variant, as in our patient, has a median age of presentation of 60 to 70 years with a slight male preponderance.[6] Its clinical manifestations are highly variable and non-specific. Systemic symptoms such as fever, weight loss and fatigue are present in half of the cases;[6] however, lymphoadenopathy is mostly absent. The majority of patients have neurological symptoms at the time of presentation,[7] including focal or diffuse cerebral signs, dementia and other confusional states and less often spinal cord syndrome, seizures, mono- and polyneuropathy or myopathy.[5,7] Besides the CNS, the skin is frequently involved (22 to 26%) but infiltration can take place in virtually any organ of the body, such as lung, liver, prostate, bone marrow and spleen.[6] IVLBCL of the adrenals is peculiar but has been reported and may be complicated by adrenal insufficiency.[8]

DIAGNOSIS

Due to its atypical and heterogeneous presentation and aggressive behaviour, the diagnosis of an IVLBCL is unfortunately made post-mortem in almost half of the cases.[9] Non-specific findings on blood examination and negative imaging studies complicate a prompt and accurate diagnosis. Laboratory findings that can be encountered include anaemia (63 to 65%), an increased LDH (80 to 90%) and an elevated ESR (43%).[4] Computed tomography (CT) is frequently not supportive because lymphoadenopathy and hepato-splenomegaly are usually absent. Only in half of the patients is CNS involvement of an IVLBCL seen on MRI as non-specific white matter lesions.[10] Cerebrospinal fluid examination can show a pleiocytosis and increased protein concentration, but no malignant lymphoma cells.[5,11] Despite these diagnostic difficulties, a representative biopsy of an organ involved is essential in diagnosing IVLBCL. In our patient a FDG-PET/CT scan was of enormous value in detecting the adrenals as one of the organs involved, which were accessible for biopsy and consequently led to the diagnosis of IVLBCL. In most types of lymphoma the combination of FGD-PET with CT is highly accurate in detecting nodal and extranodal involvement.[12] A few articles report the successful use of FDG-PET in diagnosing IVLBCL in cases of fever of unknown origin.[13,14] However, its diagnostic accuracy in IVLBCL remains unclear, as false-negative results have also been noted.[15]

TREATMENT AND PROGNOSIS

Patients with IVLBCL need to be treated with systemic chemotherapy as any disseminated malignant disease. Anthracycline-based chemotherapy is considered first-choice therapy, as results of other chemotherapeutic regimes have been disappointing.[16] In case of CNS involvement, CNS-oriented chemotherapeutic agents, such as methotrexate or cytarabine, should be added. However, despite intensive chemotherapy, recurrence of disease is seen in more than half of the cases with an ultimately poor prognosis.[4] A recent retrospective study showed promising results from the addition of rituximab, the standard therapy for CD20-positive lymphomas,[17] improving disease-free and overall survival.[18] Nevertheless, the optimal treatment of this rare distinct variant of non-Hodgkin's lymphoma, with a largely unknown biological behaviour, remains unclear.[6]

CONCLUSION

The presented case demonstrates a patient with an IVLBCL as a rare cause of initially denoted cryptogenic multifocal cerebral infarction. IVLBCL is not an obvious consideration in patients presenting with unexplained neurological symptoms. However, its aggressive behaviour

and devastating clinical course challenge the need for an early diagnosis that is crucial for the survival chances of patients. This case report illustrates the potential value of a FDG-PET/CT in unexplained neurological illness, fever and low-grade inflammation, with a small but evident chance of finding a clue to the unique diagnosis of IVLBCL.

ACKNOWLEDGEMENT

N.K. de Boer, Department of Pathology, for examination of the biopsy specimen; M.E.J. Pijl, Department of Radiology, for performing the CT-guided biopsy.

REFERENCES

1. Donnan GA, Fisher M, Macleod M, Davis SM. Stroke. Lancet. 2008;371:1612-23.
2. Guercini F, Acciarresi M, Agnelli G, Paciaroni M. Cryptogenic stroke: time to determine aetiology. J Thromb Haemost. 2008;6:549-54.
3. Pleger L, Tappeiner J. Zur Kenntnis der systemisierten Endotheliomatose der cutanen Blutgefässe. Hautartz. 1959;10:359-63.
4. Ponzoni M, Ferreri AJM, Campo E, et al. Definition, diagnosis, and management of intravascular large B-cell lymphoma: proposals and perspectives from an international consensus meeting. J Clin Oncol. 2007;25:3168-73.
5. Baumann TP, Hurwitz N, Karamitopolou E, Probst A, Herrnamm R, Steck AJ. Diagnosis and treatment of intravascular lymphomatosis. Arch Neurol. 2000;57:374-7.
6. Ferreri AJM, Campo E, Seymour JF, et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 casus, with special emphasis on the 'cutaneous variant'. Br J Haematol. 2004;127:173-83.
7. Glass J, Hochberg FH, Miller DC. Intravascular lymphomatosis. A systemic disease with neurologic manifestations. Cancer. 1993;71:3156-64.
8. Askarian F, Xu D. Adrenal enlargement and insufficiency: a common presentation of intravascular large B-cell lymphoma. Am J Hematol. 2006;81:411-3.
9. Anghel G, Pettinato G, Severino A, et al. Intravascular B-cell Lymphoma: report of two cases with different clinical presentation but rapid central nervous System Involvement. Leuk Lymphoma. 2003;44:1353-9.
10. Song DK, Boulis NM, McKeever PE, Quint DJ. Angiotropic large cell lymphoma with imaging characteristics of CNS vasculitis. Am J Neuroradiol. 2002;23:239-42.
11. Ossege LM, Postler E, Pleger B, Müller KM, Malin JP. Neoplastic cells in cerebrospinal fluid in intravascular lymphomatosis. J Neurol. 2000;247:656-8.
12. Buchmann I, Reinhardt M, Elsner K, et al. Whole body 2-[18F]-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) in the detection and staging of malignant lymphoma: a bicentral trial. Cancer. 2001;91:889-99.
13. Odawara J, Asada N, Aoki T, Yamakura M, Takeuchi M, Ohuchi T, Matsue K. 18F-Fluorodeoxyglucose positron emission tomography for evaluation of intravascular large B-cell lymphoma. Br J Haematol. 2007;136:684.
14. Hoshino A, Kawada E, Ukita T, et al. Usefulness of FDG-PET to diagnose intravascular lymphomatosis presenting with fever of unknown origin. Am J Hematol. 2004;76:236-9.
15. Shimada K, Kinoshita T, Naoe T, Nakamura S. Presentation and management of intravascular large b-cell lymphoma. Lancet Oncol. 2009;10:895-902.
16. Ferreri AJM, Campo E, Ambrosetti, et al. Anthracycline-based chemotherapy as primary treatment of intravascular lymphoma. Ann Oncol. 2004;15:1215-21.
17. Van Meerten T, Hagenbeek A. CD20-targeted therapy, a breakthrough in the treatment of non-Hodgkin's lymphoma. Neth J Med. 2009;67:251-9.
18. Ferreri AJ, Dognini GP, Bairey O, Szomor A, Montalbán C, Horvath B, et al. The addition of rituximab to anthracycline-based chemotherapy significantly improves outcome in 'Western' patients with intravascular large B-cell lymphoma. Br J Haematol. 2008;143:253-7.

Netherlands
The Journal of Medicine

CASE REPORT

Atypical Cushing's syndrome caused by ectopic ACTH secretion of an oesophageal adenocarcinoma

J.M. Baas[1*], E. Kapiteijn[1], A.M. Pereira[2], J.W.R. Nortier[1]

Departments of [1]Clinical Oncology, [2]Endocrinology and Metabolic Diseases, Leiden University Medical Centre, Leiden, the Netherlands, *corresponding author: tel.: +31 (0)71-526 34 86, fax: +31 (0)71-526 6760, e-mail: j.m.baas@lumc.nl

ABSTRACT

We present an atypical case of Cushing's syndrome caused by ectopic adrenocorticotropic hormone (ACTH) secretion in a patient with a metastasised adenocarcinoma of the oesophagus. After chemotherapy and surgery the patient developed generalised oedema, hyperpigmentation and dysphagia. Laboratory tests showed hypokalaemia, normal urinary potassium, increased 24-hour urinary free cortisol excretion and serum ACTH within the normal reference range. The diagnosis of ACTH-dependent Cushing's syndrome was made, most probably caused by ectopic production of ACTH. In addition to combined chemotherapy, treatment with ketoconazole sufficiently reduced urinary cortisol excretion and relieved the symptoms.

KEYWORDS

Cushing's syndrome, ectopic ACTH secretion, oesophagus carcinoma

CASE REPORT

A 61-year-old man was referred to our centre for further treatment with chemotherapy of an adenocarcinoma of the distal oesophagus metastasised to the liver and regional lymph nodes. He had previously been treated with six cycles of combination chemotherapy (cisplatin, epirubicin and capecitabine; ECC), radiofrequency ablation (RFA) of nine of 27 liver metastases, and resection and re-excision of a lymph node at the truncus coeliacus in two other university medical centres. After the six ECC courses the primary tumour in the oesophagus was no longer visible at endoscopy; however, a positron emission tomography (PET) scan revealed multiple small liver metastases and a regional lymph node metastasis in the coeliac region. It was decided to treat the largest liver metastases with RFA and to surgically resect the pathological coeliac lymph nodes.

Two months after the last course of chemotherapy and shortly after surgery, the patient noticed generalised oedema, hyperpigmentation of the skin and dysphagia. Laboratory tests revealed marked hypokalaemia (2.3 mmol/l; normal range (N) 3.6 to 4.8 mmol/l), mild hypoalbuminaemia (34 g/l; N 35 to 52 g/l), and metabolic alkalosis. Urine dipstick was negative for protein, urinary potassium excretion was normal: 79 mmol/l (N 5 to 90 mmol/l). A combined PET-CT scan showed signs of local recurrence of the primary tumour (confirmed during endoscopy), and new lesions in the liver. The patient was treated with potassium chloride and potassium-sparing diuretics (spironolactone).

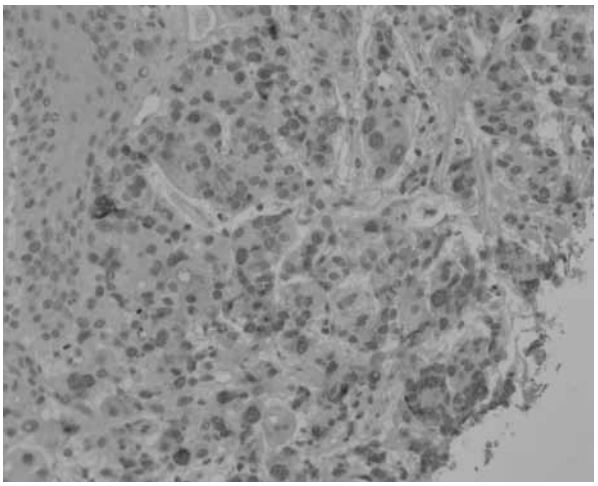
When the patient was re-evaluated to consider second-line chemotherapy, his blood pressure was 140/90 mmHg with persistent hypokalaemia and oedema. Additional tests revealed the following: serum cortisol 4.212 umol/l (N 0.1 to 0.6 umol/l), and 24-hour urinary free cortisol (UFC) excretion 30,682 nmol (N 55 to 220 nmol). ACTH was within the normal reference range: 32 ng/l (N 0 to 75 ng/l). Cortisol was not suppressed by a low dose of dexamethasone (1 mg) overnight. On CT the adrenals appeared normal. The diagnosis of ACTH-dependent Cushing's syndrome was made, most probably caused by ectopic production of ACTH. It was decided not to perform additional biochemical testing but to pragmatically treat the patient with ketoconazole as a cortisol synthesis blocking agent (starting dose 200 mg

thrice daily (tid)) in combination with spironolactone (100 mg tid). Combination chemotherapy was continued with oxaliplatin, epirubicin, and capecitabine (EOX).
Two weeks later his oedema had decreased dramatically, his blood pressure normalised (110/70 mmHg), and the serum potassium was elevated (5.6 mmol/l). Serum cortisol decreased to 0.91 µmol/l, and 24-hour UFC to 1.120 nmol. Both the oral potassium chloride supplementation and spironolactone were stopped. Eventually, 24-hour UFC normalised with a ketoconazole dose of 100 mg twice daily. Revision of the primary tumour and the resected lymph node metastases confirmed the diagnosis of a poorly differentiated non-small-cell adenocarcinoma, with some neuro-endocrine differentiation and expression of ACTH as shown by additional immunohistochemistry (*figure 1*).
After six courses of EOX and ketoconazole, the liver metastases decreased in number and size, the clinical condition of the patient markedly improved, and the symptoms of oedema and muscle weakness disappeared completely. He received additional palliative treatment of the liver metastases with microspheres labelled with Yttrium. After this, the primary tumour was no longer visible at endoscopy, though a PET-CT scan showed two new metastases in the liver. He is currently on low-dose capecitabine.

