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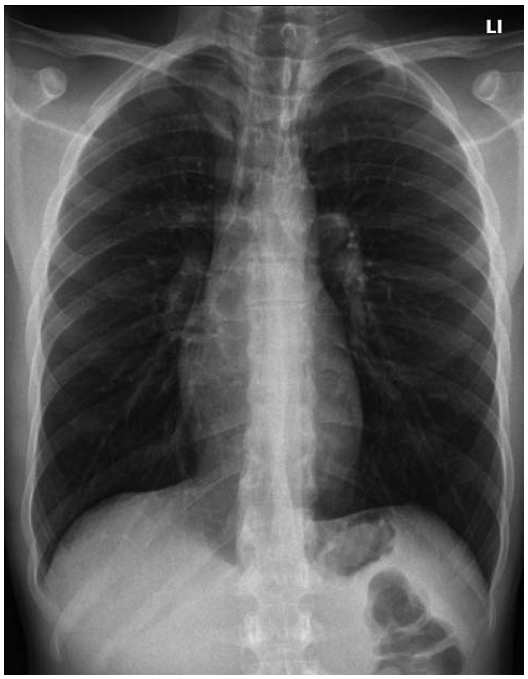


*PHOTO QUIZ: A patient with pain in the throat and chest,*
*see page 360*



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EDITORIAL

# Copper: two sides of the same coin

**R.H.J. Houwen**

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Copper is a highly toxic trace element, due to its oxidising potential, yet it is essential for proper growth and development. Both sides of this coin are shown in two inborn errors of copper metabolism: Menkes disease and Wilson's disease. In Menkes disease, due to mutations in the *ATP7A* gene, copper cannot leave the small intestinal epithelial cells after its absorption from the gut, resulting in copper deficiency throughout the body. This will primarily cause neurological dysfunction and connective tissue abnormalities, due to insufficient function of enzyme systems in which copper is an essential co-factor, such as dopamine-β-hydroxylase and lysyloxidase.[1] In Wilson's disease, due to mutations in the closely related *ATP7B* gene, copper cannot be excreted by the liver, giving a slow accumulation of copper in the liver and secondarily in other organ systems, such as the brain.[2,3] This copper overload can become symptomatic as early as at 4 years, and as late as 70 years of age. As is to be expected, patients presenting early more often have hepatic symptoms, while patients presenting later in life generally have neurological symptoms.[4]

Diagnosis of Wilson's disease can be difficult, as is described in the case report by Kok *et al.*[5] The classical biochemical abnormalities, such as a low serum ceruloplasmin and serum copper, elevated urinary copper excretion and abnormal liver copper can be partially absent. Conversely, some of these abnormalities can also be found when Wilson's disease is definitely not present, such as the low serum ceruloplasmin and serum copper frequently encountered in healthy carriers of an *ATP7B* mutation, and the increased liver copper and urinary copper excretion found in cholestatic liver disease. Therefore, a combination of several diagnostic parameters should be used in the diagnosis of Wilson's disease, for example by applying the scoring system developed by Ferenci *et al.*[6] In this scoring system some weight is given to the presence or absence of Kayser-Fleisher rings. Indeed, the presence of these pathognomonic rings, a deposit of a greenish brown copper pigment in Descemet's membrane in the cornea, is almost diagnostic for Wilson's disease. However, these rings are absent in up to 50% of the patients presenting with hepatic symptoms, as was the case in the patient described by Kok *et al.*[5] This is not surprising, as copper starts to accumulate in the liver and dissemination to other organ systems, especially the brain, of which the eyes are an extension, only develops later in the course of the disease.

Molecular analysis of the *ATP7B* gene, as done in the patients described by Kok *et al.* and Balkema *et al.*, can either confirm a diagnosis already established, or be an essential part of the diagnostic process.[5,7] However, in up to 20% of patients with an unequivocal diagnosis of Wilson's disease either one or both causative mutations can not be found. These patients probably have (a) mutation(s) in the promotor region, which is not analysed during routine analysis of the gene. So a negative genetic analysis does not exclude Wilson's disease.

In Wilson's disease the intracellular levels of copper within the liver will exceed buffering capacity after years to decades of copper accumulation. Then mitochondrial membranes will become oxidised, activating the Fas pathway, and causing apoptosis of liver cells.[8] In this process unbound copper will be released, challenging the remaining liver, and causing even more cells to go down the apoptosis route. This process can result in a rapid reduction in functioning liver mass, causing liver insufficiency and yet is characterised by a relatively modest increase in the levels of transaminases. This not very well-known characteristic of Wilson's disease may cause diagnostic delay, thereby postponing treatment. As chelating therapy is especially effective in the early stages of liver disease, any loss of time can make the difference between recovery of the patient's own liver, or a liver transplant. Whether it is indeed possible for a patient to recover with chelators only, or that an emergency liver transplant is warranted, can be reliably predicted by using the revised King's score.[9] With a score of 11, the patient described by Balkema *et al.*, came indeed close to having to be listed for a liver

transplant.[7] The phase with rapid decay of liver cells is also characterised by the release of significant amounts of unbound copper into the circulation, which can induce oxidative damage in circulating red blood cells, and may result in severe haemolysis in some patients with Wilson's disease. It can even be the presenting symptom, as in the patient described by Balkema *et al.*[7]

Interestingly, it might be possible to inhibit the most devastating consequences of oxidative cellular damage, i.e. the apoptosis displayed by liver cells, using amitriptyline. This effectively reduces apoptosis of liver cells *in vitro* by inhibiting acid sphingomyelinase, which is an essential part of the relevant signalling pathway. When applying this principle *in vivo*, by using LEC rats, an animal model of Wilson's disease, apoptosis could also be effectively blocked, thereby significantly increasing survival.[10] It seems logical to extend these findings to humans, as amitriptyline prescribed for other reasons has proven to be safe in a very large number of patients and over a wide dosage range.

Current treatment of Wilson's disease is aimed at reducing copper overload. With the availability of at least four treatment modalities to achieve this (zinc, penicillamine, trientine and tetrathiomolybdate), the best therapeutic choice continues to be a matter of debate. Unfortunately, in a recent literature search that we performed, identifying almost 1000 articles devoted to this topic, we could identify less than 20 retrospective patient series in which one of these medications was evaluated, and only one prospective trial, albeit non-randomised and non-blinded. This number was far exceeded by the number of statements and editorials claiming the superiority of one treatment over the other. Clearly, proper randomised trials with a sufficient number of patients should answer the question which therapy is the best in a specific situation, e.g. neurological presentation, mild hepatic symptoms, etc. Within EuroWilson, an EU sponsored multinational collaboration, such trials are now being developed. In the coming years the results of these trials should aid in the therapeutic choice for patients with Wilson's disease.

## REFERENCES

1. Kaler SG. Diagnosis and therapy of Menkes syndrome, a genetic form of copper deficiency. Am J Clin Nutr 1998;67:S1029-34.
2. Brewer GK, Yuzbasiyan-Gurkan V. Wilson disease. Medicine 1992;71:139-64.
3. Houwen RHJ, van Hattum J, Hoogenraad TU. Wilson disease. Neth J Med 1993;43:26-37.
4. Stapelbroek JM, Bollen CW, Ploos van Amstel JK, et al. The H1060Q mutation in ATP7B is associated with late and neurologic presentation in Wilson disease: results of a meta-analysis. J Hepatol 2004;41:758-63.
5. Kok KF, Hoevenaars B, Waanders E, Drenth JPH. Value of molecular analysis of Wilson's disease in absence of tissue copper deposits: a novel ATP7B mutation in an adult patient. Neth J Med 2008;66(8):348-50.
6. Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23:139-42.
7. Balkema S, Hamaker ME, Visser HPJ, Heine GDN, Beuers U. Haemolytic anaemia as a first sign of Wilson's disease. Neth J Med 2008;66(8):344-7.
8. Strand S, Hofmann WJ, Grambihler A, et al. Hepatic failure and liver cell damage in acute Wilson's disease involve CD95 (APO-1/Fas) mediated apoptosis. Nature Med 1998;4:588-93.
9. Dhawan A, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilsons's disease in children: 37 years experience and revised King's score for liver transplantation. Liver Transplant 2005;11:441-8.
10. Lang PA, Schenk M, Nicolay JP, et al. Liver cell death and anemia in Wilson disase involve acid sphingomyelinase and ceramide. Nature Med 2007;13;164-70.

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REVIEW

# Strategies for primary and secondary stroke prevention in atrial fibrillation

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## ABSTRACT

**Atrial fibrillation (AF) is the most common type of cardiac rhythm abnormality in adults, affecting 1 to 1.5% of the general population in the Western world and is the major risk factor for stroke with a fivefold risk compared with the general population. Pharmacological and nonpharmacological strategies are available for controlling recurrent or permanent AF as well as for prevention of AF. Prevention of recurrent AF is one of the best protections against AF-related stroke and reduces the prevalence of stroke by almost 25%. Antiplatelet compounds are indicated for CHAD scores 0-1 and reduce the risk of stroke from AF by 20 to 25%. For CHAD scores >1 oral anticoagulation with vitamin K antagonists is indicated and reduces the risk of stroke by 62%. Since inhibitors of coagulation factors Xa, VII, or IIa have either not been clinically tested for their efficacy for prevention of stroke from AF, did not show a comparable effect to well-established drugs, or had excess side effects (idraparinux, ximelagatran), and since mechanical devices are highly questionable concerning their long-term effect, there is currently no alternative to oral anticoagulation with vitamin K antagonists as primary or secondary stroke prevention in high-risk AF patients.**

## KEYWORDS

Anticoagulation, coagulation factors, stroke prevention, prophylaxis, side effects, drug safety

## INTRODUCTION

Atrial fibrillation (AF) is the most common type of cardiac rhythm abnormality in adults, affecting 1 to 1.5% of the general population in the Western world and is the major independent risk factor for stroke.[1,2] The prevalence of AF increases with age, occurring in less than 1% of the general population at age <60 years, but in almost 10% of those >80 years.[1] AF may be categorised as valvular or nonvalvular, lone or associated with other cardiac disease, permanent or paroxysmal, or as hereditary or acquired. Irrespective of the cause of AF, it is associated with a fivefold increased risk of stroke or embolism compared with patients without AF.[2] The annual risk of ischaemic stroke in patients with lone AF is 1.3% and increases to 10 to 12% in patients with previous stroke or transient ischaemic attack.[3] Strokes from AF are usually more severe and associated with an increased risk of morbidity, mortality, and poorer functional outcome than strokes from other causes.[2] The risk of stroke from AF is enhanced by the presence of additional risk factors, such as age >65 years, arterial hypertension, diabetes mellitus, heart failure, or previous stroke, as expressed by the CHAD score.[4] Age >65 years, presence of heart failure, arterial hypertension, and diabetes mellitus count 1 point each in this score, and previous stroke/embolism 2 points.[4]

## PHARMACOLOGICAL STRATEGIES FOR STROKE-PREVENTION IN ATRIAL FIBRILLATION

### Upstream therapy and risk factor modification

Pharmacological and nonpharmacological strategies for controlling AF as well as primary prevention of AF by 'upstream' therapy and risk factor modification are likely to contribute substantially to the reduction of stroke rates in the general population (*table 1*).[2] Despite recent advances and promising new approaches, prevention of recurrent AF may be one of the best protections against AF-related stroke and may reduce the prevalence of stroke by almost 25%.[5]

Antiarrhythmic drugs can approximately double the maintenance rate of sinus rhythm in recurrent AF.[6] Antiarrhythmic drugs are indicated for symptoms of short AV nodal conduction time with a high ventricular heart rate in

**Table 1.** *Strategies for primary or secondary stroke prevention in AF, clinically applicable or under clinical development*

| |
|---|
| **Pharmacological** |
| Upstream therapy and risk factor modification (ACEI, ARBs (sartans), statins, digitalis, amiodarone, β-blockers, calcium antagonists) |
| Platelet inhibitors (aspirin, clopidogrel, ticlopidine (withdrawn)) |
| Multitargeted coagulation inhibitors<br>• Vitamin-K antagonists (warfarin, phenprocoumon, acenocoumarol)<br>• Heparins (UFH or LMWH) |
| Selective inhibitors of coagulation factors<br>• Factor Xa inhibitors<br>- Short-acting, direct inhibitors (rivaroxaban (BAY 597939))<br>- Long-acting, indirect inhibitors (idraparinux, biotinylated idraparinux)<br>• Factor IIa (thrombin) inhibitors<br>- Direct oral thrombin inhibitors (ximelagatran/melagatran (withdrawn because of liver toxicity), dabigatran (BIBR-1048)) |
| **Nonpharmacological** |
| Nonpharmcological upstream measures and risk factor modification |
| Electrical cardioversion |
| Electrical ablation of right atrial conductive tissue |
| Percutaneous left atrial appendage occlusion (PLAATO) |
| Minimally invasive surgical isolation of the LAA (Maize, COX procedure) |

**ACEI = angiotensin-converting enzyme inhibitors; ARBs = angiotensin-II-receptor-blocking drugs; UFH = unfractionated heparin; LMWH = low-molecular-weight heparin; LAA = left atrial appendage.**

recurrent or sustained AF.[6] Pharmacological cardioversion is indicated if AF lasts less than one year and if the left atrium is normally sized. It is carried out by application of digitalis (in case of normal contractility), amiodarone (in case of reduced contractility), β-blockers, calcium antagonists, or propafenon (in case of normal contractility). Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor-blocking drugs (ARB) may further improve the maintenance of sinus rhythm through cardiac remodelling[6] or prevention of atrial endocardial dysfunction by rapid atrial pacing.[7] Retrospective, cross-sectional and longitudinal analysis of the SPORTIV III and V data, however, did not demonstrate a significant benefit of ACEI or ARB in AF patients, except in patients >75 years.[8] Preliminary studies suggest that also statins, such as atorvastatin, which have pleotropic effects, such as reduction of vascular inflammation, could reduce the AF incidence after cardiac surgery and the recurrence rate of AF after electrical cardioversion,[9,10] particularly together with β-blockers,[11] although other studies with paravastatin did not confirm these findings.[12]

**Platelet inhibitors**

An effective approach for reducing the risk of stroke from AF in primary stroke prevention is platelet inhibitors. If the CHAD score is <2, (low-risk patients) platelet inhibitors, such as acetylsalicylic acid (ASA), clopidogrel, or ticlopidine, are indicated as primary stroke prevention in AF.[3] Antiplatelet compounds reduce the risk of stroke from AF by 20 to 25%.[2] Although clopidogrel has proven efficacy and superiority compared with ASA to prevent systemic vascular events in at-risk patients, it currently does not play an important role in the prevention of AF-related thromboembolic events.[3] In a recent study (CHARISMA trial) clopidogrel plus ASA was not more effective than ASA alone in preventing strokes in AF patients.[13] In a substudy of the ACTIVE W trial the combination of clopidogrel plus ASA had a higher risk of bleeding compared with vitamin K antagonists (VKA), irrespective of whether patients suffered from paroxysmal or sustained AF.[14] Whether the combination of extended-release dipyridamol and ASA and the combination of clopidogrel with ASA are superior to ASA in monotherapy for stroke prevention in AF has not been investigated.[15]

**Multitargeted (acting on a number of coagulation factors)**

*Vitamin K antagonists*

Although VKA have been in clinical use for more than 50 years, they were not proven to be beneficial in primary or secondary stroke prevention until about a decade ago.[16] In high-risk patients (CHAD score >1) oral anticoagulation (OAC) with VKA (warfarin, phenprocoumon, acenocumarol) is a class I ACC/AHA indication, unless there are contraindications.[3] Pooled data from trials comparing antithrombotic treatment with placebo have shown that VKA reduce the risk of stroke from AF by 62% with an absolute reduction of about 3% per year.[3,5,16] In high-risk patients, warfarin is superior to ASA in preventing strokes, with a relative risk reduction of 36%. The Birmingham Atrial Fibrillation Treatment of the Aged (BAFTA) study additionally showed that VKA (warfarin) also reduce the risk of stroke in patients >75 years compared with ASA without increasing the bleeding risk.[17] The Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE-W) study has shown that warfarin is also superior to antiplatelet therapy with clopidogrel plus ASA in the prevention of embolic events from AF.[3,5,18] VKA were also superior over a combination of ASA and clopidogrel in a study on 70 patients with nonvalvular AF with regard to plasma markers of thrombogenesis (levels of fibrin D-dimer, β-thromboglobulin, soluble P-selectin, plasma prothrombin fragment 1 + 2).[19] The combination of ASA and clopidogrel, however, was superior over ASA alone in preventing thromboembolic events in AF.[20] In a nonrandomised study VKA also proved effective regarding the long-term prognosis of patients with AF who survived a severe, disabling stroke (modified Rankin scale 4-5).[21] Independent predictors of mortality were

increasing age, increasing handicap, and ASA *vs* VKA. Previous transitory ischaemic attack and ASA *vs* VKA were predictors of vascular recurrence. Thus, VKA lengthen survival and decrease the risk of recurrent thromboembolic events.[21] In patients under antiplatelet therapy for previous peripheral artery disease or stroke who develop AF, switching from antiplatelet therapy to VKA might be all that is required.[22] The combination of VKA and antiplatelet therapy only provides additional benefit over VKA alone in patients with prosthetic heart valves.[22] The appropriateness of a combination of VKA with antiplatelet therapy in patients with an indication for VKA (AF) who also have an indication for antiplatelet therapy (coronary heart disease) is unsolved.[22] Compared with a combination of clopidogrel and ASA, VKA also reduce the risk of stroke in AF patients with a CHAD score <2.[23]

Shortcomings of VKA include slow onset of action, numerous drug/drug and drug/food interactions, narrow therapeutic window, complexity of dose adjustment for one third of the patients, need for frequent monitoring, necessity of daily intake, genetic variation in metabolism, and the risk of bleeding.[16,24] The risk of major haemorrhage under warfarin is twice that with ASA.[25] Treatment with VKA needs to be tailored individually on the basis of age, comorbidities, and contraindications.[25] Less than 60% of patients without a contraindication to VKA actually receive them.[3] Of those who receive VKA <50% are consistently within therapeutic targets.[3] Limitations of VKA therapy prompted the development of new anticoagulants with predictable pharmacokinetics, which do not require regular monitoring.[25] VKA act nonspecifically, as they inhibit the coagulation cascade at various steps. Desired characteristics of new anticoagulants include good bioavailability, no food/food or food/drug interactions, rapid onset of action, wide therapeutic window, absent necessity of monitoring, availability of an antidote, absence of side effects, absence of interactions with other drugs, and low costs.[26,27]

*Heparins and heparinoids*

Unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) are supportive to OAC for preventing stroke in AF or if there are contraindications for OAC. The anticoagulant properties of UFH were detected in 1916 and by the 1930s their therapeutic use was evaluated.[28] UFH and LMWH act nonspecifically on a number of coagulation factors. UFH has equipotent activity against factors IIa and Xa but also acts on factors IXa, XIa, and XIIa.[29] UFH and LMWH do not require antithrombin-III as a cofactor. Disadvantages of UFH are that it can only be used intravenously, that its laboratory control is difficult, that it stimulates platelets leading to activation, aggregation, and clot formation, and that it rarely (incidence 0.2%)[30] causes heparin-induced thrombocytopenia (HIT).[28,31] Although LMWH has a predictable half-life, its subcutaneous mode of administration and long-term risks, particularly osteoporosis, mean that it is not feasible for long-term use.[3] In a study on 431 patients with acute ischaemic stroke and AF, they did not profit from LMWH (dalteparin) as compared with ASA with regard to primary outcome, measured by the International Stroke Trial scale at three months, and secondary outcome variables.[32]

**Selective inhibitors of coagulation factors**

More recent approaches to primary and secondary prevention of stroke from AF include selective inhibitors of specific coagulation factors involved in the initiation or propagation of the coagulation cascade (factors Xa, II) (*table 1*). To understand the mechanisms of action and side effects, it is important to know that coagulation factors, which are targets of the inhibitory activities of their inhibitors, also affect coagulation independent processes, such as wound healing, inflammation, immune response, tissue repair, angiogenesis, mitogenesis, tumour growth, apoptosis, and cell survival.

*Factor Xa inhibitors*

Coagulation factor Xa is an attractive target for drug development because of its position at the convergence of the intrinsic and extrinsic clotting pathways.[33] There are two different strategies for inhibiting factor Xa that are being pursued, indirect or direct inhibition, depending on whether factor Xa is inhibited with or without the mediation of antithrombin-III. Direct inhibitors without antithrombin-III mediation have a high bioavailablity, a short half-life, and are thus short-acting and orally applicable. They include rivaroxaban (BAY 597939), YM150, apixaban, razaxaban, otamixaban, DX-9065a, LY517717, DU-176b, or betrixaban (*tables 1* and *2*).[33,34] Indirect inhibitors have a low bioavailability, a long half-life, and are thus long-acting, and are subcutaneously administered. They include idraparinux, biotinylated idraparinux, and fondaparinux (SSR-126517-E). Only those compounds experimentally or clinically applied for preventing stroke/embolism from AF are further discussed.