DISCUSSION

To our knowledge, this is the first case of Cushing's syndrome caused by ectopic ACTH production by an adenocarcinoma of the oesophagus. The diagnosis of Cushing's syndrome caused by ectopic ACTH secretion was considered since laboratory tests showed an inappropriately normal urine potassium excretion in the presence of marked hypokalaemia. The relatively low level of ACTH in this patient is likely due to the assay sensitive to pituitary ACTH. Additional to the presentation of Cushing's syndrome, this patient had an unusual course of his metastasised oesophagus carcinoma. Though there is currently no international standard first-line therapy for advanced or metastatic oesophageal cancer, a combination of cisplatin or oxaliplatin, an anthracycline and 5FU/capecitabine, as was given to our patient, is considered to be most effective. However, even in patients receiving chemotherapy, median overall survival is reported to be seven to ten months.[1] Our patient has shown to be responsive to several therapies and he is now free of a local recurrence, almost two years after he was first diagnosed.
The diagnosis was not otherwise biochemically confirmed because of the clinical condition of the patient, and because the pretest likelihood of an ectopic origin of ACTH secretion was increased based on certain clinical characteristics. Effectiveness-based studies[2,3] indicated that certain clinical characteristics in Cushing's syndrome add more discriminatory power to the pretest probability in the differentiation of pituitary-dependent from an ectopic ACTH-dependent disease than biochemical testing. These characteristics for ectopic ACTH syndrome include male gender, older age, high urinary free cortisol, the presence of hypokalaemia, and ACTH levels. In addition, these studies demonstrated that the accuracy of the high-dose intravenous dexamethasone test and the corticotropin-releasing hormone test as diagnostic tools in the differential diagnosis of ACTH-dependent Cushing's syndrome was insufficient.
This case illustrates that in patients with metastasised malignancies, the clinical presentation of ectopic Cushing's syndrome can be atypical (i.e., without weight gain and truncal obesity) and only manifest with symptoms of mineralocorticoid excess, such as severe hypokalaemia and (mild) hypertension. Cushing's syndrome is caused by ectopic ACTH secretion in approximately 12% of cases. It is most frequently seen in small cell carcinoma, but also in metastasised bronchopulmonary, pancreatic and thymic carcinoids, and metastasised medullary thyroid carcinoma.[4,5] Other sporadic cases of ectopic ACTH syndrome were associated with metastasised tumours of the ovaries, colon, prostate, breast and stomach, with metastasised melanoma and squamous cell carcinoma of the oesophagus.[6-8]
The onset of Cushing's syndrome in malignancies can be either acute, with predominant features such as oedema and hypokalaemia, as seen in small cell carcinoma of the lung and in our patient, or more insidious, with a more gradual onset of classical features of Cushing's syndrome, as in Cushing's disease. The latter more gradual onset of mostly typical Cushing's syndrome is usually seen in metastasised neuroendocrine tumours such as bronchial,

Figure 1. *Oesophagus biopsy showing atypical epithelial cells with enlarged nuclei and with expression of ACTH, along with normal squamous cell epithelium*



thymic or pancreatic carcinoids and medullary thyroid carcinomas. In case of acute onset, hypercortisolism is usually severe and oedema, hypertension, hypokalaemia, metabolic alkalosis, and glucose intolerance are often present.[4] There appears to be a strong association between the presence of hypertension and hypokalaemia with Cushing's syndrome caused by ectopic ACTH secretion.[5] In addition, the presence of hypokalaemia is tightly related to excessively increased 24-hour UC excretion, which reflects a highly increased cortisol production. This is most likely due to the fact that cortisol may act as a mineralocorticoid when in excess, by saturating the 11beta-hydroxysteroid-dehydrogenase (11beta-HSD2 enzyme) that inactivates cortisol at the renal tubule. Thus, as documented in our patient, excessive cortisol levels are the principal cause of hypokalaemic alkalosis in Cushing's syndrome, rather than inhibition of the 11betaHSD2 enzyme by ACTH or the effects of adrenal steroid biosynthetic intermediaries with mineralococorticoid activity.

Resection of the ACTH-producing tumour or bilateral adrenalectomy can resolve hypercortisolism. However, complete resection of tumour mass(es) is often not possible, since most (metastasised) tumours grow invasively and are not resectable. Moreover, due to the limited life-expectancy of (metastasised) cancer patients, patients are often not considered eligible for bilateral adrenalectomy. In these cases, hypercortisolism can be controlled using agents that block adrenal corticosteroid synthesis, such as ketoconazole or metyrapone, in combination with potassium replacement and spironolactone, when necessary. Treatment can be titrated according to changes in 24-hour urinary excretion of cortisol.[8] Other pharmacological agents to treat ectopic ACTH syndrome mentioned in the literature are octreotide, and mitotane (ortho-para DDD).[4]

Although rare in adenocarcinomas, ectopic ATCH syndrome should be considered in cancer patients with oedema, hypokalaemia and alkalosis, without the presence of other classical features of Cushing's syndrome. In our patient, in addition to combined chemotherapy, treatment with ketoconazole sufficiently reduced urinary cortisol excretion and relieved symptoms of oedema and hypokalaemia.

ACKNOWLEDGEMENT

We thank Dr J.V.M.G. Bovee for her help with the pathology report and for providing the illustration.

REFERENCES

1. Wagner AD, Grothe W, Haerting J, Kleber G, Grothey A, Fleig WE. Chemotherapy in advanced gastric cancer: a systematic review and meta-analysis based on aggregate data. J Clin Oncol. 2006;24(18):2903-9.
2. Aron DC, Raff H, Findling JW. Effectiveness versus efficacy: the limited value in clinical practice of high dose dexamethasone suppression testing in the differential diagnosis of adrenocorticotropin-dependent Cushing's syndrome. J Clin Endocrinol Metab. 1997;82(6):1780-5.
3. Reimondo G, Paccotti P, Minetto M, Termine A, Stura G, Bergui M, et al. The corticotrophin-releasing hormone test is the most reliable noninvasive method to differentiate pituitary from ectopic ACTH secretion in Cushing's syndrome. Clin Endocrinol. (Oxf) 2003;58(6):718-24.
4. Orth DN. Cushing's syndrome. N Engl J Med. 1995;332(12):791-803.
5. Torpy DJ, Mullen N, Ilias I, Nieman LK. Association of hypertension and hypokalemia with Cushing's syndrome caused by ectopic ACTH secretion: a series of 58 cases. Ann N Y Acad Sci. 2002;970:134-44.
6. de Matos LL, Trufelli DC, das Neves-Pereira JC, Danel C, Riquet M. Cushing's syndrome secondary to bronchopulmonary carcinoid tumor: report of two cases and literature review. Lung Cancer. 2006;53(3):381-6.
7. Imura H, Matsukura S, Yamamoto H, Hirata Y, Nakai Y. Studies on ectopic ACTH-producing tumors. II. Clinical and biochemical features of 30 cases. Cancer. 1975;35(5):1430-7.
8. Rickman T, Garmany R, Doherty T, Benson D, Okusa MD. Hypokalemia, metabolic alkalosis, and hypertension: Cushing's syndrome in a patient with metastatic prostate adenocarcinoma. Am J Kidney Dis. 2001;37(4):838-46.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Acute abdomen and liver enzyme abnormalities

E. Nur[1,2,3*], A.B. Arntzenius[3], N. Bokani[4], W. Bruins-Slot[3]

[1]Department of Internal Medicine, Slotervaart Hospital, Amsterdam, the Netherlands, [2]Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands, Departments of [3]Internal Medicine and Gastroenterology, [4]Radiology, Spaarne Hospital, Hoofddorp, the Netherlands, *corresponding author: tel.: +31 (0)20-512 54 29, fax: +31 (0)20-691 97 43, e-mail: e.nur@amc.uva.nl

KEYWORDS

Acalculous cholecystitis, viral hepatitis, hepatitis B

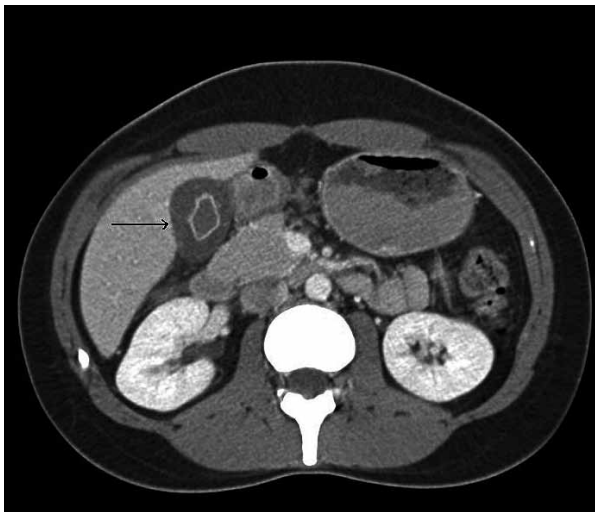
CASE REPORT

A 23-year-old woman of Turkish descent presented to our emergency department with severe pain in the epigastrium and right hypochondrium since three days. Accompanying symptoms were anorexia, nausea and vomiting. She had returned from a family visit in Turkey a few days earlier. Past medical and family histories did not reveal further relevant information.

On physical examination she was anicteric, ill, had no fever and there was right hypochondrial tenderness. There were also guarding, rebound tenderness and a positive Murphy's sign in the right upper abdomen.

Laboratory results showed elevated C-reactive protein (67 mg/l), total bilirubin (109 µmol/l), aspartate transaminase (2206 U/l), alanine transaminase (2646 U/l), lactate dehydrogenasis (1174 U/l). Marked gallbladder wall thickening was seen on a post contrast computed tomography (CT) (*figure 1*).

Figure 1. *CT-scan image of gallbladder wall thickening (arrow) during the course of an acute hepatitis B virus infection*



WHAT IS YOUR DIAGNOSIS?

See page 271 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Bloating after radiofrequency catheter ablation of atrial fibrillation

A-J. Kalsbeek[1], W.P. Beukema[2], E-J. van der Wouden[1]*

Departments of [1]Gastroenterology and Hepatology, [2]Cardiology, Isala Clinics, Zwolle, the Netherlands, *corresponding author: tel.: +31 (0)38-424 57 58, fax: +31 (0)38-424 30 56, e-mail: e.j.van.der.wouden@isala.nl

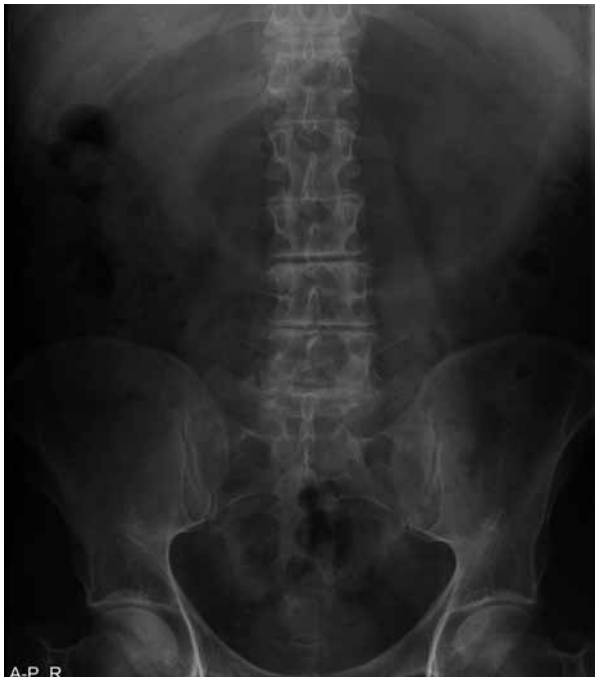
CASE REPORT

A 52-year-old man presented with progressive bloating in the epigastric region and early satiety two days after he had undergone radiofrequency pulmonary vein catheter ablation for therapy-resistant paroxysmal atrial fibrillation. The abdominal fullness increased with intake. He was suffering from nausea, but no vomiting. Defecation was normal. Apart from paroxysmal atrial fibrillation there was no significant medical history. Domperidone, prescribed by his general practitioner, gave no relief of his symptoms. Physical examination showed some hypertympanic epigastric fullness. Laboratory tests were unremarkable. Electrocardiography and transoesophageal cardiac ultrasound were normal. A plain abdominal radiography was performed (*figure 1*).

WHAT IS YOUR DIAGNOSIS?

See page 272 for the answer to this photo quiz.