Direct, short-acting factor-Xa inhibitors

Rivaroxaban is a nonpeptidic, orally bioavailable small molecule, which directly inhibits clot-associated or free Xa activity, prothrombinase activity, and reduces thrombin generation.[29,32] Rivaroxaban has a high oral bioavailability, a rapid onset of action, a half-life of five to nine hours, and predictable pharmacokinetics. Rivaroxaban has undergone extensive phase II studies for venous thromboembolism prevention after orthopaedic surgery and phase III studies have begun.[30] Rivaroxaban demonstrated superiority to enoxaparin for prophylaxis of thromboembolism after total knee arthroplasty with similar low bleeding complications.[35]

**Table 2.** *Potential or experimental strategies for primary or secondary stroke prevention in AF*

| | | |
|---|---|---|
| **Pharmacological** | | |
| *Selective inhibitors of coagulation factors* | | |
| Factor Xa inhibitors | Short-acting, direct inhibitors: | |
| | Apixaban | Experimental |
| | Razaxaban | Experimental |
| | Otamixaban | Clinical development |
| | Betrixaban | Clinical development |
| | YM-150 | Clinical development |
| | DX-9065a | Experimental |
| | LY517717 | Clinical development |
| | DU-176b | Clinical development |
| | Tick anticoagulant peptide (TAP) | Experimental |
| | Antistatin (ANT) | Experimental |
| | Antithrombin-heparin covalent complex (ATH) | Experimental |
| | JTV-803 | Experimental |
| | PRT05402 (Portola) | Experimental |
| | Long-acting, indirect inhibitors: Fondaparinux (SSR-126517-E) | Approved for VTE |
| Factor IIa (thrombin) inhibitors | Nelagatran | Experimental |
| | Argatroban | Approved for HIT, ACS |
| | Efegatran | Clinical development |
| | Desirudin | Approved for ACS |
| | Lepirudin | Approved for HIT, ACS |
| | Bivalirudin | Approved for HIT, ACS |
| | Hirudin | |
| Factor VIIa inhibitors | Nematode anticoagulant peptide (NAPc2) | Experimental |
| | Active site-blocked factor VIIa (FVIIai) | Experimental |
| | Recombinant tissue factor pathway inhibitor (rTFPI) | Experimental |
| *Stimulators of fibrinolysis* | Protein-C derivatives | Experimental |
| | Soluble thrombomodulin | Experimental |
| **Nonpharmacological** | Carotid filtering devices (emboli diverted from internal to external carotid artery) | Experimental |
| | Catheter-based isolation of the pulmonary veins[6] | Experimental |

**VTE = venous thromboembolism; HIT = heparin-induced thrombocytopenia; ACS = acute coronary syndrome.**

Rivaroxaban is currently being assessed for the treatment and secondary prevention of venous thromboembolism, prevention of stroke from AF, and secondary prevention in acute coronary syndrome.[35]

Indirect, long-acting factor-Xa inhibitors

Idraparinux is a synthetic O-sulphated, O-methylated pentasaccharide, which tightly binds to antithrombin-III and thereby and specifically induces the inactivation of the procoagulant protease, factor X.[36] Idraparinux not only differs structurally from fondaparinux for its additional methyl groups, but also for its half-life of about 80 hours, which is why it is dosed once weekly.[37] Idraparinux does not elevate liver enzymes.[38] In the AMADEUS trial idraparinux turned out to have a similar effect to warfarin but was significantly more frequently associated with bleeding events than warfarin.[39] In deep venous thrombosis, idraparinux had a similar effect to heparin plus a VKA but was less effective in patients with pulmonary embolism.[40] Idraparinux has a mechanism of action similar to that of heparin.[41] It was developed as an antithrombotic for venous and arterial thrombosis, acute coronary syndrome, stroke, or as adjunct to thrombolytic therapy.[37] The biotinylated form of idraparinux, which has avidin as an antidote, is currently being evaluated in the range of an ongoing phase III trial (BOREALIS-AF study) for its effect on stroke prevention in AF.[29]

Coagulation factor II (thrombin) inhibitors

Thrombin is a central enzyme in haemostasis exerting potent procoagulant effects and activating platelets.[31] Thrombin converts fibrinogen to fibrin and activates factors V, VIII, XI, XIII, and platelet protease-activated receptors.[29] In addition to its role in haemostasis and coagulation, thrombin exhibits numerous other biological activities affecting inflammation by controlling the expression of cytokines,[42] immune responses, tissue repair, tumour growth, apoptosis, cell survival, wound healing, endothelial cytoprotection,[43] and angiogenesis.[44] In the cerebrum thrombin induces injury of cortical neurons,[45] facilitates epileptic seizures,[46] mediates neurodegeneration or neuroprotection via protein-activated receptors,[47] induces angiogenesis,[48,49] and induces the expression of the MKP-1 gene in endothelial cells.[50] Thrombin inhibitors inhibit thrombin by directly binding to exosite I and/or the active site of thrombin and are applied orally, which is why they

are also termed direct, oral thrombin inhibitors.[29] Direct oral thrombin inhibitors include ximelagatran/melagatran, dabigatran, argatroban, efegatran, hirudin, desirudin, lepirudin, and bivalirudin (*tables 1* and *2*). Only those clinically or experimentally tested for the prevention of stroke/embolism in AF patients are further discussed.

Ximelagatran is an oral prodrug and undergoes rapid enzymatic conversion to melagatran.[51] Melagatran has poor oral bioavailability, must be given subcutaneously, and is active in the prevention and treatment of venous thromboembolic events, coronary thrombotic events,[52] or of arterial thromboembolic events from AF.[52] Ximelagatran has rapid onset of action, fixed twice-daily dosing, stable absorption, low potential to interact with other medication, does not require monitoring of drug levels or dose adjustment, and has a short plasma elimination half-life of approximately four hours.[3,51] According to the SPORTIF III and V trials, comparing ximelagatran with warfarin for stroke prevention in AF and at least one additional risk factor, ximelagatran was not inferior to warfarin.[16] Ximelagatran was also found to be as efficient as warfarin in the secondary prevention of embolic events, but had to be withdrawn because of potential liver toxicity.[2,5,53-56] Elevation of liver enzymes occurred in 5 to 10% of the included patients and was more common in older patients, particularly women.[16,54] The nonrandomised, concomitant treatment with ASA and VKA was associated with increased bleeding without indication of reducing primary outcome events.[53]

Dabigatran appears to have a better safety profile than ximilagatran and can be given without regard to age, gender, or weight and has minimal drug interactions.[2,24] Dabigatran is the only oral direct thrombin inhibitor in late-stage development. Since November 2005, a phase II trial (Re-LY trial) has been initiated, which compares the effect of dabigatran *vs* VKA for the prevention of stroke/embolism from AF.[29,31,57] Dabigatran has been proven to be equivalent to LMWH in deep venous thrombosis prophylaxis and did not show excess bleeding.[24] The plasma half-life of dabigatran is 14 to 17 hours, allowing once-daily dosing, and elimination is primarily via renal excretion.[29] In a dose-finding, warfarin-controlled study on 542 AF patients, the prevalence of stroke did not differ between the dabigatran and warfarin group.[58]

**Nonpharmacological approaches**

Electrical cardioversion is indicated when AF lasts less than one year and the left atrium is not enlarged. Even when sticking to this rule, the recurrence rate is 32% after one year.[11] Whether radiofrequency ablation is helpful for the prevention of embolic events in AF is questionable since the majority of AF patients are too old for the procedure, since candidates for ablation have a low risk of embolism, since the procedure itself may increase the embolic risk, and since it is uncertain how long the embolic risk persists after the procedure. Specific mechanical approaches to stroke prevention in AF include various models of percutaneous left atrial appendage occluders (PLAATO), minimally invasive surgical isolation of the left atrial appendage (Maize procedure, COX procedure); PLAATO was long regarded to be a safe and reasonable method for patients with contraindications to OAC or those who continue to embolise despite sufficient anticoagulation. PLAATO has already been frequently applied, but meanwhile it turned out to be more harmful than beneficial due to its strong negative influence on the regulatory function of the left atrial appendage and the frequently insufficient closure of the left atrial appendage orifice.[59]

## MONITORING

The anticoagulant effect of platelet inhibitors does not require monitoring. The effect of VKA is best monitored by determination of the international normalised ratio (INR). Sufficient oral anticoagulation in AF patients at high risk for stroke is provided if the INR is between 2 and 3. The anticoagulant effect of UFH is usually monitored by determination of the activated partial thromboplastin time (aPTT). A therapeutic effect is achieved if the aPTT is elevated two to three times the normal upper reference limit. The therapeutic effect of LMWH is usually monitored by determination of the anti-factor Xa activity. A therapeutic effect is achieved if the plasma anticoagulant level ranges between 0.6 to 1.0 U/ml. The anticoagulant effect of UFH, LMWH, and direct thrombin inhibitors can also be monitored by measuring the prothrombinase-induced clotting time (PiCT).[60] PiCT is actually the only method to measure the effect of thrombin inhibitors in contrast to the prothrombin time, aPTT, Heptest, ecarin clotting time, or chromogenic assays.[60] Fondaparinux and idraparinux prolong the coagulation time in the PiCT, Heptest, and chromogenic assays in a dose-dependent manner but not in the aPTT.[60] PiCT is a suitable test to determine the anticoagulant effect of the long-acting, indirect factor Xa inhibitors.[60] Idraparinux increases the thrombin generation time, the aPTT, the thrombin time and reduces the prothrombin fragments 1+2.[61] Lepirudin can be monitored by the aPTT, which should be maintained at 1.5 to 2.0 times baseline.[29] The anticoagulant activity of argatroban is monitored using the aPTT at 1.5 to 3.0 times baseline.[29]

## ANTAGONISTS

UFH can be antagonised by protamin. LMWH can be antagonised by protamin and prothrombin complex concentrate. VKA can be antagonised by vitamin K (slow)

or by prothrombin complex concentrate in cases of urgency. Idraparinux may be effectively antagonised by recombinant factor VIIa.[61] There is no specific antidote available for ximelagatran[61] or hirudin.

## CONCLUSIONS

Although many of the phase II and phase III studies with new anticoagulants were promising, clinical use for stroke prevention in AF, whenever approved, has been disappointing so far. Currently, there is no alternative to VKA for primary and secondary stroke prevention in patients with AF and additional risk factors. The VKA days cannot be left behind since the currently available new anticoagulants cannot be recommended for stroke prevention in AF and since new strategies require ongoing pharmacological research and clinical trials, which may last another few years before becoming available on a widespread basis. However, the ongoing basic research on new anticoagulants is promising and may be successful with time. Meanwhile, all measures should be taken to avoid under-usage of VKA for stroke prevention in AF patients at high risk for stroke embolism.[62]

## REFERENCES

1. O'Donnell M, Weitz JI. Novel antithrombotic therapies for the prevention of stroke in patients with atrial fibrillation. Am J Manag Care 2004;10(suppl 3):S72-82.
2. Savelieva I, Bajpai A, Camm AJ. Stroke in atrial fibrillation: update on pathophysiology, new antithrombotic therapies, and evolution of procedures and devices. Ann Med 2007;39:371-91.
3. Ali S, Hong M, Antezano ES, Mangat I. Evaluation and management of atrial fibrillation. Cardiovasc Hematol Disord Drug Targets 2006;6:233-44.
4. Gage BF, van Walraven C, Pearce L, et al. Selecting patients with atrial fibrillation for anticoagulation: stroke risk stratification in patients taking aspirin. Circulation 2004;110:2287-92.
5. Ederhy S, Cohen A. Optimising stroke prevention in non-valvular atrial fibrillation. Expert Opin Pharmacother 2006;7:2079-94.
6. Kirchhof P, Breithardt G. Treatment of atrial fibrillation. Internist (Berl) 2007;48:819-29;830-1.
7. Yamashita T, Sekiguchi A, Kato T, et al. Angiotensin type 1 receptor blockade prevents endocardial dysfunction of rapidly paced atria in rats. J Renin Angiotensin Aldosterone Syst 2007;8:127-32.
8. Lip GY, Frison L, Grind M. Angiotensin converting enzyme inhibitor and angiotensin receptor blockade use in relation to outcomes in anticoagulated patients with atrial fibrillation. J Intern Med 2007;261:577-86.
9. Siu CW, Lau CP, Tse HF. Prevention of atrial fibrillation recurrence by statin therapy in patients with lone atrial fibrillation after successful cardioversion. Am J Cardiol 2003;92:1343-5.
10. Ozaydin M, Varol E, Aslan SM, et al. Effect of atorvastatin on the recurrence rates of atrial fibrillation after electrical cardioversion. Am J Cardiol 2006;97:1490-3.
11. Humphries KH, Lee M, Sheldon R, et al, for the CARAF Investigators. Statin use and recurrence of atrial fibrillation after successful cardioversion. Am Heart J 2007;154:908-13.
12. Tveit A, Grundtvig M, Gundersen T, et al. Analysis of pravastatin to prevent recurrence of atrial fibrillation after electrical cardioversion. Am J Cardiol 2004;93:780-2.
13. Hart RG, Bhatt DL, Hacke W, et al, for the CHARISMA Investigators. Clopidogrel and aspirin versus aspirin alone for the prevention of stroke in patients with a history of atrial fibrillation: subgroup analysis of the CHARISMA randomized trial. Cerebrovasc Dis 2008;25:344-7.
14. Hohnloser SH, Pajitnev D, Pogue J, et al., for the ACTIVE W Investigators. Incidence of stroke in paroxysmal versus sustained atrial fibrillation in patients taking oral anticoagulation or combined antiplatelet therapy: an ACTIVE W Substudy. J Am Coll Cardiol 2007;50:2156-61.
15. Diener HC. Executive Steering Committee of the SPORTIFF III and V Investigators. Stroke prevention using the oral direct thrombin inhibitor ximelagatran in patients with non-valvular atrial fibrillation. Pooled analysis from the SPORTIF III and V studies. Cerebrovasc Dis 2006;21:279-93.
16. Donnan GA, Dewey HM, Chambers BR. Warfarin for atrial fibrillation: the end of an era? Lancet Neurol 2004;3:305-8.
17. Mant J, Hobbs FD, Fletcher K, et al; BAFTA investigators; Midland Research Practices Network (MidReC). Warfarin versus aspirin for stroke prevention in an elderly community population with atrial fibrillation (the Birmingham Atrial Fibrillation Treatment of the Aged Study, BAFTA): a randomised controlled trial. Lancet 2007;370:493-503.
18. The Active Steering Committee; ACTIVE Investigators, Connolly S, Yusuf S, Budaj A, et al. Rationale and design of ACTIVE: the atrial fibrillation clopidogrel trial with irbesartan for prevention of vascular events. Am Heart J 2006;151:1187-93.
19. Kamath S, Lip GY. Atrial fibrillation in the elderly: anticoagulation strategies and indications in the very elderly. Am J Geriatr Cardiol 2002;11:357-62.
20. Veloso HH, de Paola AA. Clopidogrel as antithrombotic therapy in atrial fibrillation. Chest 2005;128:475;
21. Vemmos KN, Tsivgoulis G, Spengos K, et al. Anticoagulation influences long-term outcome in patients with nonvalvular atrial fibrillation and severe ischemic stroke. Am J Geriatr Pharmacother 2004;2:265-73.
22. Eikelboom JW, Hirsh J. Combined antiplatelet and anticoagulant therapy: clinical benefits and risks. J Thromb Haemost 2007;5(suppl 1):255-63.
23. Healey JS, Hart RG, Pogue J, et al. Risks and Benefits of Oral Anticoagulation Compared With Clopidogrel Plus Aspirin in Patients With Atrial Fibrillation According to Stroke Risk. The Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events (ACTIVE-W). Stroke 2008;(in press).
24. Stone WM, Tonnessen BH, Money SR. The new anticoagulants. Perspect Vasc Surg Endovasc Ther 2007;19:332-5.
25. Ederhy S, Meuleman C, Hammoudi N, Janower S, Boccara F, Cohen A. Preventing cerebrovascular accidents during atrial fibrillation. Presse Med 2005;34:1315-24.
26. Bauer KA. New anticoagulants. Hematology Am Soc Hematol Educ Program 2006;:450-6.
27. Tangelder MJ, Frison L, Weaver D, et al. Effect of ximelagatran on ischemic events and death in patients with atrial fibrillation after acute myocardial infarction in the efficacy and safety of the oral direct thrombin inhibitor ximelagatran in patients with recent myocardial damage (ESTEEM) trial. Am Heart J 2008;155:382-7.
28. Lepor NE. Anticoagulation for acute coronary syndromes: from heparin to direct thrombin inhibitors. Rev Cardiovasc Med 2007;8(suppl 3):S9-17.
29. Turpie AG. New oral anticoagulants in atrial fibrillation. Eur Heart J 2008;29:155-65.
30. Smythe MA, Koerber JM, Mattson JC. The incidence of recognized heparin-induced thrombocytopenia in a large, tertiary care teaching hospital. Chest 2007;131:1644-9.
31. Francis CW. Direct thrombin inhibitors for treatment of heparin induced thrombocytopenia, deep vein thrombosis and atrial fibrillation. Curr Pharm Des 2005;11:3931-41.
32. O'Donnell MJ, Berge E, Sandset PM. Are there patients with acute ischemic stroke and atrial fibrillation that benefit from low molecular weight heparin? Stroke 2006;37:452-5.

33. Kubitza D, Haas S. Novel factor Xa inhibitors for prevention and treatment of thromboembolic diseases. Expert Opin Investig Drugs 2006;15:843-55.

34. Turpie AG. New oral anticoagulants in atrial fibrillation. Eur Heart J 2008;29:155-65.

35. Perzborn E, Kubitza D, Misselwitz F. Rivaroxaban. A novel, oral, direct factor Xa inhibitor in clinical development for the prevention and treatment of thromboembolic disorders. Hamostaseologie 2007;27:282-9.

36. Hjelm R, Schedin-Weiss S. High affinity interaction between a synthetic, highly negatively charged pentasaccharide and alpha- or beta-antithrombin is predominantly due to non-ionic interactions. Biochemistry 2007;46:3378-84.

37. Walenga JM, Jeske WP, Fareed J. Short- and long-acting synthetic pentasaccharides as antithrombotic agents. Expert Opin Investig Drugs 2005;14:847-58.

38. Reiter M, Bucek RA, Koca N, Heger J, Minar E; PERSIST. Idraparinux and liver enzymes: observations from the PERSIST trial. Blood Coagul Fibrinolysis 2003;14:61-5.

39. The Amadeus Investigators. Comparison of idraparinux with vitamin K anragonists for prevention of thromboembolism in patients with atial fibrillation: a randomised, open-label, non-inferiority trial. Lancet 2008;371:315-21.

40. Buller HR, Cohen AT, Davidson B, et al., for the van Gogh Investigators. Extended prophylaxis of venous thromboembolism with idraparinux. N Engl J Med 2007;357:1105-12.

41. Ansell J. New anticoagulants and their potential impact on the treatment of thromboembolic disease. Curr Hematol Rep 2004;3:357-62.

42. Zheng L, Martins-Green M. Molecular mechanisms of thrombin-induced interleukin-8 (IL-8/CXCL8) expression in THP-1-derived and primary human macrophages. J Leukoc Biol 2007;82:619-29.

43. Lidington EA, Steinberg R, Kinderlerer AR, et al. A role for proteinase-activated receptor 2 and PKC-epsilon in thrombin-mediated induction of decay-accelerating factor on human endothelial cells. Am J Physiol Cell Physiol 2005;289:C1437-47.

44. Sarker KP, Biswas KK, Yamaji K, et al. Inhibition of thrombin-induced vascular endothelial growth factor production in human neuroblastoma (NB-1) cells by argatroban. Pathophysiol Haemost Thromb 2005;34:41-7.

45. Fujimoto S, Katsuki H, Ohnishi M, Takagi M, Kume T, Akaike A. Thrombin induces striatal neurotoxicity depending on mitogen-activated protein kinase pathways in vivo. Neuroscience 2007;144:694-701

46. Maggio N, Shavit E, Chapman J, Segal M. Thrombin induces long-term potentiation of reactivity to afferent stimulation and facilitates epileptic seizures in rat hippocampal slices: toward understanding the functional consequences of cerebrovascular insults. J Neurosci 2008;28:732-6.

47. Luo W, Wang Y, Reiser G. Protease-activated receptors in the brain: receptor expression, activation, and functions in neurodegeneration and neuroprotection. Brain Res Rev 2007;56:331-45.

48. Caunt M, Hu L, Tang T, Brooks PC, Ibrahim S, Karpatkin S. Growth-regulated oncogene is pivotal in thrombin-induced angiogenesis. Cancer Res 2006;66:4125-32.

49. Karpatkin S. Growth regulated oncogene is pivotal in thrombin-induced angiogenesis. Thromb Res 2007;120(suppl 2):S71-4.

50. Kinney CM, Chandrasekharan UM, Mavrakis L, Dicorleto PE. VEGF and thrombin induce MKP-1 through distinct signaling pathways: role for MKP-1 in endothelial cell migration. Am J Physiol Cell Physiol 2008;294:C241-50.

51. Brighton TA. The direct thrombin inhibitor melagatran/ximelagatran. Med J Aust 2004;181:432-7.

52. Drouet L. Treatment and prevention of venous thromboembolic events: present and future antithrombotic agents. Bull Acad Natl Med 2003;187:85-96.

53. Akins PT, Feldman HA, Zoble RG, et al. Secondary stroke prevention with ximelagatran versus warfarin in patients with atrial fibrillation: pooled analysis of SPORTIF III and V clinical trials. Stroke 2007;38:874-80.

54. Ford GA, Choy AM, Deedwania P, et al, for the SPORTIF III, V Investigators. Direct thrombin inhibition and stroke prevention in elderly patients with atrial fibrillation: experience from the SPORTIF III and V Trials. Stroke 2007;38:2965-71.

55. Testa L, van Gaal W, Agostoni P, Abbate A, Trotta G, Biondi-Zoccai GG. Ximelagatran versus warfarin in the prevention of atrial fibrillation-related stroke: both sides of the story. Stroke 2007;38:e57.

56. Olsson SB; Executive Steering Committee of the SPORTIF III Investigators. Stroke prevention with the oral direct thrombin inhibitor ximelagatran compared with warfarin in patients with non-valvular atrial fibrillation (SPORTIF III): randomised controlled trial. Lancet 2003 22;362:1691-8.

57. Ieko M. Dabigatran etexilate, a thrombin inhibitor for the prevention of venous thromboembolism and stroke. Curr Opin Investig Drugs 2007;8:758-68.

58. Verheugt FW. Stroke prevention in atrial fibrillation. Neth J Med 2006;64:31-3.

59. Schneider B, Finsterer J, Stöllberger C. Effects of percutaneous left atrial appendage transcatheter occlusion (PLAATO) on left atrial structure and function. J Am Coll Cardiol 2005;45:634-5.

60. Harenberg J, Giese C, Hagedorn A, Traeger I, Fenyvesi T. Determination of antithrombin-dependent factor Xa inhibitors by prothrombin-induced clotting time. Semin Thromb Hemost 2007;33:503-7.

61. Bijsterveld NR, Vink R, van Aken BE, et al. Recombinant factor VIIa reverses the anticoagulant effect of the long-acting pentasaccharide idraparinux in healthy volunteers. Br J Haematol 2004;124:653-8.

62. Shen Q, Cordato D, Ng J, et al. Anticoagulant usage for primary stroke prevention: A general practitioner survey in local areas of metropolitan Sydney. J Clin Neurosci 2008;15:166-71.

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REVIEW

# Vascular liver disorders (I): diagnosis, treatment and prognosis of Budd-Chiari syndrome

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## ABSTRACT

**Budd-Chiari syndrome (BCS) is a venous outflow obstruction of the liver that has a dismal outcome if left untreated. Most cases of BCS in the Western world are caused by thrombosis of the hepatic veins, sometimes in combination with thrombosis of the inferior vena cava. Typical presentation consists of abdominal pain, hepatomegaly and ascites, although symptoms may vary significantly. Currently, a prothrombotic risk factor, either inherited or acquired, can be identified in the majority of patients. Moreover, in many patients with BCS a combination of risk factors is present. Myeloproliferative disorders are the most frequent underlying cause, occurring in approximately half of the patients. Recent discovery of the Janus Kinase 2 (JAK2) mutation has significantly contributed to the diagnosis of myeloproliferative disorders. Anticoagulation is indicated for all patients with BCS and additional therapy depends on the severity of symptoms and the extent of venous obstruction. A stepwise therapeutic approach is recommended, with increasing invasiveness and guided by the response to previous treatment. A transjugular intrahepatic portosystemic shunt (TIPS) is proving to be a good therapeutic option in patients with BCS, diminishing the need for surgical shunts. When all other therapy is unsuccessful or in patients with fulminant hepatic failure, a liver transplantation should be considered. Advances in diagnosis and treatment have dramatically improved the prognosis of patients with BCS. Still, many aspects of this complicated disorder remain to be clarified.**

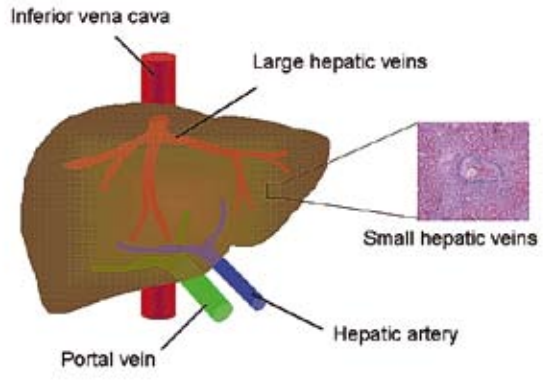
## KEYWORDS

Anticoagulation, Budd-Chiari syndrome (BCS), hepatic vein thrombosis, myeloproliferative disorder (MPD), transjugular intrahepatic portosystemic shunt (TIPS)

## INTRODUCTION

Thrombosis involving the liver vasculature is rare but constitutes a potentially life-threatening situation. Budd-Chiari syndrome (BCS) is characterised by thrombosis of the hepatic outflow tract. It is defined as a venous obstruction that can be located from the level of the small hepatic veins up to the junction of the inferior vena cava with the right atrium (*figure 1*).[1] Hepatic outflow obstruction related to right-sided cardiac failure or sinusoidal obstruction syndrome (SOS, also known as veno-occlusive disease)[2] is not included in the definition of BCS. The clinical symptoms of BCS were first described by Budd in 1845,[3] followed by Chiari's report of the underlying histopathology half a century later.[4] Over the past years, improved imaging techniques and new insights into

**Figure 1.** *Schematic overview of the vasculature of the liver*



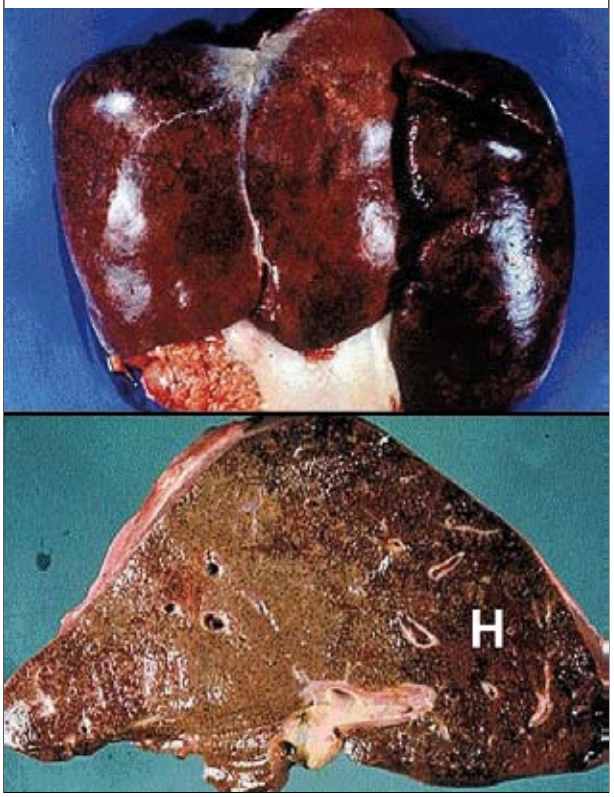

**In Budd-Chiari syndrome venous outflow from the liver is blocked, this obstruction can range from the small hepatic veins up to the inferior vena cava.**

potential causative factors have significantly contributed to the diagnosis and treatment of BCS. Nevertheless, due to the rarity of this disorder, most existing knowledge is based on data from (small) retrospective series. In this review we will give an overview of the current diagnosis, treatment and prognosis of BCS.

## CLINICAL MANIFESTATIONS OF HEPATIC VENOUS OBSTRUCTION

Obstruction of the hepatic veins gives rise to several haemodynamic changes, such as a decreased sinusoidal blood flow and an increased sinusoidal blood pressure, which can eventually lead to portal hypertension. Venous congestion also provokes ischaemia and subsequent necrosis of sinusoidal hepatocytes (*figure 2*). Significant hypoxic damage can result in a deterioration of hepatic synthetic function. Over time, hepatocytes are replaced by fibrosis, predominantly localised in the centrilobular area. Nodular regeneration is also regularly seen in patients with BCS and ultimately, cirrhosis may develop.[5] Other potential consequences of hepatic venous obstruction are portal vein thrombosis and hypertrophy of the caudate lobe. In approximately 15 to 20% of cases of BCS concomitant portal vein thrombosis is identified.[6,7] Because the caudate lobe is the only liver segment with direct venous drainage into the inferior vena cava, compensatory hypertrophy often occurs. Caudate hypertrophy itself can subsequently cause compression and stenosis of the inferior vena cava, further contributing to the already existent venous congestion.[8]

**Figure 2.** *Macroscopic view of the liver of a patient with Budd-Chiari syndrome (BCS) displaying massive congestion and patchy areas of haemorrhage (top panel) and cross-section through a liver of a BCS patient showing a clearly demarcated area of extensive haemorrhage (H) (bottom panel)*




Clinical presentation of patients with BCS is heterogeneous and ranges from the absence of symptoms to severe liver failure. The classical triad consists of abdominal pain, ascites and hepatomegaly but other possible symptoms are nausea, fever and jaundice.[9] The severity of disease is influenced by the extent of thrombosis, the rapidity of onset and the ensuing effect of compensatory mechanisms such as the formation of collateral veins. In the past years, different classifications (i.e. acute, subacute and chronic) have been used to describe patients with BCS according to the duration and severity of symptoms.[10] However, the prognostic value of these descriptive categories has not been validated. Instead, more recent studies have attempted to determine distinct prognostic classes based on the outcome of clinical and laboratory assessments.[11,12]

Despite the major haemodynamic changes involving the liver, synthetic function is often relatively spared. However, this does not preclude a late decline in general condition and liver function. During the course of the disease, portal hypertension frequently develops and may be complicated by bleeding from gastro-oesophageal varices. In a significant number of patients signs of portal hypertension, such as splenomegaly or oesophageal varices, are already present at diagnosis, implicating that an acute thrombotic event can be superimposed on a long-standing obstruction. Less common, an episode of gastrointestinal bleeding is the first presenting sign of BCS.[13,14] In contrast, ascites is an important complication of hepatic venous obstruction and a frequent cause of morbidity. Control of ascites is therefore important in the management of patients with BCS.

## AETIOLOGY

BCS can be further classified as primary or secondary, depending on the underlying cause and the type of venous obstruction. If an endoluminal venous lesion is present, such as thrombosis or an inferior vena cava web, BCS is considered primary. The secondary form consists of venous obstruction caused by external invasion or compression of the venous lumen, as is the case with malignant tumours or large cysts.[1] In Western countries, thrombosis is the most frequent cause of venous obstruction in

BCS. Whereas in the past many cases were designated as idiopathic,[10,15] it has nowadays been established that in most patients with BCS an underlying risk factor predisposing to thrombosis is present. Both inherited (e.g. Factor V Leiden mutation, deficiencies in protein C, protein S and antithrombin) and acquired (e.g. paroxysmal nocturnal haemoglobinuria, antiphospholipid syndrome) procoagulant disorders have been associated with BCS, of which myeloproliferative disorders are the most common (*table 1*).[16,17] When both overt and latent forms are taken into account, approximately 50% of patients with BCS are shown to have an underlying myeloproliferative disorder (MPD).[14,18,19] Moreover, it has become clear that in a large proportion of patients more than one risk factor can be identified.[20] In studies of BCS patients with a proven MPD, additional prothrombotic factors were found in more than 30% of the cases.[21,22]

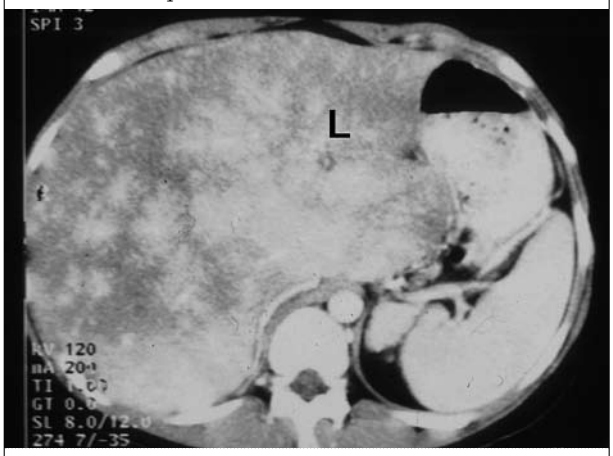
**Table 1.** *Risk factors for Budd-Chiari syndrome*

| |
|---|
| **Inherited** |
| Factor V Leiden mutation |
| Prothrombin (factor II) mutation |
| Protein C deficiency |
| Protein S deficiency |
| Antithrombin deficiency |
| |
| **Acquired** |
| Myeloproliferative disorder |
| Paroxysmal nocturnal haemoglobinuria |
| Antiphospholipid syndrome |
| Behçet's disease |
| Oral contraceptives |
| Pregnancy and puerperium |
| Hyperhomocysteinaemia |

## DIAGNOSTIC WORK-UP

Presence of hepatic venous outflow obstruction should be suspected in patients with (acute onset of) ascites and painful hepatomegaly or when refractory ascites is present, typically in combination with relatively normal liver function tests. BCS should also be considered if liver disease is observed in patients with known thrombophilia. Physical examination and laboratory investigations are usually not very specific. In most cases diagnosis can be accurately assessed with noninvasive radiological imaging. Doppler ultrasonography is the initial technique of choice and has high sensitivity and specificity.[23] Findings that support the diagnosis of BCS are absence of flow or retrograde flow in the hepatic veins and the presence of thrombosis within the hepatic veins or inferior vena cava. Other indicative features are intrahepatic or subcapsular collateral veins and failure to visualise the hepatic veins.[24,25] Computerised tomography (CT) and magnetic resonance imaging (MRI) are also frequently applied to demonstrate occlusion of the hepatic veins, inferior vena cava or both. With these techniques the liver parenchyma itself is usually better visualised to show perfusion details or necrotic areas (*figure 3*).[26] Secondary causes of BCS, such as tumoural invasion or cysts causing venous compression, can also be identified with these different imaging modalities. Invasive hepatic venography is still regarded as the reference procedure but is nowadays only performed if venous pressure measurements are required.

**Figure 3.** *Computerised tomography image showing a cross-section through the liver of a patient with Budd-Chiari syndrome*

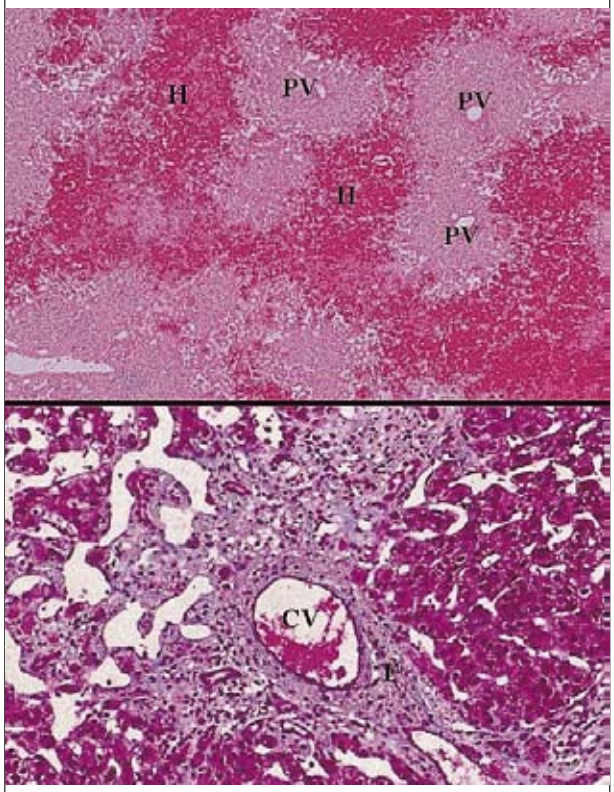



**A common finding in these patients is a patchy distribution of congestion and haemorrhage throughout the liver parenchyma.**

A liver biopsy is not required to confirm the diagnosis of BCS but can be carried out to rule out other causes. Due to the high risk of sampling error, a biopsy is insufficient to study the severity of BCS.[27] Typical histological findings of hepatic venous outflow obstruction are congestion, loss of hepatocytes and fibrosis, most often in the centrilobular area.[28] Histological abnormalities usually show an inhomogeneous distribution depending on the involved venous obstruction (*figure 4*). Other parenchymal changes that can be found in approximately 25% of patients along the course of the disease are regenerative nodules. These benign nodules are thought to develop as a result of an imbalance between arterial and portal blood flow. Usually, multiple regenerative lesions are present that can range in diameter from a few millimetres up to 7 cm.[5,29] Although malignant hepatic lesions are rarely seen in patients with BCS, it may be difficult to distinguish regenerative macronodules from hepatocellular carcinoma.[30]

An equally important part of the diagnostic work-up in patients with thrombosis of the hepatic veins is the

**Figure 4.** *Liver biopsy specimen (haematoxylin and eosin (HE) staining, x100)*



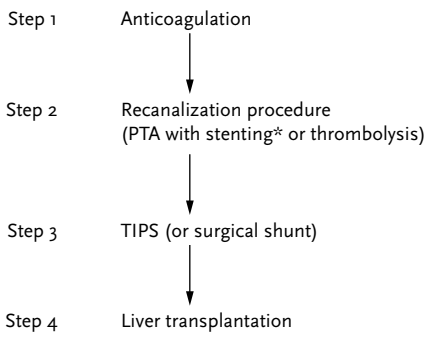

**Top panel: depicting areas of haemorrhage (H) and congestion surrounding the central veins (zone 3). The periportal area (zone 1) around the portal vein (PV) branches is relatively spared. Bottom panel: further enlarged view of liver parenchyma (HE staining, x200) depicting a central vein (CV) of a liver lobulus surrounded by an area of fibrosis (F). This so-called pericentral fibrosis is a typical finding in patients with Budd-Chiari syndrome (BCS).**

identification of underlying thrombophilic factors. As mentioned previously, in a significant number of patients multiple aetiological factors can be identified.[20] Therefore, the presence of one thrombophilic factor should not preclude further investigations of other possible risk factors. Diagnosis of an MPD can prove to be difficult in patients with BCS because in many cases typical changes in peripheral blood (i.e. high levels of haemoglobin or platelets) are absent and conventional diagnostic criteria are often not met.[31] In the past, these so-called occult or latent forms could only be detected by bone marrow biopsy or the existence of endogenous erythroid colony formation.[18,32] Recently however, the diagnosis of (occult) MPDs has been facilitated by the discovery of the Janus Kinase 2 (JAK2) mutation. This acquired gain-of-function mutation of the JAK2 tyrosine kinase can be demonstrated in the majority of patients with an MPD.[33,34] Furthermore, several studies have already pointed out that the JAK2 mutation is proving to be a reliable screening marker for MPDs in patients with BCS.[21,22,35,36] Because not all cases of MPD are JAK2 positive and further characterisation is often needed, a bone marrow biopsy will still be required in most patients.