Figure 1. *Plain abdominal radiography*



Netherlands
The Journal of Medicine

PHOTO QUIZ

An unusual groin swelling

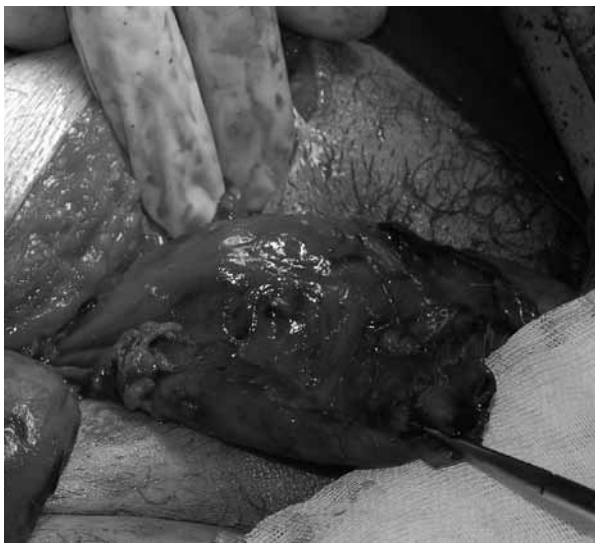
R.C. Minnee*, E.J. Nieveen-van Dijkum, J.R. Ruurda

Department of Surgery, Academic Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)20-566 78 32, fax: +31 (0)20-566 94 32, e-mail: rcminnee@hotmail.com

CASE REPORT

A 73-year-old man presented with right inguinal pain. The patient reported a gradual onset of tenderness and swelling in his right inguinal region during the past 11 days. The patient had a history of epilepsy in childhood, circumcision and cataract. He had no other medical problems, was taking no medication, and had an unremarkable social history, family history, and review of systems. The vital signs were normal. Physical examination revealed normal bowel sounds without abdominal tenderness. The right inguinal fossa showed a tender, well-circumscribed bulge of approximately 10 cm in length beginning 5 cm medial to the anterior superior iliac spine, running on a diagonal confluent with the superior aspect of the right scrotum. The right scrotum was swollen and erythematous. Reposition was attempted under the diagnosis of an incarcerated inguinal hernia, but the swelling could not be reduced. Laboratory studies showed (normal adult range): leucocytes 10.1 x 10^9/l (4 to 10), thrombocytes 327 x 10^9/l (150 to 400), and C-reactive protein (CRP) 284 mg/l (<10). The patient had a normal nonspecific urine analysis. Ultrasound showed a non-incarceration inguinal hernia containing a viable omental part. The patient underwent surgery (*figure 1*).

Figure 1.



WHAT IS YOUR DIAGNOSIS?

See page 273 for the answer to this photo quiz.

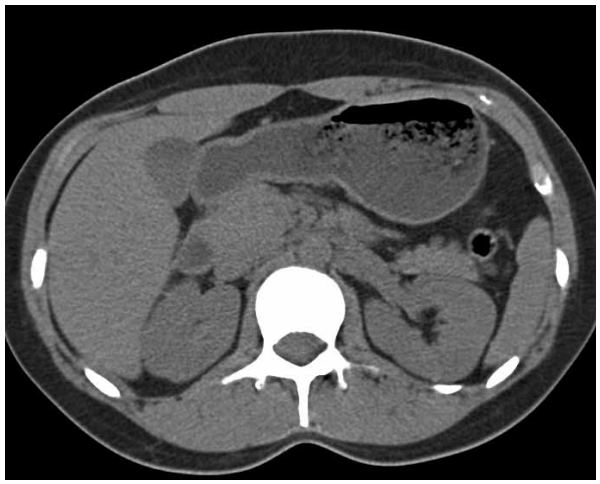
ANSWER TO PHOTO QUIZ (PAGE 268)

ACUTE ABDOMEN AND LIVER ENZYME ABNORMALITIES

DIAGNOSIS

CT imaging showed no stones in the gallbladder and there were no signs of (chronic) liver disease or dilatation of the bile duct and no splenomegaly. Viral serology revealed an acute hepatitis B virus (HBV) infection ($HBsAg^+$, $HBeAg^+$, IgM Anti-$HBcAg^+$, Anti-HBsAg 13.7 IE/l, Anti-$HBeAg^+$). The abdominal pain subsided during the hospital stay and made way for jaundice and pruritus and full clinical recovery, respectively. A second abdominal CT six weeks after discharge from hospital revealed full regression of the gallbladder wall thickening (*figure 2*). Repeated HBV serology, six months after the acute episode, showed the patient had cleared the virus and was immunised by natural infection ($HBsAg^-$, $HBeAg^-$, IgG anti-$HBcAg^+$; anti-HBsAg 67 IE/ml; anti-$HBeAg^+$). She had recently married and was probably infected by her husband as his serology showed a chronic HBV infection ($HBsAg^+$, $HBeAg^-$, IgM anti-$HBcAg^-$, IgG anti-$HbcAg^+$, Anti-$HBsAg^-$, Anti-$HBeAg^+$).

Figure 2. *Abdominal CT-scan image of the gallbladder after the acute episode of hepatitis B virus infection. Notice the complete regression of the gallbladder wall thickening*



Symptomatic disease occurs in approximately 30% of patients with HBV infection. Some degree of right hypochondrial pain and tenderness, attributed to liver inflammation, is common with acute infectious hepatitis. An acute abdominal pain due to acalculous cholecystitis is a rare presentation of hepatitis and has been described primarily during the course of acute hepatitis A virus (HAV) infection.[1] Acute HBV infection presenting as acute acalculous cholecystitis has been described only twice before.[2,3]

Though some gallbladder wall thickening is common in patients with (viral) hepatitis,[4] possibly caused by hepatocyte necrosis, direct viral invasion and infection of the gallbladder are suggested to be the cause of acute acalculous cholecystitis.[1]

REFERENCES

1. Mourani S, Dobbs SM, Genta RM, Tandon AK, Yoffe B. Hepatitis A virus-associated cholecystitis. Ann Intern Med. 1994;120(5):398-400.
2. Unal H, Korkmaz M, Kirbas I, Selcuk H, Yilmaz U. Acute acalculous cholecystitis associated with acute hepatitis B virus infection. Int J Infect Dis. 2009;13(5):e310-2.
3. Singh DS, Saxena SR, Singh AK, Gambhir IS. Acute non-calculus cholecystitis in virus B hepatitis. J Indian Med Assoc. 1996;94(2):66, 75.
4. Juttner HU, Ralls PW, Quinn MF, Jenney JM. Thickening of the gallbladder wall in acute hepatitis: ultrasound demonstration. Radiology. 1982;142(2):465-6.

ANSWER TO PHOTO QUIZ (PAGE 269)

BLOATING AFTER RADIOFREQUENCY CATHETER ABLATION OF ATRIAL FIBRILLATION

During gastroduodenoscopy a distended stomach and a contracted pylorus were seen. Gastric atony and pyloric spasm have been described in small case series as extracardiac adverse effects of catheter ablation due to iatrogenic damage of the vagal nerve.[1-3] It is suggested that nerve fibres innervating the pyloric sphincter and the gastric antrum travel within the left vagal trunk,[1] and these fibres are found to descend along the anterior aspect of the oesophagus in close proximity of the posterior left atrial wall and pulmonary veins.[4] Thus from an anatomical perspective it is not inconceivable that pulmonary vein catheter ablation could cause vagal nerve damage. Moreover, gastric dilatation was a very well-known complication of vagotomy procedures performed for peptic ulcer disease, before highly selective vagotomy came into practice.[5]

There are no evidence-based guidelines for treatment and prognosis of gastric dilatation due to vagal nerve damage after catheter ablation. Conservative treatment with antiemetics, endoscopic injection of botulinum toxin into the pyloric sphincter, and gastric bypass surgery have been described with mixed results.[1,3] We treated our patient with intrapyloric injection of botulinum toxin, antiemetics, and the recommendation to frequently eat small liquid meals. So far, our patient reported only minor improvement in symptoms.

REFERENCES

1. Shah D, Dumonceau JM, Burri H, et al. Acute pyloric spasm and gastric hypomotility: an extracardiac adverse effect of percutaneous radiofrequency ablation for atrial fibrillation. J Am Coll Cardiol. 2005;46(2):327-30.
2. Pisani CF, Hachul D, Sosa E, et al. Gastric hypomotility following epicardial vagal denervation ablation to treat atrial fibrillation. J Cardiovasc Electrophysiol. 2008;19:211-3.
3. Bunch TJ, Ellenbogen KA, Packer DL, Asirvatham SJ. Vagus nerve injury after posterior atrial radiofrequency ablation. Heart Rhythm. 2008;5(9):1327-30.
4. Sanchez-Quintana D, Cabrera JA, Climent V, Farre J, Mendonca MC, Ho SY. Anatomic relations between the esophagus and left atrium and relevance for ablation of atrial fibrillation. Circulation. 2005;112:1400-5.
5. Seymour RE, Andersen DK. Surgery for peptic ulcer disease and postgastrectomy syndromes. In: Textbook of Gastroenterology. 3rd edition. Philadelphia: Lippincott, Williams and Wilkins; 1999. p.1530-48.

ANSWER TO PHOTO QUIZ (PAGE 270)

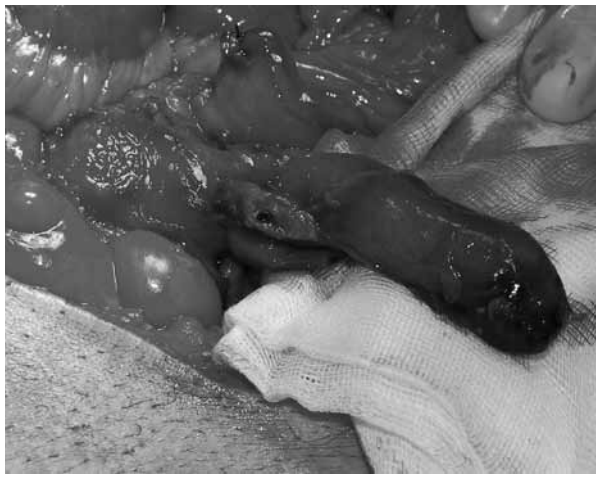
AN UNUSUAL GROIN SWELLING

DIAGNOSIS

The picture shows a large inguinal hernia with multiple adhesions and an acute appendicitis with an appendicular abscess within the hernia (*figure 2*). His appendix was removed and the hernia repaired. One week later he was discharged without any complications. An appendix within an abdominal wall defect is termed Amyand's hernia, first described by Claudius Amyand in 1735.[1]

The incidence of herniation of the appendix is approximately 1%.[2] Inflammation or perforation of such an appendix occurs less commonly. The incidence varies between 0.07 and 0.13%.[2] In cases where the clinical picture is suggestive of acute appendicitis complicating an incarcerated hernia, an ultrasound should be used preoperatively to evaluate the sac for the presence of an appendix.[3] Losanoff *et al.* suggested a four-part classification scheme for Amyand's hernias.[4] Type 1 represents a normal appendix within a hernia sac. Type 2 represents an acute appendicitis within a hernia sac. Type 3 hernias represent an acute appendicitis with abdominal sepsis and type 4 hernias are those in which there is some complicating pathology outside the hernia sac. The approach for type 1 and 2 hernias would be by herniotomy and for types 3 and 4 by laparotomy. The mortality of Amyand's hernia is approximately 5.5%.[2]

Figure 2.



REFERENCES

1. Amyand C. Of an inguinal rupture, with a pin in the appendix coeci, incrusted with stone; and some observations on wounds in the guts. Philos Trans R Soc. 1736;39:329-42.
2. D'Alia C, Lo Schiavo MG, Tonante A, et al. Amyand's hernia: case report and review of the literature. Hernia. 2003;7(2):89-91.
3. Coulier B, Pacary J, Broze B. Sonographic diagnosis of appendicitis within a right inguinal hernia (Amyand's hernia). J Clin Ultrasound. 2006;34(9):454-7.
4. Losanoff JE, Basson MD. Amyand hernia: what lies beneath--a proposed classification scheme to determine management. Am Surg 2007;73(12):1288-90.

Netherlands
The Journal of Medicine

SPECIAL ARTICLE

Regional differences in incidence of sudden cardiac death in the young

A. Hendrix[1,2*], I. Vaartjes[1], A. Mosterd[1,3,4], J.B. Reitsma[5], P.A. Doevendans[3], D.E. Grobbee[1], M.L. Bots[1]

[1]Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, the Netherlands, [2]Interuniversity Cardiology Institute of the Netherlands, Utrecht, the Netherlands, [3]Department of Cardiology, University Medical Centre Utrecht, Utrecht, the Netherlands, [4]Department of Cardiology, Meander Medical Centre, Amersfoort, the Netherlands, [5]Department of Clinical Epidemiology and Biostatistics, Academic Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)88-755 93 80, fax: +31 (0)88-755 54 85, e-mail: a.hendrix@umcutrecht.nl

ABSTRACT

Background: Observational data on sudden cardiac death (SCD) in the young are scarce, but the SCD incidence seems to differ among regions and races. The objective of this study is to examine regional differences in SCD incidence within a population among young individuals (<40 years) and to assess whether regional incidences are associated with socio-economic status (SES).

Methods: SCD cases aged <40 years were identified in 12 provinces of the Netherlands by using death certificates recorded by Statistics Netherlands during 1996-2006. Regional incidence estimates were standardised for age to the Dutch population and assessed for two age categories; 1-29 years and 30-39 years. Regional SCD incidence was related to regional SES with a Spearman correlation coefficient.

Results: The nationwide incidence of SCD at ages 1 to 40 years was 1.6 (95% CI 1.5 to 1.7) per 100,000 person-years and the incidence increased substantially after 30 years of age. Significant differences in regional incidences were assessed for both age categories (1-29 and 30-39 years). Although in the population aged 1-29 years significant differences were found in the SCD incidence between regions, no relation could be found with SES. In men aged 30-39 years, the incidence of SCD was inversely related to SES; a low socio-economic status was associated with a relatively high incidence of SCD.

Conclusion: Between regions, statistically significant differences in SCD incidence exist in young individuals. The nationwide incidence of SCD increased substantially after 30 years of age and was inversely related to SES in men aged 30-39 years.