## TREATMENT

Due to the rarity of the disorder, no controlled studies have been performed in patients with BCS. Therefore, most current knowledge and recommendations are based on case reports, retrospective studies and expert opinions. Furthermore, because experience with the treatment of this vascular liver disorder is often limited, all patients diagnosed with BCS should preferentially be referred to a specialised liver centre. The first step in the treatment of patients with BCS is initiation of anticoagulant therapy to prevent extension of the thrombosis. Although evidence remains circumstantial, lifelong anticoagulation is recommended in all patients with this life-threatening form of thrombosis, providing that there are no contraindications.[1] Underlying thrombophilic conditions should be identified and treated where possible. The next step in the management process concerns the manifestations and complications of liver disease. In the past, invasive treatment for patients with BCS was frequently applied and many patients were treated with surgical portosystemic shunts or liver transplantation.[37-40] Recently, however, a more stepwise approach has been advocated where therapeutic procedures are performed in order of increasing invasiveness and based on the response to previous treatment (*figure 5*).[41] This is supported by the finding that some patients can be adequately treated in a noninvasive manner.[19] Nevertheless, if ascites and other complications cannot be controlled with anticoagulation and diuretics alone, further (invasive) treatment steps are required. Percutaneous transluminal angioplasty (PTA) has

**Figure 5.** *Treatment algorithm for patients with Budd-Chiari syndrome*



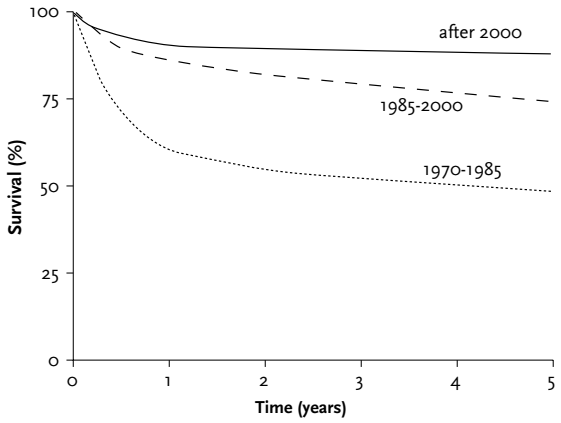

***Only possible in case of short-length stenosis. PTA = percutaneous transluminal angioplasty; TIPS = transjugular intrahepatic portosystemic shunt.**

been successful in a number of patients but should only be performed if a short-length stenosis is present.[42,43] Systemic or local thrombolytic therapy has also been attempted as a recanalisation procedure, with variable success. Recent evidence suggests that it should be executed with great caution due to the high risk of bleeding complications (unpublished data). When these recanalisation techniques are not eligible or unsuccessful at controlling symptoms of ascites and portal hypertension, a shunting procedure is indicated. Surgical portosystemic shunting has now been almost completely abandoned as a treatment modality for patients with BCS. In a recent study it was performed in less than 2% of the patients.[19] Moreover, other studies have not been able to demonstrate a survival benefit for patients treated with surgical shunts.[12,13] This could be explained by a high perioperative mortality and a risk of shunt dysfunction or thrombosis.[44,45] Instead, more patients are currently being treated with a transjugular intrahepatic portosystemic shunt (TIPS) to lower portal venous pressure and decompress the sinusoids. Over the past years it has become increasingly clear that the outcome of TIPS in patients with BCS is good. The procedure is less invasive than surgical shunting, it can be successfully performed in most patients and there are fewer complications.[46,47] Furthermore, in high-risk patients, TIPS placement may even improve survival.[48] Nevertheless, when shunting procedures do fail and clinical deterioration occurs, orthotopic liver transplantation (OLT) is the last treatment option for patients with BCS. Patients presenting with fulminant liver failure should also be considered for liver transplantation. Survival rates and graft function after OLT in patients with BCS are comparable to patients transplanted for other causes.[49,50] Additionally, previous TIPS insertion does not impair the outcome of transplantation[51] and in some cases TIPS placement can therefore serve as a bridge to liver transplantation.

## PROGNOSIS

Prognosis of patients with BCS has dramatically improved in the past decades, which could be explained by a combination of earlier diagnosis, introduction of new treatment modalities and the routine use of anticoagulation.[52] Whereas before 1985 one-year survival rates of approximately 60% were reported,[12,14,53] in more recent patient cohorts this number has increased to more than 80%. [12,14,41] Long-term survival in a large group of patients diagnosed with BCS from 1984 until 2001 was shown to be 69 and 62% at five and ten years, respectively (*figure 6*).[13] From this same cohort a prognostic score was developed (Rotterdam BCS index) that identifies three distinct groups of patients with a good, intermediate and poor prognosis. The Rotterdam BCS index is based on four different clinical parameters (encephalopathy, ascites, prothrombin time and bilirubin) and can easily be calculated at diagnosis of BCS.[13] Whether specific underlying aetiological factors influence the prognosis of patients with BCS is still unclear. Current evidence suggests that survival of patients with an MPD does not differ from patients without an underlying MPD.[21,22] Also, survival does not seem to be impaired by the recent shift in management leading to a less invasive treatment approach.[19,41] In contrast, the presence of concurrent portal vein thrombosis has been associated with a poor prognosis in patients with BCS.[6,7] Further studies are warranted to investigate the effect of different prothrombotic factors on prognosis and to identify specific patients that require early invasive treatment.

**Figure 6.** *Survival curves of patients with Budd-Chiari syndrome from different time periods*[52]




**Data on patient survival in different study periods is based on references 11-14, 19, 41 and 53.**

## REFERENCES

1. Janssen HL, Garcia-Pagan JC, Elias E, Mentha G, Hadengue A, Valla DC. Budd-Chiari syndrome: a review by an expert panel. J Hepatol 2003;38:364-71.
2. DeLeve LD, Shulman HM, McDonald GB. Toxic injury to hepatic sinusoids: sinusoidal obstruction syndrome (veno-occlusive disease). Semin Liver Dis 2002;22:27-42.
3. Budd G. On diseases of the liver. Philadelphia: Lea and Blanchard, 1846.
4. Chiari H. Ueber die selbständige Phlebitis obliterans der Haubtstämme der Venae hepaticae als Todesursache. Beitr Pathol Anat Allg Pathol 1899;26:1-18.
5. Cazals-Hatem D, Vilgrain V, Genin P, et al. Arterial and portal circulation and parenchymal changes in Budd-Chiari syndrome: a study in 17 explanted livers. Hepatology (Baltimore, Md.) 2003;37:510-9.
6. Mahmoud AE, Helmy AS, Billingham L, Elias E. Poor prognosis and limited therapeutic options in patients with Budd-Chiari syndrome and portal venous system thrombosis. Eur J Gastroenterol Hepatol 1997;9:485-9.
7. Murad SD, Valla DC, de Groen PC, et al. Pathogenesis and treatment of Budd-Chiari syndrome combined with portal vein thrombosis. Am J Gastroenterol 2006;101:83-90.
8. Holland-Fischer P, Gronbaek H, Astrup L, Keiding S, Nielsen DT, Vilstrup H. Budd-Chiari and inferior caval vein syndromes due to membranous obstruction of the liver veins: successful treatment with angioplasty and transcaval transjugular intrahepatic porto-systemic shunt. Scand J Gastroenterol 2004;39:1025-8.

9. Valla DC. Hepatic vein thrombosis (Budd-Chiari syndrome). Semin Liver Dis 2002;22:5-14.

10. Dilawari JB, Bambery P, Chawla Y, et al. Hepatic outflow obstruction (Budd-Chiari syndrome). Experience with 177 patients and a review of the literature. Medicine (Baltimore) 1994;73:21-36.

11. Langlet P, Escolano S, Valla D, et al. Clinicopathological forms and prognostic index in Budd-Chiari syndrome. J Hepatol 2003;39:496-501.

12. Zeitoun G, Escolano S, Hadengue A, et al. Outcome of Budd-Chiari syndrome: a multivariate analysis of factors related to survival including surgical portosystemic shunting. Hepatology (Baltimore, Md.) 1999;30:84-9.

13. Murad SD, Valla DC, de Groen PC, et al. Determinants of survival and the effect of portosystemic shunting in patients with Budd-Chiari syndrome. Hepatology (Baltimore, Md.) 2004;39:500-8.

14. Hadengue A, Poliquin M, Vilgrain V, et al. The changing scene of hepatic vein thrombosis: recognition of asymptomatic cases. Gastroenterology 1994;106:1042-7.

15. Ludwig J, Hashimoto E, McGill DB, van Heerden JA. Classification of hepatic venous outflow obstruction: ambiguous terminology of the Budd-Chiari syndrome. Mayo Clin Proc 1990;65:51-5.

16. Janssen HL, Meinardi JR, Vleggaar FP, et al. Factor V Leiden mutation, prothrombin gene mutation, and deficiencies in coagulation inhibitors associated with Budd-Chiari syndrome and portal vein thrombosis: results of a case-control study. Blood 2000;96:2364-8.

17. Deltenre P, Denninger MH, Hillaire S, et al. Factor V Leiden related Budd-Chiari syndrome. Gut 2001;48:264-8.

18. Valla D, Casadevall N, Lacombe C, et al. Primary myeloproliferative disorder and hepatic vein thrombosis. A prospective study of erythroid colony formation in vitro in 20 patients with Budd-Chiari syndrome. Ann Intern Med 1985;103:329-34.

19. Murad SD, Plessier A, Hernandez-Guerra M, et al. Prospective follow-up study on 163 patients with Budd-Chiari syndrome: Results from the European network for vascular disorders of the liver (EN-VIE). J Hepatol 2007;46:S4.

20. Denninger MH, Chait Y, Casadevall N, et al. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. Hepatology (Baltimore, Md.) 2000;31:587-91.

21. Smalberg JH, Murad SD, Braakman E, Valk PJ, Janssen HL, Leebeek FW. Myeloproliferative disease in the pathogenesis and survival of Budd-Chiari syndrome. Haematologica 2006;91:1712-3.

22. Kiladjian JJ, Cervantes F, Leebeek FWG, et al. Role of JAK 2 mutation detection in budd-chiari syndrome (BCS) and portal vein thrombosis (PVT) associated to MPD. Blood 2006;16;108:116a-a.

23. Bolondi L, Gaiani S, Li Bassi S, et al. Diagnosis of Budd-Chiari syndrome by pulsed Doppler ultrasound. Gastroenterology 1991;100(5 Pt 1):1324-31.

24. Millener P, Grant EG, Rose S, et al. Color Doppler imaging findings in patients with Budd-Chiari syndrome: correlation with venographic findings. AJR Am J Roentgenol 1993;161:307-12.

25. Brancatelli G, Vilgrain V, Federle MP, et al. Budd-Chiari syndrome: spectrum of imaging findings. AJR Am J Roentgenol 2007;188:W168-76.

26. Buckley O, J OB, Snow A, et al. Imaging of Budd-Chiari syndrome. Eur Radiol 2007 Aug;17:2071-8.

27. Tang TJ, Batts KP, de Groen PC, et al. The prognostic value of histology in the assessment of patients with Budd-Chiari syndrome. J Hepatol 2001;35:338-43.

28. Tanaka M, Wanless IR. Pathology of the liver in Budd-Chiari syndrome: portal vein thrombosis and the histogenesis of veno-centric cirrhosis, veno-portal cirrhosis, and large regenerative nodules. Hepatology (Baltimore, Md.) 1998;27:488-96.

29. Brancatelli G, Federle MP, Grazioli L, Golfieri R, Lencioni R. Large regenerative nodules in Budd-Chiari syndrome and other vascular disorders of the liver: CT and MR imaging findings with clinicopathologic correlation. AJR Am J Roentgenol 2002;178:877-83.

30. Vilgrain V, Lewin M, Vons C, et al. Hepatic nodules in Budd-Chiari syndrome: imaging features. Radiology 1999;210:443-50.

31. McNamara C, Juneja S, Wolf M, Grigg A. Portal or hepatic vein thrombosis as the first presentation of a myeloproliferative disorder in patients with normal peripheral blood counts. Clin Lab Haematol 2002;24:239-42.

32. Hirshberg B, Shouval D, Fibach E, Friedman G, Ben-Yehuda D. Flow cytometric analysis of autonomous growth of erythroid precursors in liquid culture detects occult polycythemia vera in the Budd-Chiari syndrome. J Hepatol 2000;32:574-8.

33. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-61.

34. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-90.

35. Janssen HL, Leebeek FW. JAK2 mutation: The best diagnostic tool for myeloproliferative disease in splanchnic vein thrombosis? Hepatology (Baltimore, Md.) 2006;44:1391-3.

36. Patel RK, Lea NC, Heneghan MA, et al. Prevalence of the activating JAK2 tyrosine kinase mutation V617F in the Budd-Chiari syndrome. Gastroenterology 2006;130:2031-8.

37. Hemming AW, Langer B, Greig P, Taylor BR, Adams R, Heathcote EJ. Treatment of Budd-Chiari syndrome with portosystemic shunt or liver transplantation. Am J Surg 1996;171:176-80; discussion 80-1.

38. Panis Y, Belghiti J, Valla D, Benhamou JP, Fekete F. Portosystemic shunt in Budd-Chiari syndrome: long-term survival and factors affecting shunt patency in 25 patients in Western countries. Surgery 1994;115:276-81.

39. Shaked A, Goldstein RM, Klintmalm GB, Drazan K, Husberg B, Busuttil RW. Portosystemic shunt versus orthotopic liver transplantation for the Budd-Chiari syndrome. Surg Gynecol Obstet 1992;174:453-9.

40. Orloff MJ, Daily PO, Orloff SL, Girard B, Orloff MS. A 27-year experience with surgical treatment of Budd-Chiari syndrome. Ann Surg 2000;232:340-52.

41. Plessier A, Sibert A, Consigny Y, et al. Aiming at minimal invasiveness as a therapeutic strategy for Budd-Chiari syndrome. Hepatology (Baltimore, Md.) 2006;44:1308-16.

42. Fisher NC, McCafferty I, Dolapci M, et al. Managing Budd-Chiari syndrome: a retrospective review of percutaneous hepatic vein angioplasty and surgical shunting. Gut 1999;44:568-74.

43. Valla D, Hadengue A, el Younsi M, et al. Hepatic venous outflow block caused by short-length hepatic vein stenoses. Hepatology (Baltimore, Md.) 1997;25:814-9.

44. Ringe B, Lang H, Oldhafer KJ, et al. Which is the best surgery for Budd-Chiari syndrome: venous decompression or liver transplantation? A single-center experience with 50 patients. Hepatology (Baltimore, Md.) 1995;21:1337-44.

45. Bachet JB, Condat B, Hagege H, et al. Long-term portosystemic shunt patency as a determinant of outcome in Budd-Chiari syndrome. J Hepatol 2007;46:60-8.

46. Perello A, Garcia-Pagan JC, Gilabert R, et al. TIPS is a useful long-term derivative therapy for patients with Budd-Chiari syndrome uncontrolled by medical therapy. Hepatology (Baltimore, Md.) 2002;35:132-9.

47. Rossle M, Olschewski M, Siegerstetter V, Berger E, Kurz K, Grandt D. The Budd-Chiari syndrome: outcome after treatment with the transjugular intrahepatic portosystemic shunt. Surgery 2004;135:394-403.

48. Raffa S, Heydtmann M, Plessier A, et al. Treatment of Budd-Chiari syndrome with tips. Long-term results in 124 patients and evaluation of prognostic factors. J Hepatol 2007;46:S36-7.

49. Mentha G, Giostra E, Majno PE, et al. Liver transplantation for Budd-Chiari syndrome: A European study on 248 patients from 51 centres. J Hepatol 2006;44(3):520-8.

50. Ulrich F, Pratschke J, Neumann U, et al. Eighteen years of liver transplantation experience in patients with advanced Budd-Chiari syndrome. Liver Transpl 2008;14:144-50.

51. Segev DL, Nguyen GC, Locke JE, et al. Twenty years of liver transplantation for Budd-Chiari syndrome: a national registry analysis. Liver Transpl 2007;13:1285-94.

52. Valla DC. Thrombosis and anticoagulation in liver disease. Hepatology (Baltimore, Md.) 2008;47:1384-93.

53. Powell-Jackson PR, Melia W, Canalese J, Pickford RB, Portmann B, Williams R. Budd-Chiari Syndrome: clinical patterns and therapy. Q J Med 1982;51:79-88.

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ORIGINAL ARTICLE

# Defective interferon-gamma production in patients with hairy cell leukaemia

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## ABSTRACT

**Background: Patients with hairy cell leukaemia (HCL) have an increased susceptibility to intracellular pathogens, such as mycobacteria and *Listeria*. Although several abnormalities of T-cell populations have been described in HCL, the effector mechanism responsible for the increased susceptibility to infections is not known.**

**Methods: Blood was collected from 11 patients with HCL and 22 age- and gender-matched volunteers. Proinflammatory cytokine production by freshly isolated mononuclear cells was stimulated with either lipopolysaccharide or various heat-killed microorganisms. Cytokine concentrations were assessed by specific ELISAs.**

**Results: We demonstrate that mononuclear cells harvested from HCL patients have a specific defect of IFNγ production when stimulated with a broad panel of bacterial stimuli. In contrast, the production of other proinflammatory cytokines, such as TNF, IL-1β and IL-6, did not differ between HCL patients and controls.**

**Conclusion: The specific defect in IFNγ production may play a role in the susceptibility of patients with hairy cell leukaemia towards intracellular pathogens.**

## KEYWORDS

Cytokines, hairy cell leukaemia, interferon-gamma, mycobacteria

## INTRODUCTION

Hairy cell leukaemia (HCL) is a chronic B-lymphocyte malignancy, in which mature neoplastic B lymphocytes show hair-like protrusions.[1] A major characteristic of HCL is splenomegaly, due to accumulation of malignant cells. In HCL, opportunistic infections, especially with facultative intracellular pathogens such as mycobacteria or *Listeria*, are prominent.[2] The occurrence of these infections suggests a serious defect in the cellular immune defence provided by T lymphocytes and macrophages.[3]

Although HCL is a B-cell malignancy, a series of abnormalities in the function of T cells have been also reported. There is abnormal T-cell activation, proliferation, clonal expansion and distribution; there is a decrease in memory T helper cells, an increase in splenic CD3-positive T cells positive for gamma delta T-cell receptors, and a restricted and skewed repertoire of the T-cell receptor family.[4-7] Although these abnormalities all point to T-lymphocyte dysfunction, the exact mechanism behind the increased susceptibility to facultative intracellular infection is not clear.

In recent years, an increased susceptibility to mycobacteria and *Salmonella* species has been described in patients with genetic defects in the interleukin-12/interferon-gamma (IL-12, IFNγ) macrophage activation route.[8] In these patients, the defects result in impaired IFNγ signalling, required to activate the microbicidal capacity of macrophages.

In the present study we have assessed the production of IFNγ by peripheral blood cells of patients with HCL, to obtain a better insight into the effector arm of cellular immunity in this disease. Since cytokines other than IFNγ are also relevant for host defence against infection, and cytokine production is regulated within what is known as the cytokine network, we measured a series of other relevant proinflammatory cytokines.

## PATIENTS AND METHODS

### Patients

Eleven patients diagnosed with HCL were included in the study (see *table 1* for clinical characteristics). Two of these patients had a mycobacterial infection at clinical

**Table 1.** *Clinical characteristics of the 11 patients with hairy cell leukemia (HCL)*

| Patient | Gender | Year of diagnosis | HCL therapy | Mycobacterial infections | Monocytes (x $10^3$/ml) |
|---|---|---|---|---|---|
| 1 | M | 1987 | IFNα | No | 70 |
| 2 | M | 1983 | IFNα<br>2-CDA | No | 56 |
| 3 | M | 1994 | 2-CDA | No | 232 |
| 4 | M | 1992 | IFNα | No | 106 |
| 5 | M | 1984 | Splenectomy<br>IFNα | No | 803 |
| 6 | M | 1995 | 2-CDA | Mycobacterial lymphadenitis | 324 |
| 7 | M | 1996 | IFNα | No | 128 |
| 8 | F | 2000 | IFNα<br>G-CSF | Disseminated *M. kansasii* | 117 |
| 9 | M | 1986 | IFNα | No | 264 |
| 10 | M | 2000 | 2-CDA | No | 406 |
| 11 | M | 1983 | Splenectomy<br>IFNα | No | 690 |

presentation. These patients had completely recovered from this infectious episode at the time of the present study. Patients had been treated previously with either cladribine (2-CDA), IFNα or a combination of both. Medication was stopped at least two weeks prior to the assessment of cytokine production. All samples were obtained between 9 am and 11 am, and for each patient, two gender-matched volunteers were concomitantly tested (n=22, 20 men and 2 women, age 26 ± 7 years).

**Ex-vivo stimulation of cytokine production**

After obtaining informed consent, venous blood was drawn from the cubital vein of patients and healthy volunteers into three 10 ml EDTA tubes (Monoject). Isolation of mononuclear cells (MNC) was performed as described,[9] with minor modifications. The MNC fraction was obtained by density centrifugation of blood diluted 1:1 in pyrogen-free saline over Ficoll-Paque (Pharmacia Biotech). Cells were washed twice in saline and suspended in culture medium (RPMI 1640 DM) supplemented with gentamicin 10 µg/ml, L-glutamine 10 mM and pyruvate 10 mM. The cells were counted in a Coulter counter (Coulter Electronics) and the number was adjusted to 5 x $10^6$ cells/ml. The MNC population consisted of approximately 80% lymphocytes and 20% monocytes, and no differences between patients and controls were apparent.

5 x $10^5$ MNC in a 100 µl volume were added to round-bottom 96-well plates (Greiner) and incubated with either 100 µl of culture medium (negative control), or one of the various stimuli: 10 ng/ml LPS (*S. typhimurium*; Sigma Chemical), or 1 x $10^6$ microorganisms/ml heat-killed (30 min, 100°C) *S. typhimurium*, *Staphylococcus aureus*, *Mycobacterium tuberculosis* or *Candida albicans*. After 24 hours (TNF, IL-1β and IL-6) or 48 hours (IFNγ) incubation at 37°C, supernatants were collected and cytokine concentrations were measured using specific ELISA kits (Pelikine, Sanquin, Amsterdam).

**Statistical analysis**

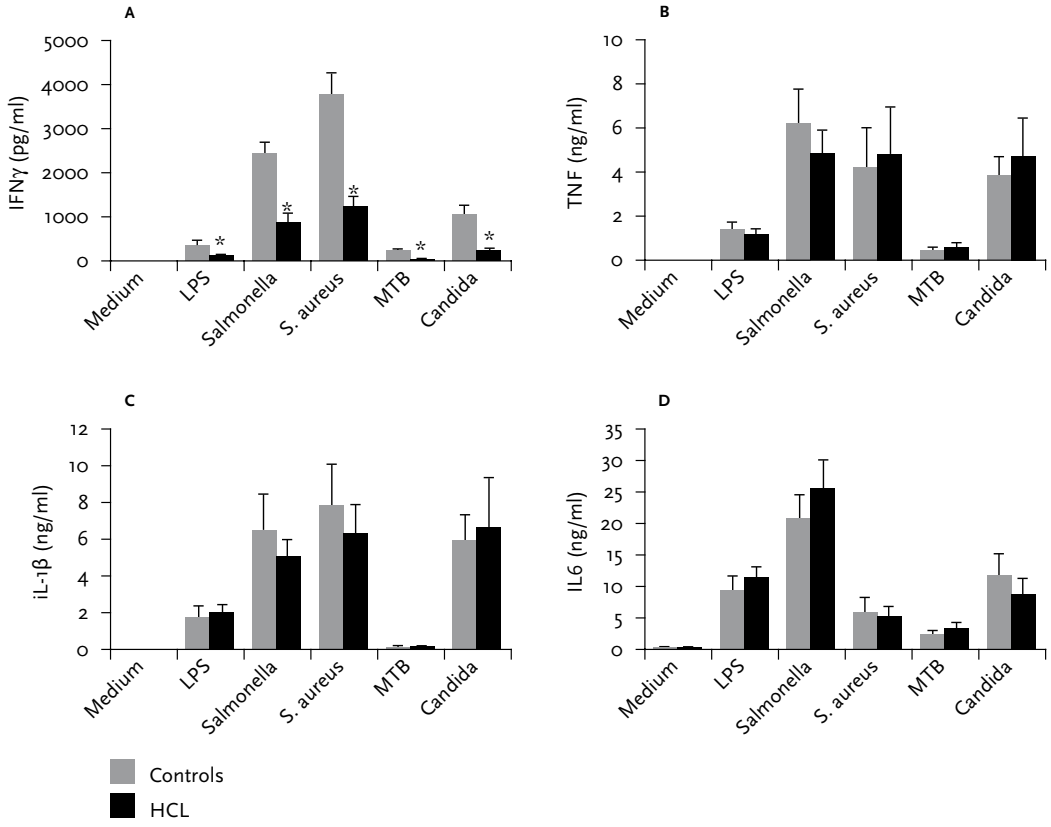
The experiments were performed in duplicate with blood obtained from patients and volunteers, and the data are presented as cumulative results of all experiments performed. The differences between groups were analysed by the Mann-Whitney U test. The level of significance between groups was set at $p<0.05$. The data are given as means ± SD.

## RESULTS AND DISCUSSION

When MNC of HCL patients were stimulated with LPS or a panel of microorganisms, the IFNγ production after 48 hours of stimulation was significantly lower compared with healthy volunteers (*figure 1A*). In contrast, the production of the proinflammatory cytokines TNFα, IL-1β and IL-6 did not differ between HCL patients and controls (*figure* 1B-D). The specific defect in IFNγ production, in contrast to the normal synthesis of monocyte products such as TNFα, IL-1β and IL-6, suggests a selective T/NK-cell defect in HCL patients, and is not part of a more general defect in cytokine production. It is tempting to assume that such defective production also occurs *in vivo* and underlies the remarkable susceptibility of HCL patients to facultative intracellular pathogens.

Our finding that TNFα production is normal is remarkable, since elevated serum concentrations of TNFα have been reported in patients with HCL.[10,11] As far as we know, defective IFNγ production has not been reported previously, despite the fact that there is extensive literature on T-lymphocyte abnormalities in this disorder.[4-7] Cytokine gene expression in patients with HCL has been reported

**Figure 1.** *Cytokine production capacity in hairy cell leukemia (HCL)*




**PBMC from healthy volunteers (n=22, open bars) and HCL patients (n=11, closed bars) were stimulated with LPS (10 ng/ml), or heat-killed *S. typhimurium, S. aureus, M. tuberculosis, or C. albicans*. The cytokine concentrations were measured in the supernatant after 48 hours (IFNγ) or 24 hours (TNF, IL-1β, and IL-6). The data are expressed as means ± SD, *p<0.05.**

by Kluin-Nelemans *et al.*[4] These authors investigated cytokine mRNA in T-cell fractions from spleens of HCL patients, and found spontaneous gene expression for IFNγ IL-2, IL-4 and granulocyte-macrophage colony stimulating factor (GM-CSF). Their finding of increased IFNγ mRNA in spleen and our report of strongly decreased IFNγ production may suggest that there is either a translational defect in these T cells, or a compartimentalised IFNγ production. Further studies are needed to elucidate this issue.

Decreased IFNγ production as a likely explanation for infections caused by facultative intracellular microorganisms, such as mycobacteria, has been reported for a number of conditions. First of all, there are hereditary disorders of the IL-12/IFNγ axis.[12,13] In CD4 lymphopenia, either as a consequence of HIV infection or idiopathic, IFNγ production is low due to the loss of CD4 cells.[14,15] In addition, in hyperIgE syndrome (HIES/Job's syndrome), deficient IFNγ production has been found when blood cells were stimulated with relevant microbial stimuli (*Staphylococcus* and *Candida* spp.).[16,17] It should be noted that in these patients, the susceptibility to infection pertains to the pathogens mentioned, rather than to facultative intracellular pathogens.[18] A similar finding has been described in chronic mucocutaneous candidiasis.[19] Finally, anti-TNFα treatment in patients with rheumatoid arthritis may lead to deficient IFNγ production and infections by both facultative intracellular pathogens and pyogenic microorganisms.[20] A consequence of our finding is that recombinant IFNγ treatment may be considered for patients with HCL suffering from serious infection, with facultative intracellular microorganisms.

## ACKNOWLEDGEMENTS

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## REFERENCES

1. Cawley JC, Burns GF and Hayhoe FJG. Hairy cell leukemia. Berlin: Springer-Verlag, 1980.
2. Bennett C, Vardiman J and Golomb H. Disseminated atypical mycobacterial infection in patients with hairy cell leukemia. Am J Med 1986;80:891-6.
3. Van der Meer JWM. Defects in host defense mechanisms. In: Rubin RH, Young LS, eds. Clinical approach to infection in the compromised host. New York: Plenum, 1994:33-66.

4. Kluin-Nelemans JC, Kester MG, Oving I, Cluitmans FH, Willemze R, Falkenburg JH. Abnormally activated T lymphocytes in the spleen of patients with hairy-cell leukemia. Leukemia 1994;8:2095-101.

5. Kluin-Nelemans HC, Kester MG, van de Corput L, et al. Correction of abnormal T-cell receptor repertoire during interferon-alpha therapy in patients with hairy cell leukemia. Blood 1998;91:4224-31.

6. Van de Corput L, Falkenburg JH, Kluin-Nelemans JC. T-cell dysfunction in hairy cell leukemia: an updated review. Leuk Lymphoma 1998;30:31-9

7. Van de Corput L, Falkenburg JH, Kester MG, Willemze R, Kluin-Nelemans JC. Impaired expression of CD28 on T cells in hairy cell leukemia. Clin Immunol 1999;93:256-62.

8. Ottenhof TH, Kumararatne D, Casanova JL. Novel human immunodeficiencies reveal the essential role of type-I cytokines in immunity to intracellular bacteria. Immunol Today 1998;19:491-4.

9. Endres S, Ghorbani R, Lonnemann G, van der Meer JWM, Dinarello CA. Measurement of immunoreactive interleukin-1 beta from human mononuclear cells: optimization of recovery, intrasubject consistency, and comparison with interleukin-1 alpha and tumor necrosis factor. Clin Immunol Immunopathol 1988;49:424-38.

10. Foa R, Massaia M, Cardona S, et al. Production of tumor necrosis factor-alpha by B-cell chronic lymphocytic leukemia cells: a possible regulatory role of TNF in the progression of the disease. Blood 1990;76:393-400.

11. Eigler A, Waller-Fontaine K, Moeller J, Hartmann G, Hacker UT, Endres S. The hairy cell leukemia cell line Eskol spontaneously synthesizes tumor necrosis factor-alpha and nitric oxide. Leuk Res 1998;22:501-7.

12. De Jong R, Altare F, Haagen IA, et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science 1998;280:1435-8.

13. Altare F, Durandy A, Lammas D, et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 1998;280:1432-5.

14. Orlikowsky T, Wang ZQ, Dudhane A, Horowitz H, Conti B, Hoffmann M. The cell surface marker phenotype of macrophages from HIV-1-infected subjects reflects an IL-10-Enriched and IFN-gamma-deprived donor environment. J Interferon Cytokine Res 1996;16:957-62.

15. Netea MG, Brouwer AE, Hoogendoorn EH, et al. Two patients with cryptococcal meningitis and idiopathic CD4 lymphopenia: defective cytokine production and reversal by recombinant interferon- gamma therapy. Clin Infect Dis 2004;39:e83-7.

16. Paganelli R, Scala E, Capobianchi MR, et al. Selective deficiency of interferon-gamma production in the hyper-IgE syndrome. Relationship to in vitro IgE synthesis. Clin Exp Immunol 1991;84:28-33.

17. Netea MG, Kullberg BJ, van der Meer JW. Severely impaired IL-12/IL-18/IFNgamma axis in patients with hyper IgE syndrome. Eur J Clin Invest 2005;35:718-21

18. Grimbacher B, Holland SM, Gallin JI, et al. Hyper-IgE syndrome with recurrent infections - an autosomal dominant multisystem disorder. N Engl J Med 1999;340:692-702.

19. Van der Graaf CAA, Netea MG, Drenth JPH, te Morsche RH, van der Meer JWM, Kullberg BJ. Candida-specific interferon-gamma deficiency and Toll-like receptor polymorphisms in patients with chronic mucocutaneous candidiasis. Neth J Med 2003;61:365-9.

20. Netea MG, Radstake T, Joosten LA, van der Meer JWM, Kullberg BJ. Salmonella septicemia in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: association with decreased interferon-gamma production and Toll-like receptor 4 expression. Arthritis Rheum 2003;48:1853-7.

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CASE REPORT

# Haemolytic anaemia as a first sign of Wilson's disease

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## ABSTRACT

**A 19-year-old female presented with haemolytic anaemia and subsequently developed liver failure. This raised suspicion of Wilson's disease, which was confirmed by Kayser-Fleischer rings, a low ceruloplasmin level, raised 24-hour urinary copper excretion and two mutations in the 'Wilson gene'. She was successfully treated with D-penicillamine and zinc.**

**In young patients with unexplained haemolysis, liver dysfunction or neuro-psychiatric symptoms, Wilson's disease should be considered.**

## KEYWORDS

Haemolysis, haemolytic anaemia, Wilson's disease

## INTRODUCTION

The differential diagnosis of haemolytic anaemia is extensive and Wilson's disease is generally not the first condition to be considered. It can be difficult to diagnose, due to the low specificity of the presenting symptoms. However, because of the potentially fatal consequences, a timely diagnosis is of utmost importance.

## CASE REPORT

A 19-year-old female student, with no prior medical history, was admitted with a Coombs negative haemolytic anaemia (haemoglobin (Hb) 3.8 mmol/l, mean cell volume (MCV) 109 fl, haptoglobin <0.1 g/l, lactate dehydrogenase (LDH) 784 U/l, aspartate aminotransferase (ASAT) 53 U/l, total bilirubin 84 µmol/l, direct bilirubin 23 µmol/l,) and an elevated gamma-glutamyl transferase (γGT) (144 U/l) with other serum liver tests in the low normal range (alanine aminotransferase (ALAT) 13 U/l, alkaline phosphatase 19 U/l.) The pathological findings were interpreted as a side effect of azithromycin treatment given for a *Chlamydia* infection. Parameters reflecting hepatic protein synthesis were not determined at that time. She was treated with prednisone and a blood transfusion and was discharged in an improved condition. After discharge, her haemoglobin level remained stable (8.0 mmol/l).

Two months later, she presented with jaundice, fatigue and subfebrile temperature. She had not used any drugs or medication, except oral contraceptives; her family history was unremarkable. On examination, she was in moderate distress and jaundiced. Her body temperature was 37.6°C; the other vital signs were normal. There were no palpable lymph nodes; liver and spleen were not enlarged.

Blood examination disclosed a macrocytic anaemia with signs of haemolysis (Hb 4.8 mmol/l, MCV 110 fl, haptoglobin <0.1 g/l, LDH 714 U/l, ASAT 105 U/l, total bilirubin 118 µmol/l, direct bilirubin 48 µmol/l, reticulocytes 0.202 x $10^{12}$/l) and a leucocytosis (17.3 x $10^{9}$/l) with young erythroid and myeloid cells, but without signs of chronic myeloid leukaemia or other myeloproliferative disorders. Her platelet count was normal. The γGT was elevated (147 U/l), ALAT was normal (22 U/l). The Coombs test was negative. Chest X-ray and abdominal ultrasound were unremarkable. Analyses for enzyme deficiencies, autoantibodies, (viral) infections and paroxysmal nocturnal haematuria were negative.

Haemolysis continued in spite of intravenous treatment with prednisone. The leucocytosis increased to 80.6 x $10^{9}$/l, presumably in part reactive and in part due to the use of prednisone. A bone marrow biopsy revealed a greatly increased erythropoiesis.

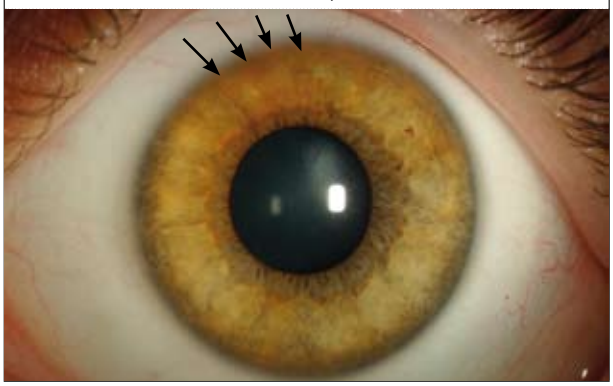
On the seventh day of admission, the patient developed ascites. The aspirate showed a transudate (serum-albumin-

ascites gradient 19 g/l), without signs of infection or malignant cells. Furthermore, the liver synthesis function was impaired (albumin 20.7 g/l, cholinesterase 1.4 kU/l, antithrombin III 24%, international normalised ratio (INR) 1.97.).

The combination of haemolysis and signs of liver failure raised suspicion of Wilson's disease. The ophthalmologist diagnosed Kayser-Fleischer rings (*figure 1*). Neurological examination revealed a slight tremor of the left hand. Serum ceruloplasmin level was low (116 mg/l, normal 200 to 600 mg/l). The 24-hour urine copper excretion was elevated more than tenfold (23.4 µmol). Liver biopsy was postponed because of the elevated INR and ascites. Diagnosis was confirmed by a mutation analysis of *ATP7B*, the gene associated with Wilson's disease, which showed two heterozygeous mutations, c.2930C>T(p.Thr977Met) and c.3207C>A (p.His1069Gln).

Medical treatment was started with D-penicillamine at low and slowly increasing doses to avoid aggravation of the tremor. Zinc was added strictly separately from the D-penicillamine. The patient tolerated treatment well. The haemolysis diminished within days, the slight tremor completely disappeared, and liver function normalised within months under continuous medical treatment.

**Figure 1.** *Image of the patient's cornea where copper depositions form Kayser-Fleischer rings (arrows), thus obscuring the underlying crypts of the iris (still visible at the lateral and medial sides)*



## PATHOGENESIS

Wilson's disease is a rare disorder of transmembrane copper transport in hepatic cells.[1] The incidence is 1:30,000 worldwide, and the usual age at presentation is 5 to 30 years.[2] However, Wilson's disease can present even after the age of 50 years. The disorder is caused by autosomal recessive mutations in the *ATP7B* gene on chromosome 13. This gene encodes a transmembrane protein in the hepatocyte that ensures copper transport in the trans-Golgi compartment for incorporation in apoceruloplasmin to form ceruloplasmin and excretion of excess copper through the billiary system.[2,3]

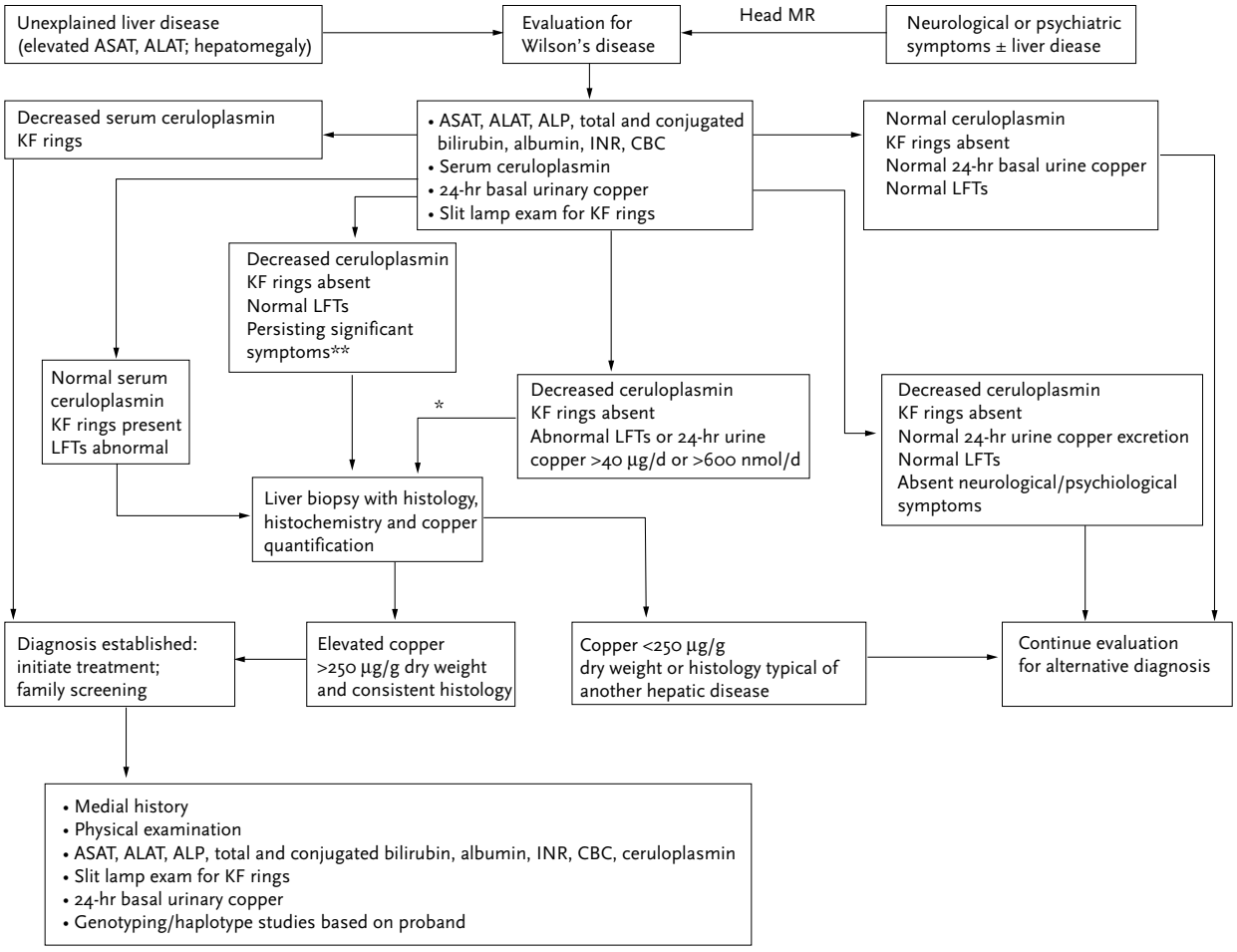
A defect in this gene leads to accumulation of copper in the hepatocyte and subsequently in all extrahepatic organs. In the liver, this leads to disturbances varying from mild elevation of serum liver tests to steatosis, chronic hepatitis and eventually cirrhosis.[2-4] Liver failure in Wilson's disease is sometimes accompanied by severe haemolysis.[2] Copper depositions in the Descemet membrane of the cornea form Kayser-Fleischer rings (*figure 1*). The nuclei lenticulares in the brain are particularly sensitive to copper accumulation. Neurological symptoms such as tremor, dysarthria and parkinsonism have been observed, as well as psychiatric symptoms such as depression and psychosis.[2-4] Haemolysis as the presenting symptom – as seen in our patient – is less common (1 to 12%).[4] It is presumably caused by excess copper released from the liver due to apoptosis/necrosis of copper-loaded hepatocytes, possibly triggered by external stimuli. This may have direct toxic effects on erythrocytes, resulting in haemolysis.[5]

## DIAGNOSIS

The diagnosis of Wilson's disease is based on a combination of several clinical and biochemical parameters (*figure 2*).[4,6,7] Kayser-Fleischer rings are identified in 50 to 100% of patients, depending on the presenting symptoms.[6,7] They are almost pathognomonic for Wilson's disease but can also be seen in chronic cholestatic diseases.[2,4] Serum ceruloplasmin levels below the normal range are found in more than 85% of patients. Low serum ceruloplasmin levels, however, are not specific for Wilson's disease, but can also be found in malnutrition, in heterozygous carriers of an *ATP7B* gene mutation, Menke's disease or in familial aceruloplasminaemia. Because ceruloplasmin is an acute-phase protein, it can also be in the normal range in patients with Wilson's disease during inflammation.[2-4,6] A normal serum ceruloplasmin may be associated with mutations that allow copper to be transported to the trans-Golgi where ceruloplasmin is formed, but not to bile. Serum liver tests are frequently abnormal. The alkaline phosphatase level will sometimes drop to zero in liver failure associated with Wilson's disease.[2-4] Although serum free copper level is less suitable for making the diagnosis, it can be used for ascertaining the effect of treatment.[3,4,8]

Release of copper from the hepatocyte increases the 24-hour urine copper excretion in nearly all symptomatic patients, although in some it can be between 600 and 1600 nmol/day.[9] Other liver diseases, particularly cholestatic diseases, can also elevate urinary copper excretion.[2-4] Liver biopsy shows nonspecific signs such as steatosis, portal and periportal lymphocytic infiltration and cirrhosis. Staining for copper is often false-negative and not reliable

**Figure 2.** *Diagnostic algorithm for Wilson's disease*[4]




**In younger patients, a penicillamine challenge test can be performed preceding liver biopsy. **Such as haemolysis, unexplained splenomegaly, or neuropsychiatric symptoms. ALP = alkaline phosphatase; ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; CBC = complete blood count; INR = international normalised ratio; LFT = liver function test; KF = Kayser-Fleischer.**

for a diagnosis of Wilson's disease. The dry copper weight is increased in 80 to 96% of patients but can be false-negative due to extensive fibrosis and false-positive in chronic cholestatic disease.[2,3,10] Radiological imaging plays a minor role in the diagnosis. In patients presenting primarily with neurological symptoms, a cerebral MRI can show abnormalities, particularly of the basal ganglia.[2,4]

Once the diagnosis is made, screening of siblings is required.[2-4,8] Therefore, genetic screening for defects in the patient's *ATP7B* gene is useful. More than 200 mutations have been described; in Europe, the most abundant is the H1069Q mutation.[2]

## TREATMENT

Wilson's disease leads to death at an early age without adequate and lifelong medical therapy. Treatment aims at lowering the copper overload in the body. This is achieved by application of (i) copper chelators such as D-penicillamine or trientine which deplete tissue copper stores and stimulate renal copper secretion, or (ii) zinc which impairs intestinal copper uptake.