KEYWORDS

Epidemiology, socio-economic status, sudden cardiac death

INTRODUCTION

The incidence of sudden cardiac death (SCD) in the young is estimated to be 0.9 to 1.6 per 100,000 person-years.[1] Often, cardiac diseases such as cardiomyopathies, primary arrhythmia syndromes, myocarditis or coronary artery disease are the underlying causes of SCD in the young.[1-3] In the SCD victims aged 30-40 years, coronary artery disease accounts for a relatively high percentage of deaths in comparison with younger SCD victims in whom monogenetic inherited diseases and congenital diseases prevail.[1,4] Coronary artery disease is attributed to both genetic factors (for example the presence of familial hypercholesterolaemia) and lifestyle factors such as smoking or inactivity. In the general population, (all ages) a low socio-economic status (SES) is positively associated with the presence of cardiovascular risk factors and the occurrence of premature cardiac death.[5-11]

Observational population-based data on SCD in the young are scarce, but it seems that the incidence of SCD (over all ages) differs among races and between regions.[11-16] However, the comparability of studies on incidence is poor as most former studies were restricted to only a single regional observation without focusing on differences across or within regions.[17,18] Multiple factors might be responsible for differences in the regional SCD incidence, among which the regional age and gender distribution and the regional prevalence of inherited cardiac diseases and coronary artery disease.[19-25] In addition, regional

differences in accessibility of care may also explain differences in out-of-hospital SCD among regions.[26,27]
The primary objective of the current study is to examine regional differences in SCD at young age (1-40 years) and to assess whether regional incidences in SCD differ between age categories (<30 years and 30-39 years) and are associated with differences in regional SES.

MATERIALS AND METHODS

Definitions

SCD is defined as sudden unexpected death due to a cardiac cause within 24 hours after the onset of symptoms.[1]

Incidence of sudden death

To estimate the regional incidence of SCD we used nationwide data on the primary cause of death recorded by Statistics Netherlands[28] over the period 1996-2006. Death certificates do not include information on the duration of preceding symptoms and the actual time of cardiac arrest, which makes it difficult to identify the 'sudden' deaths. To solve this problem, we defined sudden death as death taking place out of hospital, assuming that these deaths occurred unexpectedly and within a few hours after the onset of severe symptoms.[29,30] Because no dedicated ICD code exists for 'SCD', we reviewed the literature to define the most common causes of SCD and selected corresponding ICD-10 codes to compose a proxy for SCD as we have described earlier (*table 1*).[1]

Study regions

The study regions were defined by the boundaries of the 12 provinces of the Netherlands. Information on the regional age and gender distribution was derived from the National Cause of Death Register. For every region, scores for socio-economic status (SES) were derived from the Netherlands Institute for Social Research (SCP) over 2006. This SES score is based on information on income, unemployment and level of education per postal (zip) code. Scores were converted into factor scores by a principal component analysis as described before.[31] For the purpose of this study, the factor scores of each postal code were aggregated to the study regions weighted by the number of inhabitants.

Data analysis

The crude incidence rates (with corresponding 95% CI) of out-of-hospital SCD were reported by age, gender and study region. Age was categorised into two subgroups (1-29 years and 30-39 years). These subgroups were chosen because the percentage of deaths due to coronary artery disease increases with age (especially after 30 years of age) and a low SES is related to a high prevalence of vascular risk factors and coronary artery disease.[5-8]
Person-years were based on the number of subjects in the relevant age categories per study region from 1996-2006. In addition, comparability of the study regions was obtained by standardising the overall incidence rates to the Dutch population for age and gender.
Because aggregated data were used, a Spearman ranking correlation coefficient was chosen to describe the relation of SCD incidence and SES. Two-tailed significance levels of $p<0.05$ were used. All analyses were performed in agreement with privacy legislation in the Netherlands.[32]

RESULTS

Nationwide incidence estimates

During the study period 1996-2006, 1458 cases of SCD were reported coming from 92,374,043 person-years of follow-up. This yielded a nationwide crude incidence of SCD of 1.6 (95% CI 1.5 to 1.7) per 100,000 person-years. The incidence of SCD increased with age and was higher in men than in women (*figures 1* and *2*). A substantial increase in SCD incidence was seen especially in men after

Table 1. *Overview of selected ICD codes that were used as a proxy for SCD*

Selected causes of cardiac death	Corresponding ICD codes
Coronary artery disease (ischaemic)	E78.4, I21, I24
Conduction disorders	I45.6, I45.8, I45.9, I49.0, I49.9
Myocarditis	I40, I41
Cardiomyopathy	I42.0-I42.9
Coronary pathology (nonischaemic)	I25.4
Congenital cardiac diseases	Q20-Q24, Q87.4
Valve abnormalities	I34.1, I35.0
Other cardiovascular diseases	I46
Sudden death with unknown cause	R96

Figure 1. *Nationwide incidence (per 100,000 person-years) of SCD in persons aged 1-39 years, for men and women for the period 1996-2006*

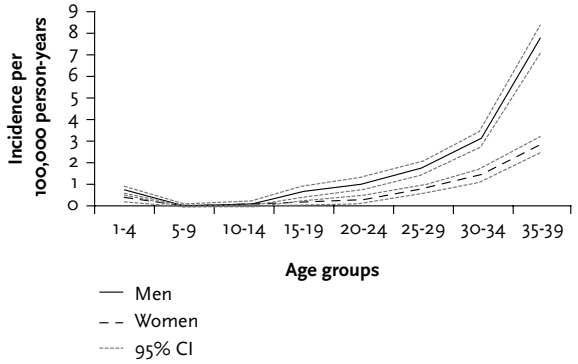


Figure 2A. *Regional incidence (per 100,000 person-years) of SCD in persons aged 1-29 years, standardised for age to the Dutch population. The regions presented at the X-axis are arranged from the north to the south of Holland*

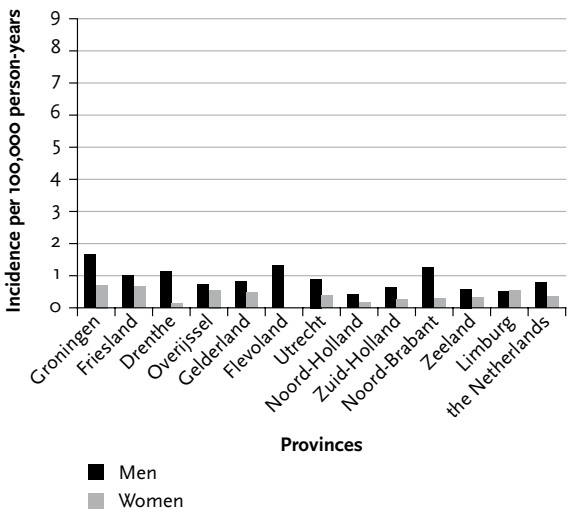
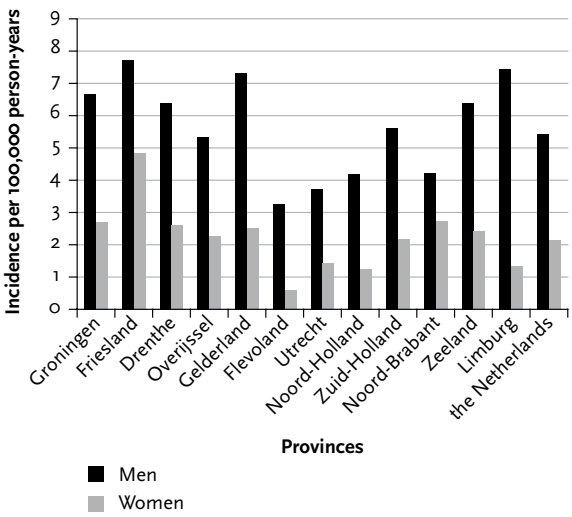


Figure 2B. *Regional incidence (per 100,000 person-years) of SCD in persons aged 30-39 years, standardised for age to the Dutch population. The regions presented at the X-axis are arranged from the north to the south of Holland*



30 years of age. The nationwide incidence of SCD in 1-29 year old men was 0.77 (95% CI 0.67 to 0.87) per 100,000 person-years and in women 0.34 (95% CI 0.28 to 0.40) per 100,000 person-years. The nationwide incidence of SCD in 30-39 year old men was 5.52 (95% CI 5.14 to 5.90) per 100,000 person-years and in women 2.17 (95% CI 1.93 to 2.41) per 100,000 person-years (*table 2*).

Regional incidence estimates

Regional incidence estimates with corresponding 95% confidence intervals are presented in *table 2*. Statistically significant differences in incidence estimates of regional SCD incidences compared with the nationwide SCD incidence were found in both age categories for both men and women. However, no clear north-south or east-west patterns emerged.

Regional incidence estimates standardised to the Dutch population are presented in *figures 2A* and *2B*. In both age categories (1-29 years and 30-39 years) the incidence varies between regions for both men and women.

Regional SES

A low factor score represents a high socio-economic status. The relations between regional incidence estimates for SCD (standardised to the Dutch population) in the young and SES are presented in *figures 3A to 3C*. In younger victims (1-29 years) no significant association could be established between the incidence of SCD and SES (in men 1-29 years, the Spearman' s rho is -0.03 (p=0.93), while in women 1-29 years, the Spearman' s rho is 0.55 p=0.07). In 30-39 year old men, an inverse relation was found between the SCD incidence and SES; a low SES was associated with a higher incidence of SCD in this age category. In women no statistically significant correlation could be established (in men >30 years, the Spearman's rho is 0.76 (p<0.01), in women >30 years, the Spearman's rho is 0.53 (p=0.08).

DISCUSSION

Disparities in SCD incidence in the young were found among study regions and age categories (1-29 and 30-39 years). In 30-39 year old men, the incidence of SCD was inversely related to SES. In men and women aged 1-29 years and in women aged 30-39 years, no significant association with SES and SCD incidence could be observed. In the young, cardiomyopathies, primary arrhythmia syndromes and congenital heart diseases are common causes of SCD.[1,4] Clustering of (inherited) cardiac diseases in populations (and regions) can be responsible for differences in SCD incidence, as is previously described by others.[23,25,33] An example is the high prevalence of a primary arrhythmia syndrome similar to the Brugada syndrome in the southeast of Asia that is responsible for a high number of SCDs in that region.[23,33] In the Netherlands, a founder mutation that causes hypertrophic cardiomyopathy in the majority of cases is predominantly detected in the north-western part of the country.[25] Furthermore, a haplotype (associated with familiar idiopathic ventricular fibrillation) was recently identified, which originates from the centre of the Netherlands.[20] In the current study, no clear patterns in SCD incidence in

Table 2. *Incidence (per 100,000 person-years) of SCD in young persons (1-39 years) in the Netherlands, by age (1-29 and 30-39 years), sex and province. Period 1996-2006*

		Men		Women	
	Age (years)	**Incidence**	**95% CI***	**Incidence**	**95% CI***
Groningen	1-29	1.77[2]	1.63-191	0.71[2]	0.62-0.80
	30-39	6.56[2]	6.14-6.98	2.80[2]	2.52-3.08
Friesland	1-29	0.98[2]	0.88-1.09	0.65[2]	0.56-0.74
	30-39	7.70[2]	7.25-8.15	4.93[2]	4.56-5.30
Drenthe	1-29	0.98	0.87-1.09	0.12[1]	0.08-0.16
	30-39	6.57[2]	6.15-6.99	2.84[2]	2.56-3.12
Overijssel	1-29	0.72	0.63-0.81	0.54[2]	0.46-0.62
	30-39	5.44	5.06-5.82	2.34	2.09-2.59
Gelderland	1-29	0.81	0.71-0.91	0.47	0.39-0.55
	30-39	7.37[2]	6.93-7.81	2.62	2.35-2.89
Flevoland	1-29	1.18[2]	1.06-1.30	0.00[1]	0.00-0.00
	30-39	3.45[1]	3.15-3.75	0.62[1]	0.49-0.75
Utrecht	1-29	0.89	0.79-0.99	0.38	0.31-0.45
	30-39	3.69[1]	3.38-4.00	1.53[1]	1.32-1.74
Noord-Holland	1-29	0.40[1]	0.33-0.47	0.16[1]	0.12-0.20
	30-39	4.12[1]	3.79-4.45	1.26[1]	1.07-1.45
Zuid-Holland	1-29	0.65	0.56-0.74	0.25	0.19-0.31
	30-39	5.59	5.20-5.98	2.17	1.93-2.41
Noord-Brabant	1-29	1.25[2]	1.13-1.37	0.33	0.27-0.39
	30-39	4.27[1]	3.93-4.61	2.72[2]	2.45-2.99
Zeeland	1-29	0.54[1]	0.46-0.62	0.29	0.23-0.35
	30-39	6.28	5.87-6.69	2.37	2.11-2.63
Limburg	1-29	0.97	0.86-1.08	0.49[2]	0.41-0.57
	30-39	7.75[2]	7.30-8.20	1.47[1]	1.27-1.67
The Netherlands	1-29	0.77	0.67-0.87	0.34	0.28-0.40
	30-39	5.52	5.14-5.90	2.17	1.93-2.41

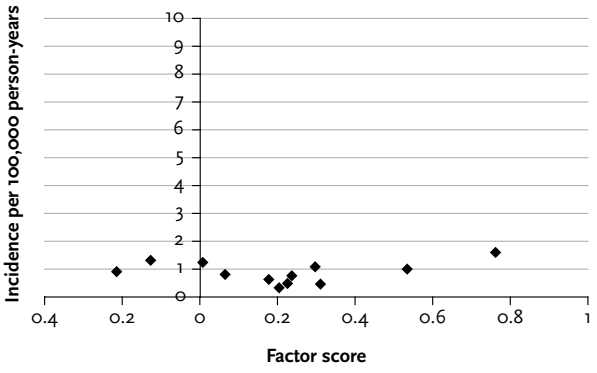
*** CI = confidence interval; [1]Incidence statistically significantly lower than nationwide incidence, [2]Incidence statistically significantly higher than the nationwide incidence.**

the young (1-29 years) were found that might be explained by this regional clustering of inherited diseases. However, the low incidence rates of SCD and the presence of other factors that are associated with the occurrence of SCD might have obscured such an association.