In symptomatic patients with Wilson's disease, chelation therapy is an effective treatment strategy. The most frequently used drugs are D-penicillamine and trientine. Both bind copper and are subsequently excreted by the kidneys, thereby providing an alternative route for the defective biliary copper excretion. D-penicillamine is more effective, but also more toxic than trientine: 10 to 20% of patients experience side effects such as allergic reactions, neutropenia, thrombopenia, proteinuria and eventually renal toxicity.[2,8] Furthermore, 20 to 50% of patients with neurological symptoms experience worsening of symptoms during treatment, which can be irreversible.[11]

In asymptomatic patients with Wilson's disease, zinc is a safe and effective treatment when administered at adequate doses. Zinc induces intracellular metallothionein formation in enterocytes (and hepatocytes). Metallothionein binds copper in the cytosol of enterocytes and, thereby, lowers systemic copper uptake. Normal intestinal epithelial turnover leads to shedding of copper-loaded enterocytes resulting in a net copper loss.[2-4] Zinc is administered as a monotherapy in pre-symptomatic patients and as maintenance therapy following effective chelation treatment.[2,8]

Medical treatment must be continued lifelong to prevent disease progression and death. In general, monotherapy with chelators or zinc separate from meals is the recommended form of treatment depending on disease stage and symptoms. If administration of both zinc and a chelator is considered, both drugs need to be administered at different times of the day to avoid drug interactions.

The need for dietary measures in Wilson's disease is debatable; clear evidence for its effectiveness is lacking. Still, patients are informed to avoid copper-rich nutrients such as shellfish, organ meats, nuts, or chocolate.[8]

If the treatment compliance is good, the prognosis is excellent in most cases.[2,4,8] However, if treatment is not tolerated, if the disease is too far advanced before treatment is started, or if patients present with fulminant liver failure, the only option may be a liver transplantation. In patients with only hepatic manifestations, liver transplantation may cure the disease. In patients with primarily neurological symptoms, a transplantation does not guarantee complete remission of symptoms.[12] A relatively new chelator, ammonium tetrathiomolybdate, has shown good treatment results in these patients, but is not yet generally available.[13]

## CONCLUSION

In patients with haemolytic anaemia, liver dysfunction or neuropsychiatric symptoms of unknown cause, Wilson's disease should be considered. A timely diagnosis can be life-saving.

## REFERENCES

1. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 1912;34:295-507.
2. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet 2007;369:397-408.
3. Ferenci P. Diagnosis and current therapy of Wilson's disease. Aliment Pharmacol Ther 2004;19:157-65.
4. Roberts EA, Schilsky ML. A practice guideline on Wilson disease. Hepatology 2003;37:1475-92.
5. Attri S, Sharma N, Jahagirdar S, Thapa BR, Prasad R. Erythrocyte metabolism and antioxidant status of patients with Wilson disease with hemolytic anemia. Pediatr Res 2006;59:593-7.
6. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease. Gut 2007;56:115-20.
7. Taly AB, Meenakshi Sundaram S, Sinha S, Swamy HS, Arunodaya GR. Wilson's disease: description of 282 patients evaluated over 3 decades. Medicine 2007;86:112-21.
8. Brewer G, Askari F. Wilson's disease: clinical management and therapy. J Hepatol 2005;42:S13-21.
9. Müller T, Koppikar S, Taylor RM, et al. Re-evaluation of the penicillamine challenge test in the diagnosis of Wilson's disease in children. J Hepatol 2007;47:270-6.
10. Ferenci P, Steindl-Munda P, Vogel W, et al. Diagnostic value of quantitative hepatic copper determination in patients with Wilson disease. Clin Gastroenterol Hepatol 2005;8:811-8.
11. Brewer GJ, Terry CA, Aisen AM, Hill GM. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. Arch Neurol 1987;44:490-3.
12. Medici V, Mirante VG, Fassati LR, et al. Liver transplantation for Wilson's disease. Liver Transpl 2005;11:1056-63.
13. Brewer GJ, Askari F, Lorincz MT, et al. Treatment of Wilson disease with ammonium tetrathiomolybdate IV. Arch Neurol 2006;63:521-7.

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CASE REPORT

# Value of molecular analysis of Wilson's disease in the absence of tissue copper deposits: a novel *ATP7B* mutation in an adult patient

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## ABSTRACT

**Wilson's disease (WD) is a disorder of copper metabolism leading to copper accumulation in the liver and in extrahepatic organs, such as brain and cornea. We present a patient with liver disease who did not fulfil the biochemical criteria for WD. Mutational analysis was necessary to make the diagnosis and show a new mutation. Our case supports the use of mutation analysis in cases with unclear liver disease and suggests that the spectrum of WD is broader than currently assumed.**

## KEYWORDS

Copper, hepatitis, mutation analysis, Wilson's disease

## INTRODUCTION

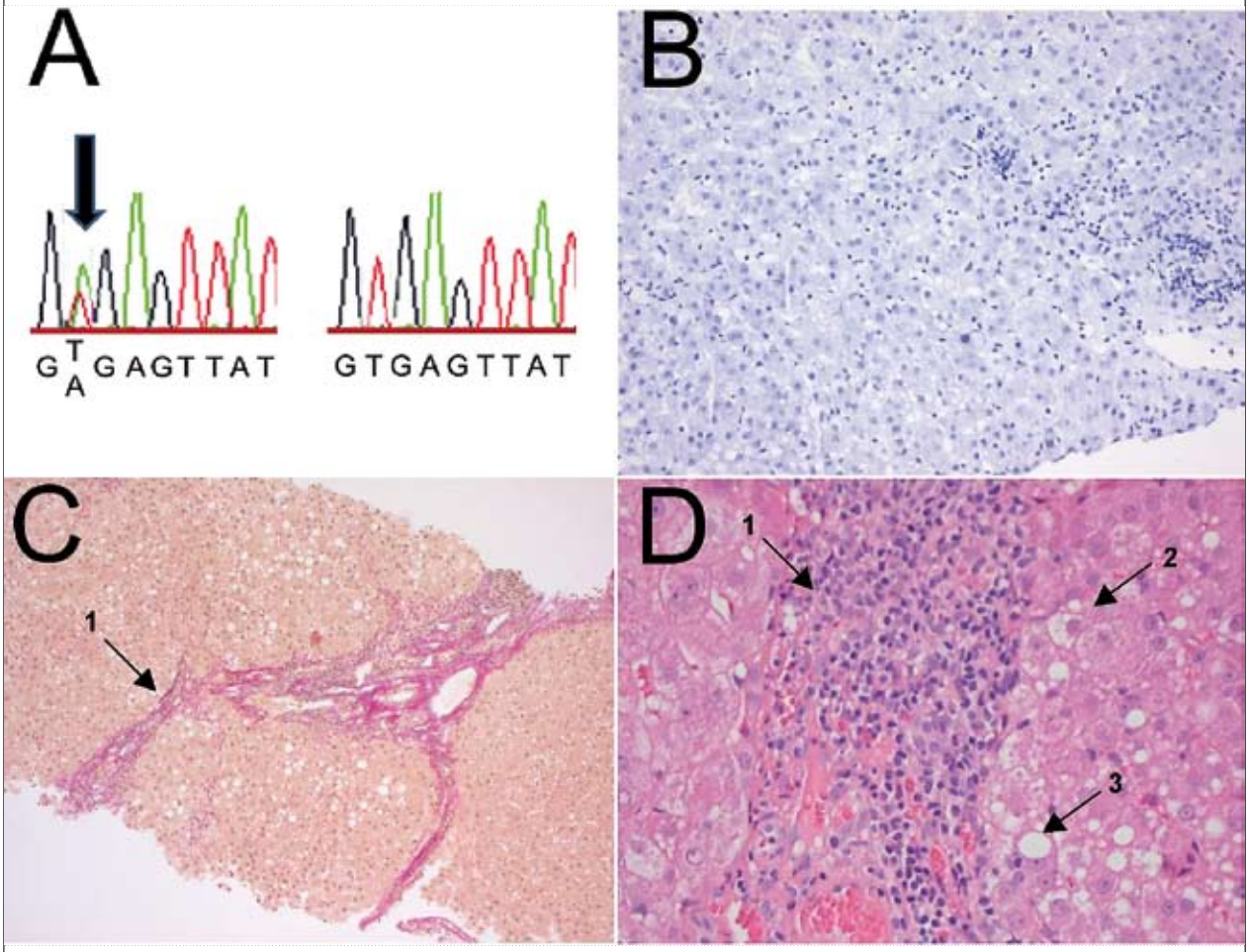
Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism. The ATP7B protein is an important transporter of copper and is responsible for WD; dysfunction can lead to copper accumulation in the liver and in extrahepatic organs, such as brain and cornea. This can lead to liver disease but also to (neuro)psychiatric disorders and Kayser-Fleischer (KF) rings around the iris. The diagnosis of WD is based on the results of several clinical and biochemical tests (decreased serum ceruloplasmin concentration, elevated 24-hour urinary copper excretion or elevated liver copper concentration). A liver biopsy with copper deposits is considered to be 'the gold standard' for the diagnosis.

WD is usually diagnosed in childhood and most physicians are aware of the association of the above-mentioned clinical findings with WD. However, in the absence of 'typical' clinical findings, WD is a diagnosis that can be hard to make. We would like to present a patient in whom we were only able to make the diagnosis of WD at adult age aided by molecular testing of the WD gene, *ATP7B*.[1,2]

## CASE REPORT

A 28-year-old woman visited our outpatient's clinic because of cryptogenic liver disease. At the age of 10 years she presented with disturbed liver enzymes without signs of viral hepatitis, autoimmune hepatitis, toxic hepatitis or metabolic liver diseases such as alpha-1 antitrypsin deficiency or hereditary haemochromatosis. Biochemistry demonstrated a decreased ceruloplasmin (0.13 g/l, normal 0.2 to 0.5 g/l), and mildly increased urinary copper excretion (2.31 umol/24 h, normal <1.5 umol/24 h). Repeated ophthalmological examination never showed Kayser-Fleischer rings. A liver biopsy at the age of 13 years showed steatosis and fibrosis. Copper staining was negative, reason to reject a diagnosis of WD at that time. At reassessment 15 years later, WD was reconsidered. The transaminases were still elevated (alanine aminotransferase 138 U/l; aspartate aminotransferase 63 (normal <45 U/l)). Serum copper was normal, most probably because the major copper binding protein in the plasma, ceruloplasmin, was only mildly decreased (0.17 g/l). In the absence of another explanation for the liver disease and in view of the decreased ceruloplasmin with increased urinary copper excretion of 2.9 umol/24 h we proceeded to perform mutation analysis of the *ATP7B* gene. Upon sequencing we detected compound heterozygosity for c.3207C>A (p.H1069Q) and c.2447+2T>A (*figure 1*, panel A). The p.H1069Q mutation is the most commonly detected

**Figure 1.** *A) The sequence analysis of ATP7B gene on an electrospherogram, B-D) Liver biopsy specimen of a 28-year-old patient with Wilson's disease*




**A) The left insert shows the mutant sequence in the patient. There is a c .2447+2T>A mutation at the splice donor site of intron 9 that affects splicing pre-messenger-RNA from *ATP7B*.**
**B) Copper staining is absent (rhodanine staining) (10x).**
**C) Elastic-van Giesons's staining on a representative liver section compatible with bridging fibrosis (5x).[1]**
**D) Haematoxylin eosin staining, original magnification (20x): there is 1) periportal hepatitis with 2) ballooning of hepatocytes and 3) moderate steatosis.**

mutation in WD; the second mutation c.2447+2T>A is a novel frame-shift mutation. We analysed the c.2447+2T>A variant for potential effects on the splice site using the splice site prediction by neural network (SSPNN; http://www.fruitfly.org/seq_tools/splice.html). The programme predicts the theoretical strength of donor and acceptor splice sites. The original splice site of intervening sequence 9 (IVS9) reaches the maximum probability score of 1.00 indicating that when the c.2447T>A variant is inserted in the sequence, the splice site is abolished.

We proceeded to perform a new liver biopsy which was consistent with periportal hepatitis, ballooning of hepatocytes, steatosis and bridging fibrosis (Metavir 2); again copper staining was negative (*figure 1*, panel B-D) The Metavir score assesses liver fibrosis on a five-point scale (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, 4 = cirrhosis). Given the positive molecular diagnosis of WD, we started her on chelating therapy with zinc sulphate (200 mg three times/day).

## DISCUSSION

WD is characterised by a decreased biliary copper excretion and a defective incorporation of copper into ceroloplasmin, leading to copper accumulation in liver and brain. The prevalence is about 1 in 30,000. The range of clinical symptoms in Wilson's disease is wide; hepatic disease can lead to elevated transaminases, chronic hepatitis, cirrhosis and fulminant liver failure. Brain involvement is associated with neurological symptoms such as tremor, chorea, parkinsonism, pseudobulbar symptoms, dystonia and seizures and even psychiatric disease. The archetypical

ocular symptoms are KF rings and cataract. In rare cases other organs such as heart (cardiomyopathy) can be involved.

There is not a single specific test for the diagnosis of WD; symptoms are often nonspecific. KF rings are often present, especially in patients with predominantly neurological disease (85 to 98%), in contrast to patients with predominantly liver disease where KF were detected in only 52%.[1,2] Quantitative copper concentration measurement remains the best biochemical evidence for WD, but due to a considerable sampling error, normal hepatic copper content does not exclude WD.[3] Histochemical staining by rhodanine is not a substitute; less than 10% of confirmed cases had proteinaceous copper deposits in hepatocytes.[4] To simplify the difficult diagnosis a diagnostic scoring system for WD has been proposed recently. This score includes data on the presence of KF rings, neuropsychiatric symptoms, haemolytic anaemia, urine copper, quantitative liver copper, rhodanine staining, serum ceruloplamin and mutation analysis.[5] Prior to mutational analysis our patient scored 2 points (decreased ceruloplasmin and increased urine copper excretion); after introduction of mutational analysis in the algorithm 4 additional points were gained: this made the diagnosis of WD 'highly likely'. Although it aids in making the diagnosis, we need to emphasise that this scoring system has not been assessed prospectively.

The gene that is defective in WD is *ATP7B* which encodes a transmembrane protein ATPase which functions as a copper-dependent P-type ATPase. The ATP7B transporter has synthetic and excretory roles, facilitating transport of copper into the trans-Golgi compartment, for incorporation into ceruloplasmin and into bile. When copper excess is present, the transporter adopts an excretory role by increasing biliary copper excretion. The *ATP7B* gene was cloned in 1993; since then more than 380 mutations have been reported in patients with WD from many different populations (http://www.medgen.med.ualberta.ca/database.html). The p.H1069Q mutation is the most commonly detected mutation in WD, and has an allele frequency of up to 72% among WD patients. This mutation is seen in patients with late manifestations of WD and is associated with only a mildly disturbed copper metabolism. In compound heterozygotes, the phenotype of WD to a small extent depends on the type of mutation coinherited with p.H1069Q. Homozygosity for frameshift mutations in *ATP7B* is associated with severe disturbances of copper metabolism and presentation at young age. The effect of compound heterozygosity of a mild (p.H1069Q) and severe (c.2447+2T>A) mutation is less well established although these patients develop WD at a later stage than carriers of two severe mutations.[6]

Our case history highlights several important aspects of WD. First, this case expands the clinical phenotype of Wilson's disease, showing that advanced WD can be present without readily detectable tissue copper staining. This apparently contradicts the concept that readily detectable liver copper accumulation should be present to diagnose WD. Negative copper staining results may be explained by heterogeneous liver copper distribution (sampling error) and differences in sensitivity of rhodanine for free copper and copper binding proteins, but might also suggest that other, hitherto unknown, cofactors might be present in order to cause liver fibrosis. It also shows the importance of molecular testing in cases with equivocal biochemical results as this showed the presence of two pathogenic *ATPB7* mutations consistent with WD. Lastly, the novel mutation adds to the mutational spectrum of WD.

Our data support the use of mutation analysis in cases with unclear liver disease with negative copper staining results and suggest that the spectrum of WD is broader than currently assumed.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet 2007;369:397-408.
2. Merle U, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut 2007;56:115-20.
3. McDonald JA, Snitch P, Painter D, Hensley W, Gallagher ND, McCaughan GW. Striking variability of hepatic copper levels in fulminant hepatic failure. J Gastroenterol Hepatol 1992;7:396-8.
4. Ludwig J, Moyer TP, Rakela J. The liver biopsy diagnosis of Wilson's disease. Methods in pathology. Am J Clin Pathol 1994;102:443-6.
5. Ferenci P, Caca K, Loudianos G, et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003;23:139-42.
6. Gromadzka G, Schmidt HH, Genschel J, et al. Frameshift and nonsense mutations in the gene for ATPase7B are associated with severe impairment of copper metabolism and with an early clinical manifestation of Wilson's disease. Clin Genet 2005;68:524-32.