Little is known about the relation between SES and SCD in the young. In the older age categories (>30 years), coronary artery disease is responsible for a high proportion of SCD, especially in men.[4,34,35] Because a low SES is related to a high prevalence of vascular risk factors and prevalence of coronary artery disease, this might explain the increased incidence of SCD at older age in regions with a relatively low SES.[5-8] Our findings correspond with the findings from other studies. Huff and coworkers reported that SES (by deprivation index which was based on unemployment, household, overcrowding, car ownership and home ownership) was inversely associated with all-cause mortality in 0-74 year olds and cardiovascular disease mortality.[7] In people aged 25 to 64 years a low SES is associated with a higher rate of myocardial infarction and death due to coronary artery disease.[6] We investigated the association between SES and SCD in the young. Yet, below 30 years of age, no significant association could be established between SES and SCD incidence.

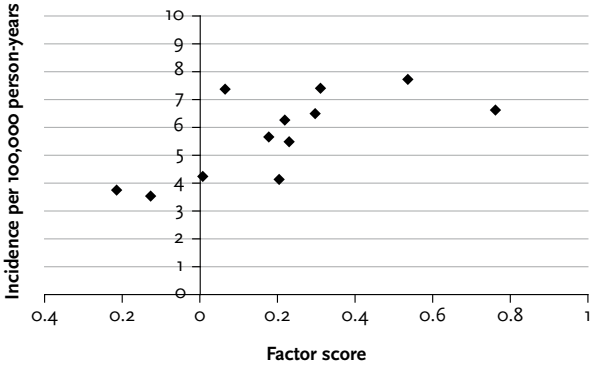
The strength of the current study is the nationwide approach leading to large number of cases of sudden cardiac death. The study also has some limitations that need to be addressed. First, ecological fallacy might have weakened the associations of SCD incidence and SES. No individual information on SES was available; scores assessed for each postal code were aggregated to regions which might have lead to an underestimation of the reported associations. Secondly, racial dispersion was not taken into account in the current study, although this might be partly incorporated in SES. Racial differences are associated with the prevalence of hypertension and obesity, and the development of coronary artery disease.[5] As mentioned before, also inherited cardiac diseases that underlie SCD in the young may cluster among races.[23,25,33] Thirdly, we were not able to collect information on the survival rates of cardiac arrest in the young in the Netherlands, while survival rates might be influenced

Figure 3A. *Regional incidence of SCD (per 100,000 person-years) and regional SES (factor score), in men aged 1-29 years*



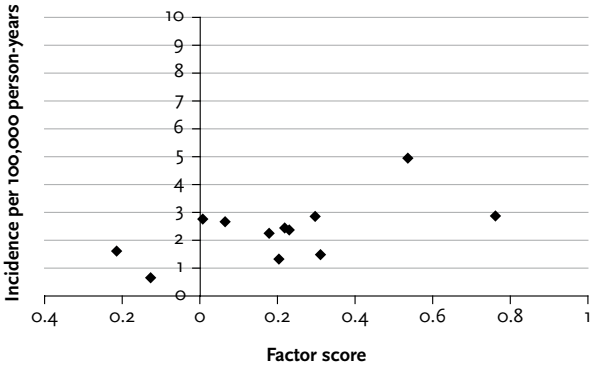
*** A low factor score represents a high SES, and a high factor score indicates a low SES. Zero represents a mean SES.**

Figure 3B. *Regional incidence SCD (per 100,000 person-years) and regional SES (factor score), in men aged 30-39 years*



*** A low factor score represents a high SES, and a high factor score indicates a low SES. Zero represents a mean SES.**

Figure 3C. *Regional incidence SCD (per 100,000 person-years) and regional SES (factor score), in women 30-39 years*



*** A low factor score represents a high SES, and a high factor score indicates a low SES. Zero represents a mean SES.**

by the presence of a witness or by the mean response times of emergency services.[26,27] However, in contrast to other countries, the differences in mean response times of emergency services are relatively small in the Netherlands.[36,37] We do not expect that this affected the observed difference in SCD rate across study regions.

The current study was primarily designed to investigate differences in incidence of SCD in the young across regions. More studies are needed to investigate the underlying mechanisms that are responsible for disparity in regional SCD incidences. A nationwide disease-specific registry of all SCD cases at young age might be helpful to identify predictors or causal factors that are related to the occurrence of SCD. This information may be of value for the future development of preventive strategies directed to high-risk populations or regions.

In conclusion, significant differences in the SCD incidence in young individuals across study regions were observed. The nationwide incidence of SCD substantially increases after 30 years of age, especially in men. The SCD incidence in men aged >30 years seems to be inversely related to SES. This might be due to the increasing percentage of deaths due to coronary artery disease with age.

ACKNOWLEDGEMENTS

Grant support : ICIN, Utrecht.

A. Hendrix was supported by a grant from the Interuniversity Cardiology Institute of the Netherlands (grant number 07601) and I. Vaartjes was supported by a grant of Netherlands Heart Foundation (grant number 3163250). No conflicts of interest were reported.

REFERENCES

1. Vaartjes I, Hendrix A, Hertogh EM, et al. Sudden death in persons younger than 40 years of age: incidence and causes. Eur J Cardiovasc Prev Rehabil. 2009;16:592-6.
2. Jongbloed RJ, Wilde AA, Geelen JL, et al. Novel KCNQ1 and HERG missense mutations in Dutch long-QT families. Hum Mutat. 1999;13:301-10.
3. Doevendans PA. Hypertrophic cardiomyopathy: do we have the algorithm for life and death? Circulation. 2000;101:1224-6.
4. Zheng ZJ, Croft JB, Giles WH, Mensah GA. Out-of-hospital cardiac deaths in adolescents and young adults in the United States, 1989 to 1998. Am J Prev Med. 2005;29:36-41.
5. Mensah GA, Mokdad AH, Ford ES, Greenlund KJ, Croft JB. State of disparities in cardiovascular health in the United States. Circulation. 2005;111:1233-41.
6. Morrison C, Woodward M, Leslie W, Tunstall-Pedoe H. Effect of socioeconomic group on incidence of, management of, and survival after myocardial infarction and coronary death: analysis of community coronary event register. BMJ. 1997;314:541-6.
7. Huff N, Macleod C, Ebdon D, Phillips D, Davies L, Nicholson A. Inequalities in mortality and illness in Trent NHS Region. J Public Health Med. 1999;21:81-7.

8. de Backer G, Thys G, de Craene I, Verhasselt Y, de Henauw S. Coronary heart disease rates within a small urban area in Belgium. J Epidemiol Community Health. 1994;48:344-7.

9. Singh GK, Siahpush M. Increasing inequalities in all-cause and cardiovascular mortality among US adults aged 25-64 years by area socioeconomic status, 1969-1998. Int J Epidemiol. 2002;31:600-13.

10. Reinier K, Stecker EC, Vickers C, Gunson K, Jui J, Chugh SS. Incidence of sudden cardiac arrest is higher in areas of low socioeconomic status: a prospective two year study in a large United States community. Resuscitation. 2006:70:186-92.

11. Soo L, Huff N, Gray D, Hampton JR. Geographical distribution of cardiac arrest in Nottinghamshire. Resuscitation. 2001;48:137-47.

12. Nichol G, Thomas E, Callaway CW, et al. Regional variation in out-of-hospital cardiac arrest incidence and outcome. JAMA. 2008;300:1423-31.

13. Hua W, Zhang LF, Wu YF, et al. Incidence of sudden cardiac death in China: analysis of 4 regional populations. J Am Coll Cardiol. 2009;54:1110-8.

14. Galea S, Blaney S, Nandi A, et al. Explaining racial disparities in incidence of and survival from out-of-hospital cardiac arrest. Am J Epidemiol. 2007;166:534-43.

15. Becker LB, Han BH, Meyer PM, et al. Racial differences in the incidence of cardiac arrest and subsequent survival. The CPR Chicago Project. N Engl J Med. 1993;329:600-6.

16. Ong ME, Tan EH, Yan X, et al. An observational study describing the geographic-time distribution of cardiac arrests in Singapore: what is the utility of geographic information systems for planning public access defibrillation? (PADS Phase I). Resuscitation. 2008;76:388-96.

17. Corrado D, Basso C, Pavei A, Michieli P, Schiavon M, Thiene G. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. JAMA. 2006;296:1593-601.

18. Molander N. Sudden natural death in later childhood and adolescence. Arch Dis Child. 1982;57:572-6.

19. Vatta M, Dumaine R, Varghese G, et al. Genetic and biophysical basis of sudden unexplained nocturnal death syndrome (SUNDS), a disease allelic to Brugada syndrome. Hum Mol Genet. 2002;11:337-45.

20. Alders M, Koopmann TT, Christiaans I, et al. Haplotype-sharing analysis implicates chromosome 7q36 harboring DPP6 in familial idiopathic ventricular fibrillation. Am J Hum Genet. 2009;84:468-76.

21. Corrado D, Basso C, Schiavon M, Thiene G. Screening for hypertrophic cardiomyopathy in young athletes. N Engl J Med. 1998;339:364-9.

22. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. Circulation. 2009;119:1085-92.

23. Tatsanavivat P, Chiravatkul A, Klungboonkrong V, et al. Sudden and unexplained deaths in sleep (Laitai) of young men in rural northeastern Thailand. Int J Epidemiol. 1992;21:904-10.

24. Roberts R, Brugada R. Genetics and arrhythmias. Annu Rev Med. 2003;54:257-67.

25. Alders M, Jongbloed R, Deelen W, et al. The 2373insG mutation in the MYBPC3 gene is a founder mutation, which accounts for nearly one-fourth of the HCM cases in the Netherlands. Eur Heart J. 2003;24:1848-53.

26. Larsen MP, Eisenberg MS, Cummins RO, Hallstrom AP. Predicting survival from out-of-hospital cardiac arrest: a graphic model. Ann Emerg Med. 1993;22:1652-8.

27. Finn JC, Jacobs IG, Holman CD, Oxer HF. Outcomes of out-of-hospital cardiac arrest patients in Perth, Western Australia, 1996-1999. Resuscitation. 2001;51:247-55.

28. Centraal bureau voor de statistiek. Den Haag. 2009. Available from: https://statline.cbs.nl.

29. Fox CS, Evans JC, Larson MG, et al. A comparison of death certificate out-of-hospital coronary heart disease death with physician-adjudicated sudden cardiac death. Am J Cardiol. 2005;95:856-9.

30. Iribarren C, Crow RS, Hannan PJ, Jacobs DR, Luepker RV. Validation of death certificate diagnosis of out-of-hospital sudden cardiac death. Am J Cardiol. 1998;82:50-3.

31. Knol FA.Van hoog naar laag; van laag naar hoog. De sociaal-ruimtelijke ontwikkeling van wijken tussen 1971-1995. 1998. Available from: http://www.scp.nl.

32. Reitsma JB, Kardaun JW, Gevers E, de Bruin A, van der Wal J, Bonsel GJ. Possibilities for anonymous follow-up studies of patients in Dutch national medical registrations using the Municipal Population Register: a pilot study. Ned Tijdschr Geneeskd. 2003;147:2286-90.

33. Tungsanga K, Sriboonlue P. Sudden unexplained death syndrome in north-east Thailand. Int J Epidemiol. 1993;22:81-7.

34. Wisten A, Forsberg H, Krantz P, Messner T. Sudden cardiac death in 15-35-year olds in Sweden during 1992-99. J Intern Med. 2002;252:529-36.

35. Chugh SS, Jui J, Gunson K, et al. Current burden of sudden cardiac death: multiple source surveillance versus retrospective death certificate-based review in a large U.S. community. J Am Coll Cardiol. 2004;44:1268-75.

36. Byrne R, Constant O, Smyth Y, et al. Multiple source surveillance incidence and aetiology of out-of-hospital sudden cardiac death in a rural population in the West of Ireland. Eur Heart J. 2008;29:1418-23.

37. Ambulancezorg Nederland. Ambulances in zicht. 2008.

Netherlands
The Journal of Medicine

SPECIAL ARTICLE

Changing morbidity pattern in oesophagus, stomach and duodenum in Turkish patients: a time-trend analysis

S.M.L.A. Loffeld, R.J.L.F. Loffeld*

Department of Internal Medicine, Zaans Medisch Centrum Zaandam, the Netherlands,
*corresponding author: tel.: +31 (0)656 50 27 79, fax: +31 (0)75-650 23 79, e-mail: loffeld.r@zaansmc.nl

ABSTRACT

Background: From an epidemiological point of view it is interesting to study changing morbidity patterns in disease, especially in upper gastrointestinal diseases. It was previously noted that there was a difference in yield of upper gastrointestinal endoscopy in consecutive years. It was also noticed that there was a difference in occurrence of reflux disease and peptic ulcer disease when comparing Turkish immigrants and native Dutch patients.

Aim: To determine the yield of upper gastrointestinal endoscopy in patients of Turkish descent living in the Zaanstreek region (the Netherlands) in consecutive years.

Material and Methods: All Turkish patients undergoing an upper gastrointestinal endoscopy from 1992 until 2009 where included in this study. Findings of endoscopy were retrieved from the files.

Results: The yearly number of Turkish patients undergoing endoscopy varied from 8 to 15% of the total number of upper gastrointestinal endoscopies. An increase in Turkish patients undergoing endoscopy was seen; this was mainly due to an increase in Turkish women. A decrease was seen in the prevalence of ulcer disease; however, hiatus hernia and reflux oesophagitis increased. The number of patients with nodular gastritis decreased while the number of patients with erosive gastritis increased. In this study period there where two patients with oesophageal cancer and nine patients with gastric cancer; no conclusions can be drawn here.

Conclusion. From the present study it is concluded that in the Turkish population living in the Zaanstreek region, the prevalence of peptic ulcer disease is decreasing, while the prevalence of reflux disease is rising.

KEYWORDS

Gastroscopy, epidemiology, diagnostic yield, peptic ulcer disease, reflux disease

INTRODUCTION

Studying morbidity patterns is interesting from an epidemiological point of view. For instance, acute rheumatic disease was once a major problem in the Western world leading to significant heart disease. Nowadays, mainly due to better hygiene and antibiotics, this disease has almost been eradicated. Also the morbidity pattern of diabetes is changing. Its incidence rises.[1] The prevalence of reflux disease is increasing in the Western world.[2]

Upper gastrointestinal symptoms frequently occur in general as well as in gastroenterological practice. Upper gastrointestinal endoscopy is applied routinely for diagnostic purposes. The most important endoscopic diagnoses are reflux oesophagitis, peptic ulcer disease and cancer of the oesophagus or stomach. Upper abdominal symptoms, and especially gastro-oesophageal reflux disease, are associated with a huge economic burden and decrease the quality of life.[3] Some data report on changes in findings of upper gastrointestinal endoscopy.[4] For instance, peptic ulcer disease used to be one of the major abnormalities in gastroenterology. Nowadays this condition is becoming rare, at least in the Western world.[5]

In earlier studies done in the Zaanstreek region it was noticed that there were clear differences in the occurrence of reflux disease and peptic ulcer when Turkish immigrants were compared with native Dutch patients.[6] It was also seen

that there was a change in the yield of upper gastrointestinal endoscopy in consecutive years.[4] Given these results, the question rises whether changes in yield of upper gastrointestinal endoscopy also occur in patients of Turkish descent.

For this reason the results of upper gastrointestinal endoscopy in patients of Turkish descent in consecutive years were determined.

PATIENTS AND METHODS

All consecutive upper gastrointestinal endoscopies from 1992 until 2009, done in the endoscopy department of the Zaans Medical Centre, the regional hospital of the Zaanstreek region in the Netherlands, were included.

In the Zaanstreek region, there is a large population (about 10% of the total population) originating from Turkey (first, second as well as third generation). Only patients of Turkish descent were included in the present study. All patients were identified by their well-known Turkish family names. Endoscopies done because of direct follow-up after a previous diagnosis were excluded.

All endoscopies were carried out with Olympus endoscopes: in the beginning of the 1990s with fibreoptic endoscopes, from 1993 on with the video systems EVIS 100, Exera 140 and 180 video endoscopes. All endoscopy reports were handwritten in a standardised format. From 2003 a customised version of Endobase™ system from Olympus was used. Endoscopies were done on the request of the general practitioner or the specialist, mostly internist or gastroenterologist, sometimes surgeon or cardiologist. Trend lines were plotted in the individual figures.

RESULTS

The yearly number of Turkish patients undergoing endoscopy from 1992 until 2009 varied from 8% of the total number of upper gastrointestinal endoscopies in 1992 to a maximum of 13% in 2005. This yearly number showed a steady fluctuation and reflects the percentage of Turkish people living in the Zaanstreek region (*figure 1*).

Figure 2 shows the absolute number of Turkish patients in the consecutive years, with the number of endoscopies without macroscopic abnormalities. The number of Turkish patients increased, while the number of endoscopies without abnormalities showed a parallel increase. *Figure 3* shows the number of Turkish men and women. The percentage of women increased.

In *figure 4* the yearly percentage of endoscopies showing duodenal or gastric ulcers is shown. There was a clear trend in decreasing prevalence of ulcer disease. This was mainly due to changes in duodenal ulcer. However, the percentage of gastric ulcers, although less strong, also decreased.

Figure 5 shows the increase in two major signs of reflux disease, namely reflux oesophagitis and hiatus hernia. Both increased.

Figure 6 shows the prevalence of two major signs of endoscopic gastritis, namely nodular gastritis and erosive gastritis. While nodular gastritis decreased, erosive gastritis increased.

Figure 1. *The percentage of Turkish patients undergoing upper gastrointestinal endoscopy in the consecutive years*

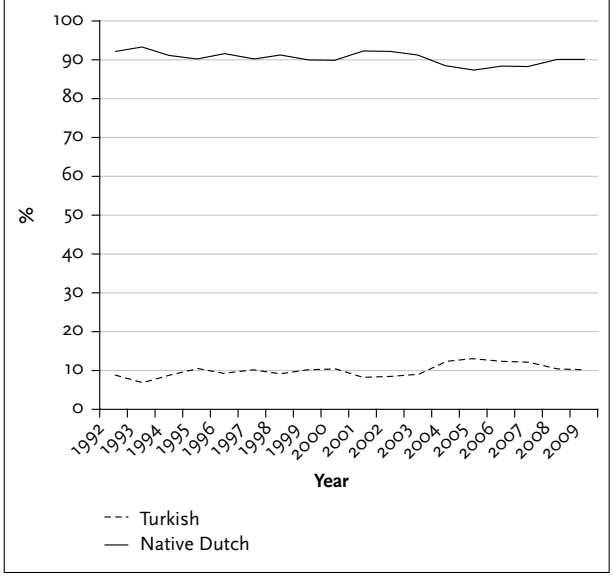


Figure 2. *The absolute number of Turkish patients each year with the number of patients showing no abnormalities during endoscopy*

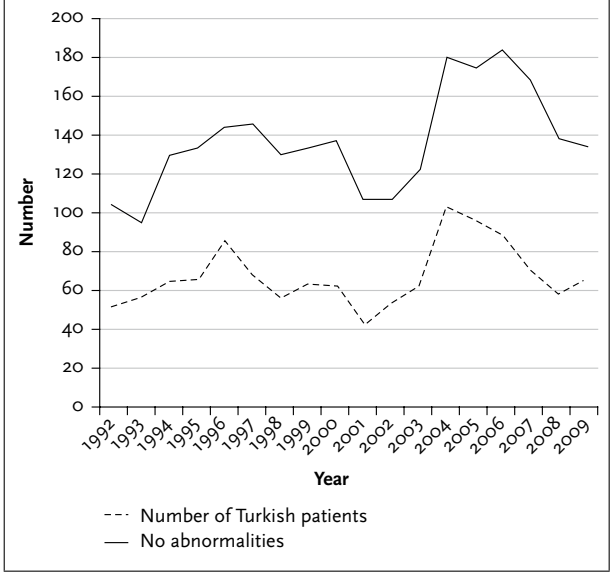


Figure 3. *The percentage of Turkish men and women in the consecutive years*

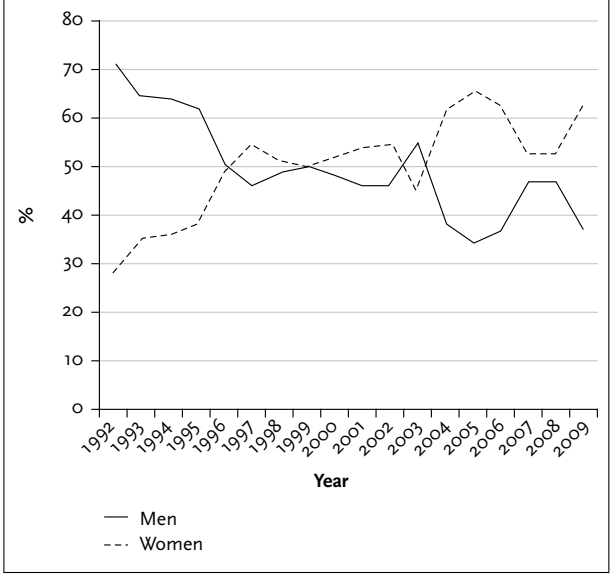


Figure 5. *The prevalence of hiatus hernia and reflux oesophagitis in Turkish patients in the consecutive years*

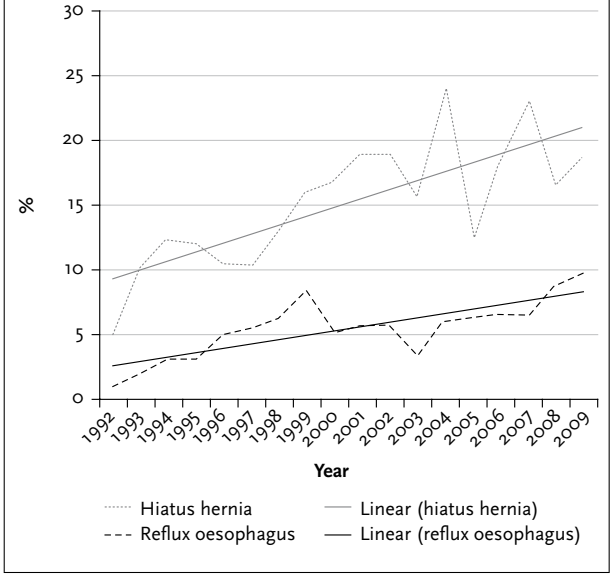


Figure 4. *The yearly prevalence of ulcer disease in the Turkish patients*

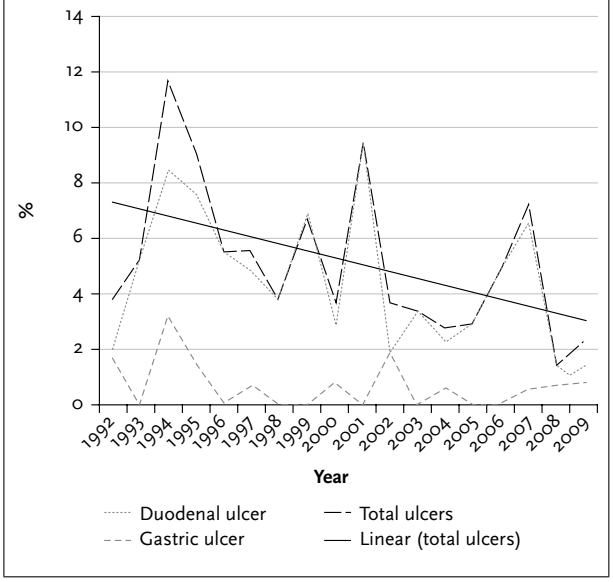
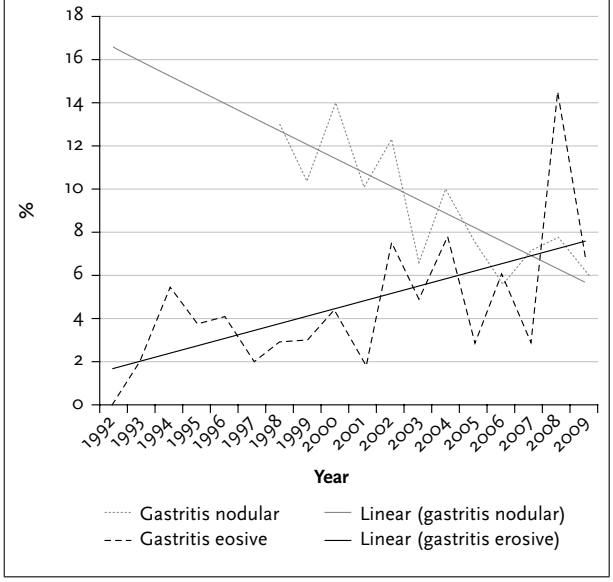


Figure 6. *The yearly prevalence of gastritis*



In the total study period, two oesophageal and nine stomach cancers were diagnosed in the Turkish patients. This number is too small to warrant any analysis.

DISCUSSION

For clinical purposes it is interesting to study prevalence of diseases in different populations. The problem in studying upper abdominal symptoms, and more specifically reflux disease, is that there are cultural differences in the registration of symptoms. There are language barriers in understanding common terms of reflux.[2] In this respect the present study is unique in that only objective parameters such as the presence of oesophagitis and hiatus hernia or ulceration, are used in order to establish the correct endoscopic diagnosis. As people from different ethnicities or races often have very different cultural habits such as eating habits, food storage, hygiene, and socio-economic standards, it is always plausible that there are also differences in

morbidity patterns. It is also interesting to study changes in the morbidity patterns in consecutive years.