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CASE REPORT

# Guilty as charged: unmeasured urinary anions in a case of pyroglutamic acidosis

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## ABSTRACT

**A patient developed an unexplained metabolic acidosis with the characteristics of renal tubular acidosis. By correcting the serum anion gap for hypoalbuminaemia and analysing the urinary anions and cations, the presence of unmeasured anions was revealed. The diagnosis of pyroglutamic acidosis, caused by a combination of flucloxacillin and acetaminophen, was established. Strategies for solving complex cases of metabolic acidosis are discussed.**

## KEYWORDS

Anion gap, flucloxacillin, hypernatraemia, hypokalaemia, metabolic acidosis, urine osmolal gap

## CASE REPORT

A 72-year-old woman (body weight 58 kg, height 170 cm) presented to the emergency room with back pain, fever and confusion. She had a previous history of lung emphysema, hepatitis A and B, breast cancer (treated with surgery and radiotherapy), and, more recently, a vertebral laminectomy for lumbar spinal canal stenosis. Her only medication consisted of bronchodilators and analgesics, including acetaminophen, non-steroidal anti-inflammatory drugs and morphine. At presentation, both vital signs (blood pressure 130/63 mmHg, pulse 94 beats/min and temperature 37.0°C) and physical examination were unremarkable. However, she had an infection (C-reactive protein 276 mg/l, leucocytes 19.3 x $10^9$/l) and was admitted for further analysis. Magnetic resonance imaging showed an epidural abscess, and subsequent computed tomography revealed bilateral abscesses in the psoas muscles. *Staphylococcus aureus* was isolated from blood cultures and from one of the psoas abscesses. Treatment with long-term and high-dose (two grams six times daily) intravenous flucloxacillin was commenced. Her only additional medication was acetaminophen (one gram four times daily). During her recovery, she became somnolent on the 43rd day of admission. Her laboratory results now showed a metabolic acidosis, hypokalaemia, and hypernatraemia (*table 1*). The following parameters were also measured: γ-glutamyltransferase 45 U/l, alkaline phosphatase 139 U/l, serum phosphate 0.80 mmol/l, serum uric acid 0.20 mmol/l; urine was negative for ketones, glucose and protein. Initially, renal tubular acidosis was suspected. However, two days later, this diagnosis was revised, after a high serum anion gap became apparent and after an analysis of measured and unmeasured urinary anions and cations was performed, revealing a considerable

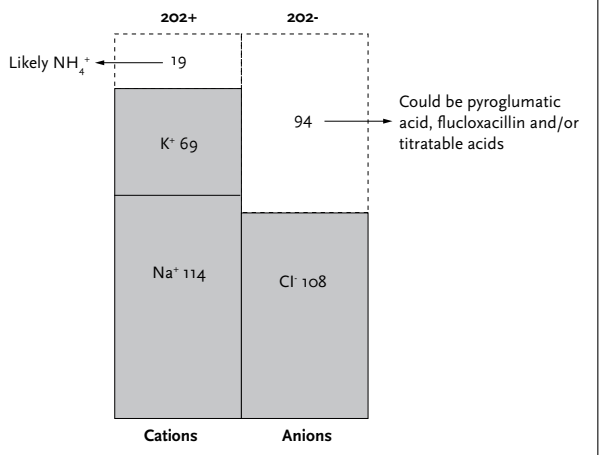
**Table 1.** *Measurements and calculations in serum and urine*

| | Serum | | Urine | |
|---|---|---|---|---|
| | Day 43 | Day 45 | Day 43 | Day 45 |
| C-reactive protein, mg/l | 74 | 63 | - | - |
| Leucocytes, $10^9$/l | 16.5 | 18.1 | - | - |
| Creatinine, µmol/l<br>mmol/l | 46 | 50 | - | <br>2.2 |
| Urea, mmol/l | 4.6 | 4.2 | - | 96 |
| Osmolality, mOsm/kg | 308 | - | - | 500 |
| $Na^+$, mmol/l | 150 | 151 | 66 | 114 |
| $K^+$, mmol/l | 3.3 | 3.5 | 79 | 69 |
| $Cl^-$, mmol/l | 120 | 123 | 87 | 108 |
| pH | 7.36 | 7.33 | 6.0 | 6.0 |
| $pCO_2$, kPa (mmHg) | 3.2 (25) | 2.5 (19) | - | - |
| $HCO_3^-$, mmol/l | 16 | 10 | - | - |
| Albumin, g/l | 25 | 25 | Negative | Negative |
| Lactate, mmol/l | 0.9 | 0.6 | - | - |
| Anion gap, mEq/l* | 14 | 18 | + 58 | + 75 |
| Corrected anion gap, mEq/l | 18 | 22 | - | - |

*Reference range for the serum anion gap in our centre is 8 to 16 mEq/l.

concentration of unmeasured urine anions (202-108 = 94 mEq/l, *figure 1*). Pyroglutamic acid was suspected to be this unmeasured anion, because several risk factors (flucloxacillin, acetaminophen, ongoing infection, female gender) for pyroglutamic acidosis were present. Indeed, urine pyroglutamic acid was elevated (90.9 mmol/mmol creatinine, reference <0.1 mmol/mmol creatinine, absolute concentration 201.6 mmol/l). The serum acetaminophen concentration was not very high (2 µg/ml). She was transferred to the intensive care unit, where all medication was discontinued and where she was treated with sodium bicarbonate. After her acid-base and electrolyte disorders had been corrected, she recovered without sequelae and was discharged to a nursing home for further recovery.

**Figure 1.** *Measured and unmeasured urinary anions and cations in a case of pyroglutamic acidosis*




**The number of anions and cations was calculated on day 45 using the following formula: (urine osmolality-urine urea)/2 = (500-96)/2 = 202. The measured cations and anions are depicted in light grey boxes with solid lines; the unmeasured cations and anions are depicted in white boxes with dashed lines.**

## DISCUSSION

Pyroglutamic acidosis is increasingly being recognised as an important cause of high anion gap metabolic acidosis.[1] Because Kortmann *et al.* have already covered the pathophysiology of pyroglutamic acidosis in this issue of the Journal,[2] our objective in this discussion is to focus on the diagnostic challenges associated with metabolic acidosis due to an unmeasured anion.

Initially, we diagnosed our case as renal tubular acidosis, because of the apparent hyperchloraemic non-anion gap metabolic acidosis, high urine pH, positive urine anion gap, presence of hypokalaemia, and the absence of common disorders causing high-anion gap acidosis.[3] However, a critical reappraisal, which consisted of appropriately adjusting the serum anion gap for hypoalbuminaemia and an analysis of urinary cations and anions (*figure 1*), suggested unmeasured anions. This reappraisal generated several teaching points (summarised in *table 2*).

**Table 2.** *Teaching points*

- Pyroglutamic acidosis is a cause of high anion gap metabolic acidosis
- Pyroglutamic acid can be 'overproduced' in the setting of glutathione depletion (acetaminophen, sepsis, liver dysfunction, malnutrition) or 'undersecreted' due to inhibition of its enzyme (flucloxacillin, vigabatrin)
- When hypoalbuminaemia is present, the serum anion gap should be adjusted downward (2.5 mEq decrease in anion gap for each 10 g/l decrease in albuminaemia)
- The urine anion and osmolal gaps can be used to assess urinary ammonium excretion:
  - Urine anion gap: urine $Na^+ + K^+ - Cl^-$
  - Urine osmolal gap: measured minus calculated (2* [$Na^+ + K^+$] + urea + glucose) urine osmolality
  - Estimated urinary $NH_4^+$ excretion: (urine osmolal gap)/2
- The urine anion gap cannot differentiate between renal tubular acidosis and metabolic acidosis due to an unmeasured anion
- Analysis of urine cations and anions can be useful to detect the presence of unmeasured anions when the serum anion gap is inconclusive

First, the serum anion gap must be adjusted downward in patients with hypoalbuminaemia, because the negative charges on the serum proteins primarily determine the serum anion gap.[4] Nevertheless, even the adjusted serum anion gap may be difficult to interpret, because its expected normal values range so widely (~8 to 16 mEq/l when not including $K^+$) and also depend on the measurement characteristics of the laboratory.[5]

Second, in cases in which the serum anion gap is 'borderline' and the cause of the acidosis is not obvious from the clinical context, the analysis of the urinary composition may be useful. Traditionally, three urinary tests have been utilised to differentiate metabolic acidosis, including the urine pH, the urine anion gap and the urine osmolal gap.[6] Although the urine pH is expected to be high (generally >5.5) in renal tubular acidosis (due to urine bicarbonate loss or impaired ammoniogenesis), this test is limited by the use of semiquantitative dipsticks, dietary factors, and presence of urinary pathogens.[7]

Both the urine anion and osmolal gaps provide an estimate of urinary ammonium excretion.[8,9] The urinary ammonium excretion can be used to assess whether the kidneys are attempting to 'rid the acid' (high urinary ammonium excretion) or if the problem resides within the kidneys (failure to excrete ammonium), as for example in distal renal tubular acidosis.

The urine anion gap is an indirect measure of urine ammonium excretion, because ammonium is usually excreted as ammonium chloride.[8] However, the urine anion gap cannot differentiate between renal tubular acidosis and metabolic acidosis due to unmeasured anions.[6,8] In both of these settings the urine chloride concentration will be relatively low and therefore the anion gap positive, but for different reasons. In renal tubular acidosis, ammonium excretion is impaired and therefore little ammonium chloride is excreted. In metabolic acidosis due to an unmeasured anion, the kidneys will respond appropriately to the acidosis by increasing urinary ammonium excretion, but ammonium will be excreted with the unmeasured anions instead of with chloride.

The urine osmolal gap is a direct and semiquantitative index of the urinary ammonium concentration, because it estimates the concentration of this unmeasured cation (*figure 1*, *table 2*).[9] The estimated urinary ammonium excretion is our patient was 19 mEq/l, which is approximately the cut-off value between an appropriate and an inappropriate renal response to metabolic acidosis.[9] Although one would expect this value to be higher, the recent onset and relatively mild degree of metabolic acidosis likely produced the modest urine osmolal gap. More convincingly, however, was the large proportion of unmeasured anions, which prompted us to pursue the search for unmeasured anions. In theory (and for added complexity), these anions could also be urine bicarbonate, for example in proximal renal tubular acidosis; although this concentration of urine bicarbonate would seem rather high, there were no other signs of proximal tubular dysfunction, and treatment with bicarbonate would not have resolved the acidosis so easily.

Finally, we propose the following explanations for the hypokalaemia and hypernatraemia. Hypokalaemia could have been caused by a non-reabsorbable anion, which can stimulate potassium secretion in the renal collecting duct. Both pyroglutamic acid and flucloxacillin can act as non-reabsorbable anions,[10,11] and flucloxacillin may therefore also have contributed to the unmeasured urinary anions (*figure 1*). Hypernatraemia may have been caused by a positive sodium balance (large sodium content administered with flucloxacillin[12]) and/or a solute diuresis combined with reduced water intake.[13]

## CONCLUSION

We have presented yet another case of pyroglutamic acidosis to illustrate the importance of having an index of suspicion for this diagnosis in unexplained cases of a high anion gap metabolic acidosis, especially because discontinuation of the offending factors can easily reverse this condition. Moreover, this case was presented to illustrate the utility of analysing unmeasured urinary anions to assist in the diagnosis of challenging cases of metabolic acidosis.

## REFERENCES

1. Dempsey GA, Lyall HJ, Corke CF, Scheinkestel CD. Pyroglutamic acidemia: a cause of high anion gap metabolic acidosis. Crit Care Med 2000;28:1803-7.
2. Kortmann W, Agtmael MA, Diessen J, Kanen BL, Jakobs C, Nanayakkara PW. 5-Oxoproline as a cause of high anion gap metabolic acidosis: an uncommon cause with common risk factors. Neth J Med 2008;66(8):354-7.
3. Rodríguez Soriano J. Renal tubular acidosis: the clinical entity. J Am Soc Nephrol 2002;13:2160-70.
4. Gabow PA. Disorders associated with an altered anion gap. Kidney Int 1985;27:472-83.
5. Kraut JA, Madias NE. Serum anion gap: its uses and limitations in clinical medicine. Clin J Am Soc Nephrol 2007;2:162-74.
6. Halperin ML, Vasuvattakul S, Bayoumi A. A modified classification of metabolic acidosis. A pathophysiologic approach. Nephron 1992;60:129-33.
7. Richardson RM, Halperin ML. The urine pH: a potentially misleading diagnostic test in patients with hyperchloremic metabolic acidosis. Am J Kidney Dis 1987;10:140-3.
8. Batlle DC, Hizon M, Cohen E, Gutterman C, Gupta R. The use of the urine anion gap in the diagnosis of hyperchloremic metabolic acidosis. N Engl J Med 1988;318:594-9.
9. Halperin ML, Margolis BL, Robinson LA, Halperin RM, West ML, Bear RA. The urine osmolal gap: a clue to estimate urine ammonium in "hybrid" types of metabolic acidosis. Clin Invest Med 1988;11:198-202.
10. Lipner HI, Ruzany F, Dasgupta M, Lief PD, Bank N. The behavior of carbenicillin as a nonreabsorbable anion. J Lab Clin Med 1975;86:183-94.
11. Hoorn EJ, Zietse R. Severe hypokalaemia caused by flucloxacillin. J Antimicrob Chemother 2008;61:1396-8.
12. Brunner FP, Frick PG. Hypokalaemia, metabolic alkalosis, and hypernatraemia due to "massive" sodium penicillin therapy. BMJ 1968;4:550-2.
13. Palevsky PM, Bhagrath R, Greenberg A. Hypernatremia in hospitalized patients. Ann Intern Med 1996;124:197-203.

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CASE REPORT

# 5-Oxoproline as a cause of high anion gap metabolic acidosis: an uncommon cause with common risk factors

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## ABSTRACT

**High anion gap metabolic acidosis might be caused by 5-oxoproline (pyroglutamic acid). As it is very easy to treat, it might be worth drawing attention to this uncommon and probably often overlooked diagnosis. We present three cases of high anion gap metabolic acidosis due to 5-oxoproline seen within a period of six months.**

## KEYWORDS

Acidosis, high anion gap, oxoproline

## CASE REPORTS

### Case 1

A 72-year-old woman was treated with flucloxacillin (8 g/day) and lavage for septic arthritis of the right shoulder caused by *S. aureus*. Acetaminophen 4 g/day was prescribed to relieve the pain. She had documented osteoarthritis, frequent urinary tract infections and hypertension-induced renal failure. She was recovering when around the 10th day of treatment her condition started to deteriorate. Within a few days her breathing was laboured (Kussmaul breathing) and her consciousness became diminished (E3M5V4). Blood pressure (116/70 mmHg), pulse (81 beats/min) and temperature (36°C) were normal. Her renal function (Cockcroft-Gault clearance of 20 ml/min) was stable, and the cholestatic liver enzymes were slightly elevated (alkaline phosphatase (ALP) 191 U/l, gamma-glutamyl transferase (γGT) 117 U/l). The arterial blood gas analysis showed a pH of 7.12, a $pCo_2$ of 11 mmHg (1.47 kPa), a bicarbonate of 3.5 mmol/l, a base excess of -25 and a $pO_2$ of 175 mmHg (23.3 kPa) with oxygen. The anion gap was 30.75 mEq/l (corrected for serum albumin of 15 g/l). The serum lactate was repeatedly normal (0.7 mmol/l) and no ketones could be demonstrated in the urine. Ingestion of a substance such as ethylene glycol, methanol or salicylate was very unlikely. The acetaminophen level in the blood was therapeutic (3.2 mg/l). Although her body mass index (BMI) was 27 kg/m$^2$ she was malnourished. She was not artificially fed. A rapid Google search led us to the probable diagnosis of 5-oxoproline as a cause of this high anion gap metabolic acidosis in this malnourished patient with renal and lever insufficiency, who was taking acetaminophen and flucloxacillin. Acetaminophen was stopped and flucloxacillin was replaced by clindamycin. We treated her with bicarbonate (8.4%) and acetylcysteine (600 mg/8 hours). Her general condition improved rapidly. By gas chromatography-mass spectrometry[4,13] we demonstrated a highly elevated 5-oxoproline in urine (16,623 µmol /mmol creatinine (normal <100 µmol /mmol creatinine))[7,11] and plasma (6573 µmol/l (normal 15 µmol/l)). Two weeks later 5-oxoproline was undetectable in urine.

### Case 2

A 56-year-old HIV-positive woman came to the outpatient clinic because of shortness of breath. She was treated with antiretroviral therapy (didanosine, lamivudine and efavirenz) for HIV (undetectable viral load and CD4 of 430/µl). In the past she had developed renal failure probably due to tenofovir, which was replaced by didanosine. A week ago she was treated for a urinary tract infection due to *E. coli* with norfloxacin (400 mg twice daily). She used alcohol and methadone chronically and had been taking acetaminophen 2 g/day since the urinary tract infection. The physical examination was unremarkable, except for

a weight of 47 kg and some shortness of breath while undressing. Capillary blood gas analysis showed a pH of 7.20, $pCo_2$ of 27 mmHg (3.6 kPa), and a bicarbonate of 10 mmol/l. We calculated an anion gap of 28 mEq/l (corrected for serum albumin of 31 g/l). Lactic acidosis caused by didanosine was suspected but the serum lactate was repeatedly normal (1.3 mmol/l). No ketones could be demonstrated in the urine. The serum creatinine had increased to 155 µmol/l (Cockcroft-Gault clearance of 27 ml/min), the cholestatic liver enzymes were elevated (ALP 382 U/l, γGT 534 u/l) and the osmolal gap was 0.9 mOsm/kg. The acetaminophen level was therapeutic (12 mg/l). Our experience with the first case made us think of 5-oxoproline as a cause of this high anion gap metabolic acidosis due to the combination of acetaminophen, malnourishment, renal failure and alcohol abuse. She recovered after discontinuation of acetaminophen. Again we demonstrated a high 5-oxoproline in plasma (2292 µmol/l) and urine (4184 µmol /mmol creatinine) which returned to normal.

**Case 3**

A 79-year-old woman with chronic obstructive pulmonary disease (COPD) and osteoporosis was treated with flucloxacillin 12 g/day for a spondylodiscitis due to *S. aureus*. She had been taking acetaminophen 3 g/day for two months. After three weeks of treatment she developed shortness of breath without signs of an exacerbation of COPD. Besides a tachypnoea (respiratory rate of 30/min) the physical examination was unremarkable. Her BMI was 23 kg/m². The chest X-ray did not show any new pathology. Arterial blood gas analysis revealed a metabolic acidosis: pH 7.29, $pCO_2$ 23 mmHg (3.1 kPa), bicarbonate 10.9 mmol/l, base excess -15.7, $pO_2$ 81 mmHg (10.8 kPa). The normal serum lactate of 1.6 mmol/l could not explain the whole anion gap of 29 mEq/l. She had a serum creatinine of 183 µmol/l (Cockcroft-Gault clearance of 28 ml/min). The most likely cause of the renal failure was a combination of diabetes, use of gentamycin in the past and urinary tract infections. The cholestatic liver enzymes were elevated (ALP 391 U/l, γGT 523 u/l). Before the diagnosis of spondylodiscitis was made, she had been ill for quite a while and suffered from a severe bleed from a duodenal ulcer. Tube feeding was initiated but the patient repeatedly removed the nasogastric tube. Because of the possibility of 5-oxoproline as a cause of high anion gap metabolic acidosis, flucloxacillin and acetaminophen were stopped and she was treated with bicarbonate (8.4%) and acetylcysteine (600 mg/8 hours) infusion. The patient and her husband did not want her to be transported to the ICU and unfortunately she died of respiratory insufficiency. Autopsy was not permitted. In the plasma a very high 5-oxoproline was demonstrated.

**Table 1.** *Laboratory results*

| | Patient 1 | Patient 2 | Patient 3 |
|---|---|---|---|
| pH | **7.12** | **7.20** | **7.29** |
| $HCO3^-$ (22-26 mmol/l) | **3.5** | **10** | **10.9** |
| Anion gap (8-16 mEq/l) | **24.5** | **26** | **24.1** |
| Anion gap corrected for albumin (mEq/l) | **31.75** | **28.25** | **29.1** |
| Lactate (< 2.2 mmol/l) | 0.7 | 1.3 | 1.6 |
| Albumin (35-52 g/l) | **15** | **31** | **20** |
| Creatinine (60-110 µmol/l) | **241** | 155 | 189 |
| Creatinine clearance CG (125-135 ml/min) | **20** | **27** | **28** |
| $Na^+$ (136-146 mmol/l) | 143 | 141 | 142 |
| K (3.6-4.8 mmol/l) | 3.7 | **3.4** | 3.6 |
| $PO_4^{3-}$ (0.7-1.4 mmol/l) | **2.21** | 1.11 | 1.2 |
| $Ca^{2+}$ (2.2-2.6 mmol/l) | 2.1 | 2.15 | 1.9 |
| $Cl^-$ (98-108 mmol/l) | **115** | 105 | 107 |
| C-reactive protein (<8 mg/l) | **76** | **12** | **92** |
| Leucocytes (4.0-10.0 10 x 9/l) | **14.6** | 8.5 | 9.1 |
| Bilirubin (<20 µmol/l) | 6 | 4 | 7 |
| Alkaline phosphatase (<120 U/l) | **191** | **382** | **391** |
| γ-GT (<40 U/l) | **117** | **534** | **523** |
| ASAT (<30 u/l) | 20 | 14 | 24 |
| ALAT (<35 u/l) | 74 | 15 | **41** |
| Prothrombin time (0.8-1.2 INR) | **1.65** | 1.05 | 1.10 |
| TSH (0.3-4.5 mu/l) | 3.1 | 3.9 | 4.1 |
| Plasma oxoproline (≤15 µmol/l) | **6573** | **2292** | **1050** |
| Urinary oxoproline (<100 µmol/mmol creat) | **16,623** | **4184** | - |

**CG = Cockcroft-Gault; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase; TSH = thyroid stimulating hormone; INR = international normalised ratio. Bold indicates abnormal values.**

## DISCUSSION

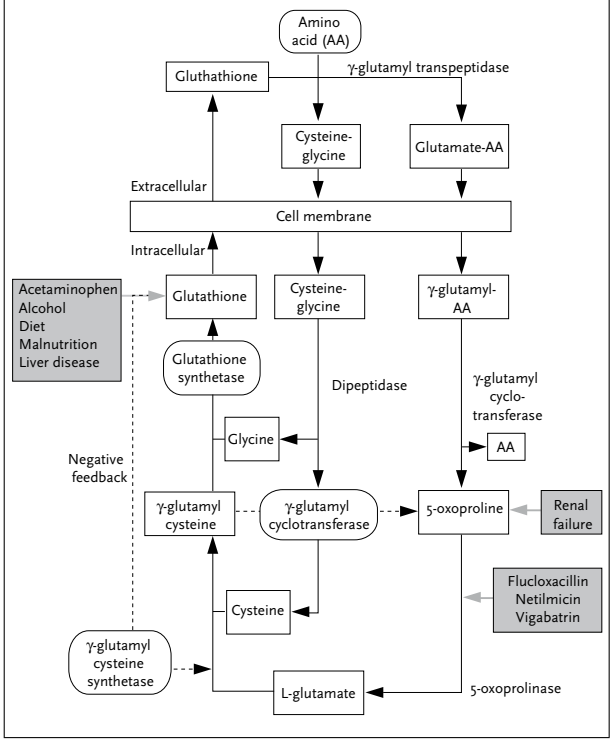
Pyroglutamic acidaemia is a rare cause of high anion gap metabolic acidosis.