The number of Turkish women undergoing upper gastrointestinal endoscopy steadily increased in the consecutive years. The reason for this rise is not obvious. All people in the Netherlands have a mandatory health insurance, hence health care is accessible to everyone.

Peptic ulcer disease had a tremendous effect on morbidity and mortality until the last decades of the 20th century, when epidemiological trends started to point to an impressive fall in its incidence. Two important developments are associated with the decrease in rates of peptic ulcer disease: the discovery of effective and potent acid suppressants, and of *Helicobacter pylori*.[7] In general the number of patients with active ulcer disease is decreasing in the Zaanstreek region.[4] In addition, there is a strong difference in occurrence of peptic ulcer disease in people of Turkish descent when compared with the native Dutch population. The pattern of peptic ulcer disease in Turkish patients resembles the same pattern as 50 years ago in the Netherlands.[8] In the present study it is shown that the prevalence of peptic ulcer disease, though much higher, also decreases in the Turkish population. This is probably due to a decrease in acquisition of *H. pylori*.[9] In another large study from the Netherlands it was confirmed that the incidence of duodenal ulcers declined while the incidence of gastric ulcers was stable over time. The authors think that test and treatment regimens for *H. pylori* have contributed to this decline.[5] In a Brazilian study a decrease in duodenal ulcer prevalence was also observed, 1.3% a year in ten years of follow-up.[10] Decreasing prevalence of *H. pylori* infection has been reported in some countries. In another Brazilian study only children were examined. Prevalence of *H. pylori* was 60.% in the first year of the study and 30% in the last.[11]

Figure 6 shows that data on nodular gastritis were first recorded in 1998. The reason for this was the introduction of new endoscopes with a higher resolution. Nodular gastritis strongly correlates with *H. pylori* gastritis.[12] The symptoms of nodular gastritis and endoscopic features regress significantly after *H. pylori* therapy with a proton pump inhibitor and two antibiotics. Why erosive gastritis increased in the Turkish population is not obvious. While erosive gastritis is also correlated with *H. pylori* gastritis, it is also possible that non-steroidal anti-inflammatory drug use is responsible for this increase. The only possible explanation for the finding that nodular gastritis decreased from 1998 is the decline in the incidence of *H. pylori*.

El-Serag did an extensive study on the epidemiology of gastro-oesophageal reflux disease. He applied a Poisson regression model on population-based studies reporting on reflux symptoms. Population-based studies reporting the prevalence of symptoms at two time points in the same source population were reviewed. And, finally, he studied longitudinal studies that noted the prevalence of symptoms and oesophagitis in primary and secondary care. The Poisson model revealed a significant ($p<0.0001$) trend for an increase in the prevalence of reflux symptoms in the general population over time. Separately, significant increases with time were found for North America ($p=0.0005$) and Europe ($p<0.0001$) but not Asia ($p=0.49$). Studies of the same source population over time indicated an increase in the prevalence of reflux in the US, Singapore and China but not Sweden. An increase in the prevalence of reflux symptoms or oesophagitis was found in the majority of longitudinal studies. There is evidence that the prevalence of reflux symptoms has increased during the past two decades.[13] In accordance with these results, it was already shown that the prevalence of reflux oesophagitis and hiatus hernia is also increasing in consecutive years in the Zaanstreek region.[4] Reflux oesophagitis was diagnosed in only 5.8% of Turkish patients. Turkish men suffered more often from reflux oesophagitis (81 *vs* 19%, $p<0.0001$), and hiatus hernia (58 *vs* 42%, $p<0.0001$) than women. Women on the other hand more often showed no abnormalities ($p<0.0001$).[14] The present study shows that prevalence of reflux oesophagitis and hiatus hernia, although lower than in native Dutch, increases in consecutive years in the Turkish population. This rise was only seen in Turkish men (data not shown).[14] The reason for this increase is not obvious. Unfortunately no data are present on body mass index and specific dietary habits. On the other hand it has already been postulated that the decreasing prevalence of *H. pylori* infection can be responsible for an increase in reflux disease. In a large population-based study it was seen that amongst 1640 patients diagnosed with reflux oesophagitis only 9.7% were of Turkish descent. *H. pylori* was present in 60.6% of Turkish patients and in 18.5% of Dutch patients. All Turkish patients only suffered from mild oesophagitis.[6] Immigrants with reflux disease are significantly younger than native Dutch patients, mean age 42 *vs* 57 years, respectively ($p<0.0001$).[15] Reflux disease is not very common in Turkey. Atug *et al.* did a multicentre study in order to test the efficacy of esomeprazole. It took 52 collaborators to include a total of 235 patients.[16] In a study in 630 subjects randomly selected out of 8857 adults in Turkey it appeared that the prevalence for heartburn was 10% and for acid regurgitation 15.6%.[17] Bor *et al.* conducted a population-based survey on the prevalence of reflux disease in Turkish people in a low-income region and concluded that there was no difference with developed countries. However, reading the paper carefully it appeared that reflux was detected in a low prevalence and that most people suffered from dyspepsia. The prevalence of heartburn was only 10%.[18]

From the present study it is concluded that in the Turkish population living in the Zaanstreek region, the prevalence of peptic ulcer disease is decreasing, while the prevalence

of reflux disease, although still lower than in the native Dutch patients, is rising. Possible explanations could be the decreasing acquisition of *H. pylori*. If this trend continues than the burden of peptic ulcer disease and its complications such as bleeding and perforation decrease, but it could contribute to the increasing incidence of more serious complications associated with reflux, such as oesophageal adenocarcinoma, as well as costs to healthcare systems and employers.

REFERENCES

1. Patterson CC, Dahlquist GG, Gyürüs E, Green A, Soltesz G, for the Eurodiab Study Group. Incidence trends for childhood type 1 diabetes in Europe during 1989-2003 and predicted new cases 2005-2-: a multicentre prospective registration study. Lancet. 2009;373:2027-33.
2. Sharma P, Wani S, Romero Y, Johnson D, Hamilton F. Racial and geographic issues in gastroesophageal reflux disease. Am J Gastroenterol. 2008;103:2669-80.
3. Wahltqvist P, Reilly MC, Barkun A. Systemic review: the impact of gastro-oesophageal reflux disease on work productivity. Aliment Pharmacol Ther. 2006;24:259-72.
4. Loffeld RJLF, van der Putten ABMM. The yield of upper gastrointestinal endoscopy: a study of a ten-year period in the "Zaanstreek". Neth J Med. 2003;61:18-22.
5. Groenen MJ, Kuipers EJ, Hansen BE, Ouwendijk RJ. Incidence of duodenal ulcers and gastric ulcers in a Western population: back to where it started. Can J Gastroenterol. 2009;23:604-8.
6. Loffeld RJ. H. pylori and reflux esophagitis in Turkish patients living in the Zaanstreek region in the Netherlands. Dig Dis Sci. 2003;48:1846-9.
7. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet. 2009;374:1149-61.
8. Loffeld RJLF, van der Putten ABMM. The occurrence of duodenal or gastric ulcer in two different populations living in the same region: a cross-sectional endoscopical study in consecutive patients. Neth J Med. 2001;59:209-12.
9. Loffeld RJLF, van der Putten ABMM. Changes in prevalence of Helicobacter pylori infection in two groups of patients undergoing endoscopy and living in the same region. Scand J Gastroenterol. 2003;38:938-41.
10. Saul C, Teixeira CR, Pereira-Lima JC, Torresini RJ. Prevalence and reduction of duodenal ulcer: a Brazilian study. (Retrospective analysis in the last decade: 1996-2005). Arq Gastroenterol. 2007;44:320-4.
11. Kawakami E, Machado RS, Ogata SK, Langner M. Decrease in prevalence of Helicobacter pylori infection during a 10-year period in Brazilian children. Arq Gastroenterol. 2008;45:147-51.
12. Dwivedi M, Misra SP, Misra V. Nodular gastritis in adults: clinical features, endoscopic appearance, histopathological features, and response to therapy. J Gastroenterol Hepatol. 2008;23:943-7.
13. El-Serag HB. Time trends of gastroesophageal reflux disease: a systematic review. Clin Gastroenterol Hepatol. 2007;5:17-26.
14. Wegman AI, Loffeld RJ. Gastroscopy in immigrants of Turkish descent. J Gastroenterol Hepatol. 2009;24:1187-90.
15. Loffeld RJ, van der Putten AB. Prevalence of gastroesophageal reflux disease in immigrants living in the Zaanstreek region in the Netherlands. Dis Esophagus. 2004;17:87-90.
16. Atug O, Girla A, Kalayci C, Doalr E, Isitan F, Oguz D, et al, Turkish HEMANEX study group. Esomeprazole in acute and maintenance treatment of reflux oesophagitis: a multicentre prospective study. Adv Ther. 2008;25:552-66.
17. Kitapcioglu G, Mandiaracioglu G, Caymaz Bor C, Bor S. Overlap of symptoms of dyspepsia and gastrooesophageal reflux in the community. Turk J Gastroenterol. 2007;18:14-9.
18. Bor S, Mandiracioglu A, Kitapcioglu G, Caymaz-Bor C, Gilbert RJ. Gastroesophageal reflux disease in low-income region on Turkey. Am J Gastroenterol. 2005;100:759-65.

Netherlands
The Journal of Medicine

Pancreatitis associated with the use of itraconazole

J.L.M. Passier[1]*, E.P. van Puijenbroek[1], G.J.P.M. Jonkers[2], A.C. van Grootheest[1]

[1]Netherlands Pharmacovigilance Centre Lareb, 's-Hertogenbosch, the Netherlands, [2]Rijnland Hospital, Department of Internal Medicine, Leiderdorp, the Netherlands, *corresponding author
tel.: +31 (0)73-646 97 18, fax: +31 (0)73-642 61 36, e-mail: a.passier@lareb.nl

ABSTRACT

Background: We call attention to the assumed association between itraconazole and pancreatitis by presentation of four Dutch case reports.

Methods and results: The Netherlands Pharmacovigilance Centre Lareb received four reports of pancreatitis associated with the use of itraconazole, all reported by health professionals. The diagnosis of pancreatitis was confirmed by diagnostic tests. All four patients had been using relatively high doses of itraconazole. In two of these cases, recurrent use of itraconazole resulted in recurrent symptoms. We describe these four cases and discuss the possible mechanism.

Conclusions: The presented cases suggest a causal relation between itraconazole and pancreatitis. Given the often mild indication for the use of itraconazole and the seriousness of this possible adverse drug reaction, it is essential that more data are obtained in order to strengthen the causality of this association. Physicians are invited to report their experiences on the subject.

KEYWORDS

Adverse drug reaction, itraconazole, pancreatitis

INTRODUCTION

Acute pancreatitis, pathophysiology

Acute pancreatitis is a relatively rare, but serious clinical disorder. The acute inflammation of the pancreas is believed to be caused by inappropriate intra-pancreatic activation of digestive enzymes, which leads to subsequent auto-digestion. Acute pancreatitis arises when intracellular protective mechanisms to prevent trypsinogen activation or to reduce trypsin activity are overwhelmed. It is characterised by the presence of acute and constant pain in the epigastric area or the right upper quadrant. Pain might last for several days, radiate to the back, and be associated with nausea and vomiting. Amylase and lipase are released from acinar cells during acute pancreatitis, and their concentration in the serum is used to support the diagnosis. Serum amylase concentrations exceeding three times the normal upper limit confirm acute pancreatitis. Serum amylase rises within hours after the onset of symptoms and returns to normal values within three to five days. Serum lipase concentrations remain high for a longer period of time. Contrast-enhanced CT or MRI can be performed to confirm the diagnosis of pancreatitis.[1]

Possible causes

In developed countries, the most frequent causes of acute pancreatitis are alcohol abuse and cholelithiasis. In 10 to 30% of cases, the cause is unknown, although recent studies have suggested that up to 70% of cases of idiopathic pancreatitis are secondary to biliary microlithiasis. Other aetiologies include autoimmune diseases, inflammatory bowel diseases, infections, genetic disorders, toxins, trauma, postoperative complications, hyperlipidaemia, hypercalcaemia and exposure to specific drugs.[1-3] Overall, drugs are a rare cause of acute pancreatitis.[4]

Itraconazole-induced pancreatitis

Itraconazole is a triazole antifungal agent.[5] Gastrointestinal symptoms are the most commonly occurring adverse drug reactions (ADRs). To our knowledge, pancreatitis is not described as a possible ADR of itraconazole in the product information or in international literature, except for one single case report, which was based on one of the

Lareb cases included in this study.[6] It should be noted that pancreatitis is listed as an ADR in the official product information for two other antifungal triazole derivatives: posaconazole and voriconazole.[7,8]

The Netherlands Pharmacovigilance Centre Lareb has received four reports on pancreatitis in association with itraconazole. In this paper, we present a short overview of these reports.

MATERIALS AND METHODS

The Netherlands Pharmacovigilance Centre Lareb maintains the voluntary adverse drug reaction reporting system in the Netherlands on behalf of the Dutch Medicines Evaluation Board. Physicians and pharmacists have been reporting adverse drug reactions to Lareb since 1985. Patients may report ADRs since April 2003. The Lareb reports are sent to the European Medicines Agency (EMEA) and are included in the worldwide database of the World Health Organisation (WHO).

RESULTS

Dutch Lareb case reports

In the period from November 1999 to February 2010, the Netherlands Pharmacovigilance Centre Lareb received four reports of pancreatitis in association with the use of itraconazole. Details on these reports are summarised in *table 1*.

CASE REPORT A

This case was reported to Lareb and published by the reporting internist in 2001.[6]

A 50-year-old woman, who neither smoked nor used alcohol, took itraconazole pulse therapy for onychomycosis. The patient used itraconazole 200 mg twice daily for a week. Seven days later she experienced abdominal pain, anorexia, vomiting and high fever. These symptoms disappeared spontaneously over time. After a medication-free interval of two weeks she took itraconazole for another two weeks. Nine days after starting this second course, the patient suffered from more severe abdominal pain, high fever and malaise. She was admitted to a hospital. The erythrocyte sedimentation rate (ESR) was 80 mm/h, leucocytes 10.1 x 10^9/l, serum amylase 438 U/l (normal (N) 50 to 220 U/l), and amylase in urine 4325 U/l (N 140 to 1500 U/l). Liver and kidney function were normal. Ultrasound showed a normal pancreas with normal biliary ducts, without gallstones. The patient was diagnosed with pancreatitis. Itraconazole use was discontinued and the patient recovered.

The dosage scheme for this patient was more intense than recommended for onychomycosis: the medication free

Table 1. *Reports of pancreatitis associated with the use of itraconazole*

Patient, sex, age	Suspect drug, indication for use	Dose, duration of treatment	Concomitant medication	Suspected adverse drug reaction	Time to first symptoms, outcome	Lab tests*
A F,50**	Itraconazole for onychomycosis (pulse therapy)	2 dd 200 mg, pulse, 1 week + 2 weeks	None	Gastroenteritis Pancreatitis	7 days after start first week of treatment/ 9 days after start second course, recovered	Serum amylase 438 U/l Urine amylase 4325 U/l
B M,55	Itraconazole for tinea pedis	2 dd 200 mg, 17 days	Budesonide Betamethasone	Pancreatitis	Several days after start Not recovered (9 days after discontinuation)	Serum amylase 492 U/l Urine amylase 2173 U/l Lipase 531 U/l
C M,15	Itraconazole for tinea pedis	1 dd 100 mg, 7 weeks / 1 dd 250 g, 10 weeks	Ketoconazole cream	Recurrent pancreatitis	7 weeks after start of 100 mg treatment/10 weeks after start of 250 mg treatment No full recovery (5 months after discontinuation)	Serum amylase 2812 U/l Lipase 2925 U/l
D F,67	Ciprofibrate for hyperlipidaemia Itraconazole*** for onychomycosis (pulse therapy)	1 dd 100 mg 2 dd 200 mg, pulse 1 week	Simvastatin Psyllium seed Captopril Chlortalidone Beclometasone	Necrotising pancreatitis Death	Several days after start of ciprofibrate/ 2 weeks after start of itracon Fatal	Amylase 1728 U/l Lipase 13241 U/l CT abdomen: picture fits necrotising pancreatitis

*Measurements were repeated in time; only peak values are presented here; **Published by reporting specialist in 2001;[6] ***Originally reported as concomitant medication.

period was two weeks instead of three, and the second treatment period was two weeks instead of one.

CASE REPORT B

A 50-year-old male used itraconazole 200 mg twice daily for tinea pedis. He experienced abdominal symptoms within days, leading to admission to the hospital. Laboratory values of 492 U/l serum amylase (N 70 to 300 U/l), 2173 U/l urine amylase (N 200 to 3500 U/l) and 531 U/l lipase (N 23 to 200 U/l) were measured. Based on these lab values in combination with his clinical presentation, the reporting internist diagnosed pancreatitis in this patient. The itraconazole was discontinued after 17 days of use. Nine days later, at the time of notification, the patient had not yet recovered. The daily dose of 400 mg was higher than the recommended dose (100 mg daily) for this indication.

CASE REPORT C

Patient C refers to a 15-year-old boy. After taking 100 mg itraconazole daily for seven weeks (for tinea pedis), he suffered from stomach ache. He restarted the itraconazole 1.5 months later: 250 mg daily for a period of ten weeks. He then experienced more severe symptoms of abdominal pain and vomiting and discontinued the itraconazole. He was admitted to hospital and was diagnosed with serious necrotising pancreatitis, complicated by pseudocyst formation (shown by MRI). Gallstones or an anatomic deviation of the pancreas or choledochal duct were excluded. Maximal serum amylase was 2812 U/l, and maximal lipase was 2925 U/l. Virology for hepatitis B and C, CMV, Epstein-Barr virus, parvo B19, enterovirus, herpes, varicella zoster and *Mycoplasma* was negative. The patient had no history of frequent alcohol use, hypercalcaemia or hypertriglyceridaemia. At the time of discharge, the patient had a PEG feeding tube. Five months after discontinuation of itraconazole he had not yet fully recovered.
The dosage of 100 mg for seven weeks and 250 mg daily for ten weeks was higher than recommended (100 mg daily during four weeks) for this indication.

CASE REPORT D

A 67-year-old woman, who neither smoked nor used alcohol, had a medical history of recurrent cystitis, hypertension, coronary artherosclerosis, combined hyperlipidaemia and diabetes. The patient was on ciprofibrate, simvastatin, psyllium seed, captopril, chlortalidone and beclometasone nasal spray and had recently taken itraconazole for a week. Two weeks after the start of itraconazole treatment, several days after starting ciprofibrate, the patient suffered from a swollen, hard and painful abdomen which aggravated over time. One month after the first symptoms the patient was admitted to ICU. Serum values of 1728 U/l amylase and 13241 U/l lipase were measured. CT scanning showed an oedematous pancreas and severe liver and spleen necrosis, reported as 'a picture fitting with acute necrotising pancreatitis'. The patient had severe liver failure and metabolic acidosis and died, two days after admittance. Autopsy was not performed.
The reporter indicated cipofibrate (100 mg once daily for hyperlipoproteinaemia) as suspect drug, because the symptoms appeared soon after starting this drug. However, in retrospect, she had used itraconazole 200 mg twice daily for a week (pulse therapy for onychomycosis), two weeks prior to the first symptoms. Moreover, from the medication history of this patient it appeared that she had taken itraconazole pulse therapy six months before as well: itraconazole 200 mg once daily for one week, followed by a medication free week and another two weeks of treatment with itraconazole 200 mg once daily. After both periods of treatment she complained of mild abdominal pain, treated with antacids. It cannot be excluded that these symptoms were caused by a mild pancreatitis as well. The close temporal relationship with the use of itraconazole for both the recent and the past episode is suggestive for a causal relationship between this drug and the occurrence of pancreatitis.
For this patient the medical history of hyperlipidaemia plus the use of simvastatin for this condition may have contributed to the development of acute pancreatitis.[2,9,10] Besides, a CYP3A4 interaction between itraconazole and simvastatin may have lead to increased risk of simvastatin-induced ADRs. There seems to be little support (from literature) for the role of ciprofibrate.
The dosage scheme used six months ago was more robust than the recommended scheme for onychomycosis: the medication free period was one week instead of three, while the treatment period was two weeks instead of one.

Case reports worldwide

The database of the World Health Organisation Collaborating Centre (accessed 22 February 2010) contained a total of 42 reports of pancreatitis in patients on itraconazole, including the four Dutch cases. In 33 of these cases itraconazole was reported to be the only suspect drug.

DISCUSSION

Drugs are a relatively rare cause of acute pancreatitis, with an estimated incidence of 0.1 to 2%. Certain subpopulations such as children, women, the elderly and patients with advanced HIV infection or inflammatory

bowel disease may be at higher risk.[2] In literature reviews e.g. Balani *et al*. Dhir *et al*., Badalov *et al*. and Bergholm *et al*. [2,4,9,10] various different drugs have been associated with pancreatitis. Literature on itraconazole-induced pancreatitis is as far as we know limited to only one Dutch case report, based on case A in *table 1*.[6]

Mechanism of drug-induced pancreatitis

Few data exist on the mechanism of drug-induced pancreatitis. Various mechanisms have been proposed, which differ for each individual drug.[4]

In general, drugs associated with tissue-specific injury can be divided in those with intrinsic toxicity for the organ and those that cause injury as a result of an idiosyncratic reaction. Intrinsic toxicity (type A) is usually characterised by reproducibility, dose dependence and a short, consistent latency.[9] With respect to drug-induced pancreatitis in general, there is little evidence for intrinsic toxicity of drugs being the causative factor: few drugs have been associated with acute pancreatitis in the setting of an overdose. An idiosyncratic reaction is considered more likely.[9]

Idiosyncratic drug reactions, also known as type B reactions, are drug reactions which occur rarely and unpredictably amongst the population. Idiosyncratic drug reactions do not appear to be directly dose related. Clinical symptoms of idiosyncratic drug reactions are different than the pharmacological effect of the drug. The proposed mechanism of idiosyncratic drug reactions is not certain, but may involve a reactive metabolite of the drug binding to proteins, thereby causing a response from the immune system (hapten hypothesis). This response may be triggered by cell injury or cell stress (danger hypothesis). Stressed cells produce danger signals that stimulate an immune response, by co-stimulation of T cells. In the absence of this second signal, the response would be tolerance.[11,12] An alternative theory is the pharmacological interaction hypothesis, which suggests a direct binding of the drug (not the metabolite) to the MHC-T cell receptor complex, causing an immune response. In practice, it appears that a clear separation between an immune and a nonimmune mechanism may not be possible: a cytotoxic agent may well cause cell damage that provokes an immune response and the immune response may contribute to the damage caused by a cytotoxic agent.[12]

Mechanism of itraconazole-induced pancreatitis

With respect to the Lareb cases on itraconazole and pancreatitis, the low incidence and poor predictability make an idiosyncratic reaction a plausible cause. The relatively short time period for the onset of pancreatitis in cases A, B and D and the rapid recurrence of symptoms after recurrent use of itraconazole in patients A and D are in line with an immune response.

On the other hand, relatively high doses of itraconazole were used in all four cases, which would be in favour of an accumulation of a toxic metabolite.[4]

FINAL REMARKS

The diagnosis of acute pancreatitis was medically confirmed by the treating internist in all four cases, based on diagnostic tests in combination with the clinical presentation of the patient.

In general, acute pancreatitis is seldom caused by drugs, which makes it important to rule out more common causes.[4] The cases we have presented here do not provide conclusive evidence for the causative role of itraconazole. However, the reporting internists of cases A, B and C specifically indicated that there were no other possible causes involved besides the use of itraconazole.

The recurrence of symptoms in patient C, despite discontinuation of itraconazole, may be explained by the fact that many patients with idiopathic pancreatitis experience spontaneous recurrent attacks of acute pancreatitis.[9]

Confounding by indication is considered unlikely for these four patients, given the indication for use (onychomycosis and tinea pedis).

CONCLUSION

The presented cases suggest a causal relation between itraconazole and pancreatitis. Given the often mild indication for the use of itraconazole and the seriousness of this possible adverse drug reaction, it is essential that more data are obtained in order to strengthen the causality of this association. Physicians are invited to report their experience on the subject.

REFERENCES

1. Frossard JL, Steer ML, Pastor CM. Acute pancreatitis. Lancet 2008;371(9607):143-52.
2. Balani AR, Grendell JH. Drug-induced pancreatitis : incidence, management and prevention. Drug Saf. 2008;31(10):823-37.
3. Gardner TB, Berk BS, Yakse P. Pancreatitis, acute. http://emedicine medscape com/article/181364-overview 2008 June 10 [cited 2009 Jul 6].
4. Dhir R, Brown DK, Olden KW. Drug-induced pancreatitis: a practical review. Drugs Today (Barc). 2007;43(7):499-507.
5. Dutch SmPC Trisporal®. http://db cbg-meb nl/IB-teksten/h13224 pdf 2005 September 14 [cited 2008 Aug 5].
6. Langers AM, Jonkers GJ. [Pancreatitis ascribed to the use of itraconazole]. Ned Tijdschr Geneeskd. 2001;145(23):1127-8.
7. EPAR Vfend®. http://www emea europa eu/humandocs/PDFs/EPAR/vfend/H-387-PI-en pdf 2007 March 21 [cited 2009 Jul 6].
8. EPAR Noxafil®. http://www emea europa eu/humandocs/PDFs/EPAR/noxafil/H-610-PI-en pdf 2005 October 25 [cited 2009 Jul 6].

9. Badalov N, Baradarian R, Iswara K, Li J, Steinberg W, Tenner S. Drug-induced acute pancreatitis: an evidence-based review. Clin Gastroenterol Hepatol. 2007;5(6):648-61.

10. Bergholm U, Langman M, Rawlins M, Gaist D, Andersen M, Edwards IR, et al. Drug-induced acute pancreatitis. Pharmacoepidemiol Drug Saf. 1995;4:329-34.

11. Knowles SR, Uetrecht J, Shear NH. Idiosyncratic drug reactions: the reactive metabolite syndromes. Lancet. 2000;356(9241):1587-91.

12. Uetrecht J. Idiosyncratic drug reactions: current understanding. Annu Rev Pharmacol Toxicol. 2007;47:513-39.~

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.