Calculation of the anion gap might be helpful in the differential diagnosis of metabolic acidosis. The anion gap is the difference between the plasma concentration of the major cations ($Na^+$) and the major measured anions ($Cl^-$ and $HCO_3^-$) and is the difference between the unmeasured anions and the unmeasured cations.[1] In our patients the anion gap was markedly elevated (normal between 8 and 16 mEq/l). A fall in cations might cause just a small rise in anion gap, so the cause should be found in elevated unmeasured anions. The normal value for the anion gap must be adjusted downwards in patients with hypoalbuminaemia (2.5 mEq/l for every 10 g/l decline in the plasma albumin concentration).[1] In all three cases the lactate was either normal or slightly elevated. No ketones could be demonstrated in the urine. Ingestion of, for example, ethylene glycol, methanol and salicylate was very unlikely in the two patients in the

hospital and could be excluded in the other patient because of the normal osmolal gap. A normal creatinine kinase in all three patients excluded rhabdomyolysis as a cause. The renal function in all patients was diminished but could not explain the whole anion gap. Acidosis in renal failure is principally due to an accumulation of acids and a reduction in ammonium production due to decreased nephron mass. Acute renal failure typically presents with a combination of hyperchloraemic acidosis and high anion gap metabolic acidosis. Bicarbonate levels usually remain >15 mmol/l, and the anion gap does not usually exceed 20 mEq/l. In all three cases we demonstrated very high levels of 5-oxoproline which caused the acidosis. The fact that the levels of oxoproline in the three cases differ more than the level of the anion gap might be explained by the fact that measurements were not taken at the same moment in all cases and the anion gap might have been higher than our measurements indicated.

In the γ-glutamyl cycle the main tripeptide glutathione (glutamic acid, cysteine and glycine) plays an important role in immunomodulation, amino acid transport and detoxification. The enzymes glutathione synthetase and γ-glutamyl cysteine synthetase produce glutathione. A negative feedback of glutathione on γ-glutamyl cysteine synthetase regulates the activity of this enzyme.[2] Depletion of glutathione activates the enzyme in producing γ-glutamyl cysteine out of cysteine and glutamate. Gamma-glutamyl cysteine can be converted to glutathione by glutathione synthetase. With a high level of γ-glutamyl cysteine, γ-glutamyl cyclotransferase converts it directly to 5-oxoproline.[3] 5-Oxoproline is oxidised to glutamate by 5-oxoprolinase. This is a rate-limiting step and with a high level of 5-oxoproline, accumulation of 5-oxoproline occurs in the blood which causes acidosis.[4] Glutathione is found in all cells but mainly in the liver. Depletion of glutathione might be caused by acetaminophen,[5,6] diets,[7] severe sepsis, chronic alcohol abuse and diminished liver function. Renal failure causes diminished clearance of 5-oxoproline.[8] Some drugs such as flucloxacillin, vigabatrin, and netilmicin might inhibit the oxidation of 5-oxoproline.[7] Almost all case reports in the literature about transient 5-oxoproline concern women,[9] probably due to the difference in activity of certain enzymes between men and women. Besides the known inherited causes of high 5-oxoproline (glutathione synthetase and 5-oxoprolinase deficiency), the cause of transient 5-oxoprolinaemia is multifactorial.[4] It is unknown whether the symptoms are fully explained by acidosis. Oxoproline might cause symptoms as well. Treatment consists of withdrawing the causes and bicarbonate infusion might be considered in a severe acidosis (e.g. pH <7.0). Acetylcysteine might restore glutathione levels by cysteine.[8,10] In an unexplained metabolic acidosis it is worth calculating the anion gap. In a patient with the above-mentioned risk factors and an unexplained high anion gap metabolic acidosis, the possibility of 5-oxoproline as a cause should always be considered. It is easy to treat and might prevent unnecessary diagnostic tests and mortality. If clinicians do not consider the possibility of 5-oxoproline as a cause of a high anion gap metabolic acidosis, the real incidence of this condition will never be known.

**Figure 1.** *Gamma-glutamyl cycle*




## CONCLUSION

Our advice to clinicians is to consider the possibility of 5-oxoproline induced metabolic acidosis in patients with an unexplained high anion gap and the above-mentioned conditions, such as renal insufficiency, malnutrition in combination with the use of the above-mentioned drugs.

## REFERENCES

1. Moe OW, Fuster D. Clinical acid-base pathophysiology: disorders of plasma anion gap. Best Practice & Research: Clin Endocrinology and Metabolism 2003;17:559-74.
2. Creer MH, Lau BW, Jones JD, Chan KM. Pyroglutamic acidemia in an adult patient. Clin Chem 1989;35:684-6.
3. Croal BL, Glen ACA, Kelly CJG, Logan RW. Transient 5-oxoprolinuria (pyroglutamic aciduria) with systemic acidosis in an adult receiving antibiotic therapy. Clin Chem 1998;44:336-40.
4. Tailor P, Raman T, Garganta CL, et al. Recurrent High Anion Gap Metabolic Acidosis Secondary to 5-Oxoproline (Pyroglutamic Acid). Am J Kid Dis 2005;46:E4-10.

5. Mizock BA, Belyaev S, Mecher C. Unexplained metabolic acidosis in critically ill patients: the role of pyroglutamic acid. Intensive Care Med 2004;30:502-5.

6. Takayesu KJ, Bazari H, Linshaw M. Case 7-2006 - A 47-Year-Old Man with Altered Mental Status and Acute Renal Failure. N Engl J Med 2006;354:1065-72.

7. Fenves AZ, Kirkpatrick HM, Patel VV, Sweetman L, Emkmett M. Increased anion gap metabolic acidosis as a result of 5-oxoproline (pyroglutamic acid): a role for acetaminophen. Clin J Am Soc Nephrol 2006;1:441-7.

8. Humphreys BD, Forman JP, Zandi-Nejad K, Bazari H, Seifter J, Magee CC. Acetaminophen-Induced Anion Gap Metabolic Acidosis and 5-oxoprolinuria (Pyroglutamic Aciduria) Acquired in Hospital. Am J Kid Dis 2005;46:143-6.

9. Pitt JJ, Hauser S. Transient 5-oxoprolinuria and high anion gap metabolic acidosis: clinical and biochemical findings in eleven subjects. Clin Chem 1998;44:1497-503.

10. Dempsey GA, Lyall HJ, Corke CF, Scheinkestel CD. Pyroglutamic academia: A cause of high anion gap metabolic acidosis. Crit Care Med 2000;28:1803-7.

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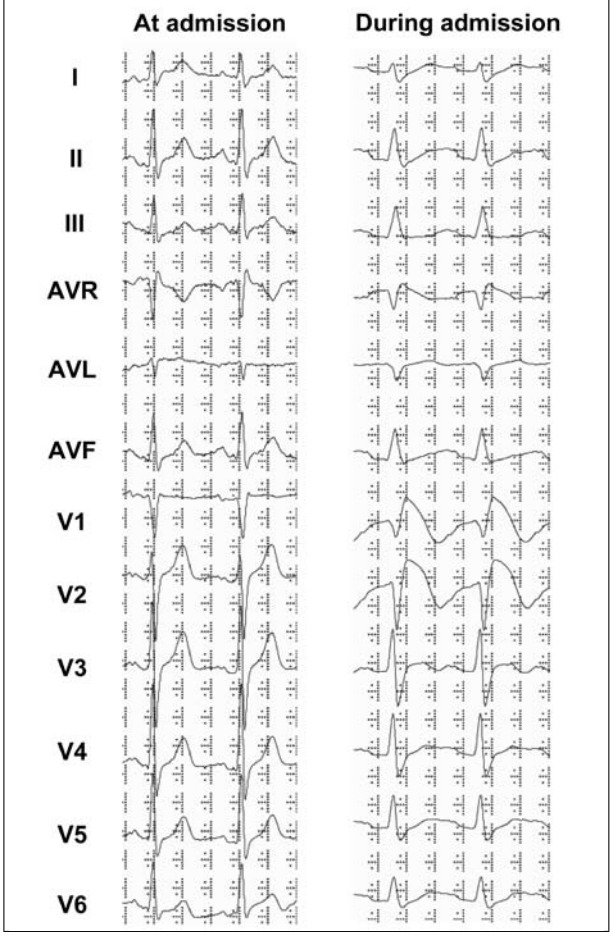
# Brugada syndrome induced by amitriptyline toxicity

Dear Editor,

A 52-year-old woman was found comatose with an empty box labelled olanzapine by her side. She was taking olanzapine for psychotic depression, but had no cardiac history or family history of sudden death. Her other vital signs, physical examination, and routine laboratory tests were normal. The ECG showed sinus tachycardia and intraventricular conduction delay with right bundle branch block-like configuration (QRS width 128 ms, *figure 1*; left panel). Such ECG abnormalities had not been previously documented. She was admitted for observation for suspected olanzapine overdose. Several hours later, she sustained pulseless ventricular tachycardia. After resuscitation, the ECG showed that QRS complexes had widened further and merged with typical ST elevations in $V_1$ and $V_2$ (*figure 1*, right panel). Such ST elevations in right precordial leads (>2 mm J-point elevation smoothly descending into a negative T wave),[1] with ventricular tachyarrhythmias unrelated to myocardial infarction or structural heart disease, were consistent with Brugada syndrome. It was found that amitriptyline had been prescribed previously. Serum analysis revealed amitriptyline overdose (serum level 2.3 µM, therapeutic level 0.18 to 0.72 µM, toxic level >1.8 µM). She recovered without arrhythmia recurrence, but declined cardiological analysis or follow-up.

**Figure 1.** *ECG of abnormalities*




Brugada syndrome is an autosomal-dominant disease associated with sudden death following ventricular tachyarrhythmias. Mutations in *SCN5A*, the gene which encodes the cardiac sodium channel that initiates cardiac excitation, are found in ~30%. Other disease-causing genes await discovery. Brugada syndrome may revolve around impaired depolarisation (excitation), abnormal repolarisation, and/or additional derangements.[3] Mutant sodium channels conduct reduced current,[3] explaining conduction slowing. Cardiac sodium channel blockers, e.g., class I antiarrhythmic drugs, evoke lethal arrhythmias through excessive conduction slowing, and are used diagnostically to unmask silent disease carriers.[4] Various drugs prescribed for noncardiac disease also block cardiac sodium channels, e.g., cyclic antidepressants (amitriptyline), lithium, and some anticonvulsants. Accordingly, these drugs may provoke life-threatening arrhythmias in Brugada syndrome patients, or unmask silent carriers. Some patients may have variants in depolarisation-controlling genes that render them vulnerable to proarrhythmia induced by cardiac sodium channel blockers. Brugada syndrome ECGs were reported in 15 of 98 patients with tricyclic antidepressant intoxication.[5] These ECGs normalised when serum levels dropped to <1 µM. On admission, our patient did not have a Brugada syndrome ECG. Possibly, plasma amitriptyline levels were still rising from continued resorption from the gastrointestinal tract. Clearly, repeated ECG recording and rhythm monitoring are required if intoxication with cardiac sodium channel

blockers is suspected. Sodium bicarbonate is the drug of choice for ventricular dysrhythmias following tricyclic antidepressant poisoning.[6] How it unblocks cardiac sodium channels is unresolved.

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## REFERENCES

1. Wilde AAM, Antzelevitch C, Borggreve M, et al. Proposed diagnostic criteria for the Brugada syndrome. Consensus report. Circulation 2002;106:2514-9.
2. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. J Am Coll Cardiol 1992;20:1391-6.
3. Meregalli PG, Wilde AAM, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder or more? Cardiovasc Res 2005;67:367-78.
4. Meregalli PG, Ruijter JM, Hofman N, et al. Diagnostic value of flecainide testing in unmasking SCN5A-related Brugada syndrome. J Cardiovasc Electrophysiol 2006;17:857-64.
5. Goldgran-Toledano D, Sideris G, Kevorkian JP. Overdose of cyclic antidepressants and the Brugada syndrome N Engl J Med 2002;346:1591-2.
6. Albertson TE, Dawson A, de Latorre F, et al. Tox-ACLS: toxicologic-oriented advanced cardiac life support. Ann Emerg Med 2001;37(4 suppl):S78-90.

## MONTHLY NJM ONLINE HITLIST

The table lists online hits for all articles pulbished in the May issue of the Netherlands Journal of Medicine 2008 (available online on PubMed since 19 May 2008).

| Article | Hits |
|---|---|
| EDITORIAL | |
| Pyogenic liver abscess – predicting failure to improve outcome | 118 |
| REVIEW | |
| Reality of severe metformin-induced lactic acidosis in the absence of chronic renal impairment | 183 |
| ORIGINAL ARTICLES | |
| Chronic hepatitis B and C dual hepatitis has a poor response to pegylated interferon and ribavirin therapy | 95 |
| Predictors of mortality in patients with pyogenic liver abscess | 125 |
| CASE REPORTS | |
| Colchicine-induced neuromyopathy in a patient with chronic renal failure: the role of clarithromycin | 163 |
| Community-onset *Clostridium difficile*-associated diarrhoea not associated with antibiotic usage – Two case reports with review of the changing epidemiology of *Clostridium difficile*-associated diarrhoea | 143 |
| PHOTO QUIZZES | |
| Undulating blood pressure | 82 |
| An unexpected finding in bacterial pneumonia | 87 |
| LETTERS TO THE EDITOR | |
| Anaemia and haemolysis in pregnancy due to rapid folic acid and vitamin B12 depletion | 161 |
| Bacteraemia following rubber band ligation for non-bleeding oesophageal varices in a patient with alcoholic liver cirrhosis | 119 |
| Current problems in the diagnosis of Henoch-Schönlein purpua according to the ACR or EULAR/PReS criteria | 88 |
| SPECIAL REPORTS | |
| Aldosterone-to-renin ratio as a screening test for primary aldosteronism – The Dutch ARRAT Study | 112 |
| The *Netherlands Journal of Medicine*: the Utrecht years | 59 |
| **Total** | **1535** |

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PHOTO QUIZ

# A patient with pain in the throat and chest

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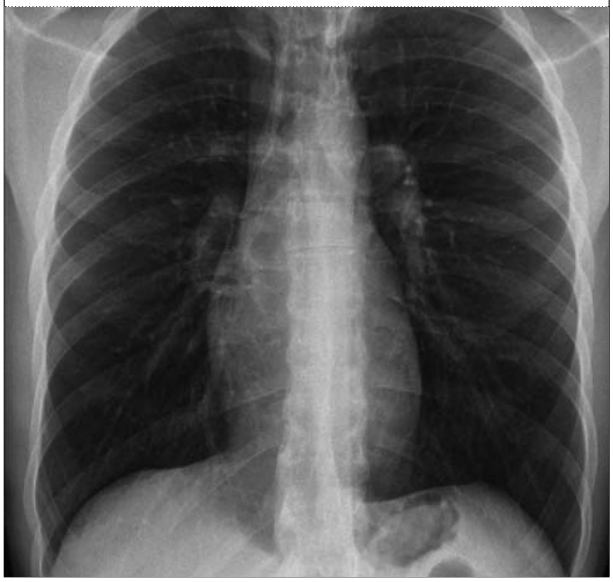
## CASE REPORT

An 18-year-old male with a history of asthma presented to the emergency room with pain in his throat and chest lasting two days, which worsened with inspiration. He had no risk factors for venous thromboembolism and he denied trauma or any activity that may result in a Valsalva manoeuvre, such as coughing, sneezing or vomiting. On admission we saw a tall thin young man. Physical examination revealed no abnormalities. Percutaneous oxygen saturation was 100% while breathing room air. Laboratory investigations, inclusive D-dimer, were normal.
A chest radiograph was obtained (*figure 1*) and we were directly called by a concerned radiologist.

**Figure 1.** *Chest radiograph on admission*



## WHAT IS YOUR DIAGNOSIS?

See page 361 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 360)

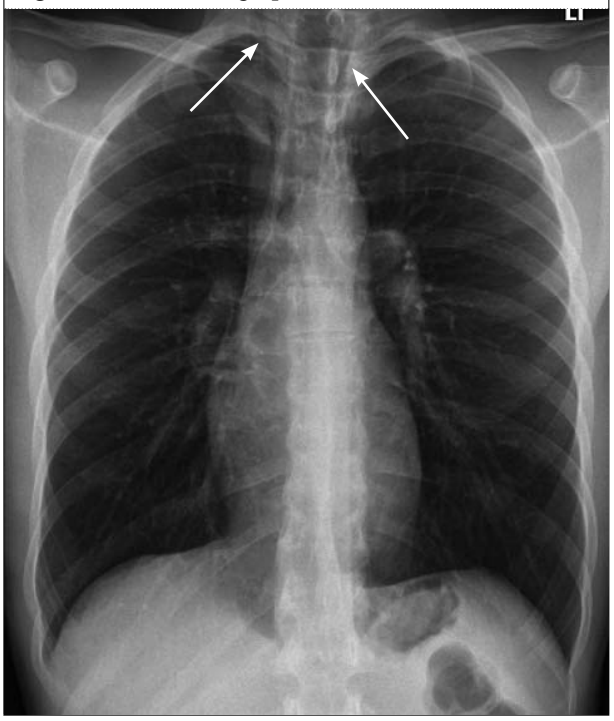
# A PATIENT WITH PAIN IN THE THROAT AND CHEST

## DIAGNOSIS

The chest radiograph (*figure 1*) demonstrated a pneumomediastinum without subcutaneous emphysema. The patient was admitted because of the spontaneous pneumomediastinum and observed for four days. He recovered rapidly, his symptoms disappeared without any intervention other than analgesics, bed rest and reassurance. Within one week he was seen as an outpatient and his chest X-ray appeared to be normal.

The first report of a spontaneous pneumomediastinum was by Hamman in 1939.[1,2] Hamman's sign (crunching sound or crepitation synchronous with the heart beat on chest auscultation) is the pathognomonic sign of a spontaneous pneumomediastinum.[1,2] Physical examination can also reveal subcutaneous emphysema, which was not present in our patient.

**Figure 1.** *Chest radiograph on admission*



The pathophysiology of this disorder was described by Macklin and Macklin.[1,2] An alveolar rupture caused by overdistension or increased alveolar pressure is the most relevant underlying factor. Alveolar rupture allows bubbles of gas to dissect along the pulmonary vasculature towards the hilum and subsequently the mediastinum.[1,2]

Spontaneous pneumomediastinum is a rare (1 in 30,000 emergency department referrals), self-limiting and benign condition that usually occurs in young men who present without an apparent precipitating event.[1,2] An asthma history has been reported in up to half of the cases.[1] Therefore chest pain in an asthma patient can be caused by a spontaneous pneumomediastinum. This condition may be underdiagnosed.[3] It is important to exclude other serious conditions such as a Boerhaave syndrome in persons with chest pain after vomiting or bronchial rupture by endoscopy or contrast investigations (oesophagogram).[1,3]
If a healthy young person complains about chest pain, spontaneous pneumomediastinum should be considered as the cause of the symptoms (differential diagnosis).[2]

## CONCLUSION

Spontaneous pneumomediastinum.

## REFERENCES

1. Newcomb AE, Clarke CP. Spontaneous pneumomediastinum - A benign curiosity or a significant problem? Chest 2005;128:3298-302.
2. Gerazounis M, Athanassiadi K, Kalantzi N, Moustardas M. Spontaneous pneumomediastinum: A rare benign entity. J Thorac Cardiovasc Surg 2003;126:774-6.
3. Momin AU, Chung DA, John LCH. Childhood asthma predisposes to spontaneous pneumomediastinum. Emerg Med J 2004;21:630-1.

# 50 years *Netherlands Journal of Medicine*

Over the years, the terminology for the various types of publications has differed.

Terms as invited review (or article), current practice, special section (or report), brief report, (lustrum) lectures, proceedings and symposium presentations were reclassified into the categories as indicated in *table 1*. All meeting abstracts were omitted, as were reports from a number of societies.

**Table 1.** *Number of publications*

| Type of publication | Rough estimate of the number over the 50 years |
|---|---|
| Editorials | 350 |
| Reviews | 770 |
| Original articles | 990 |
| Case reports | 780 |
| Letters to the editor | 90 |

**Figure 1.** *Number and category of publications in the indicated years*

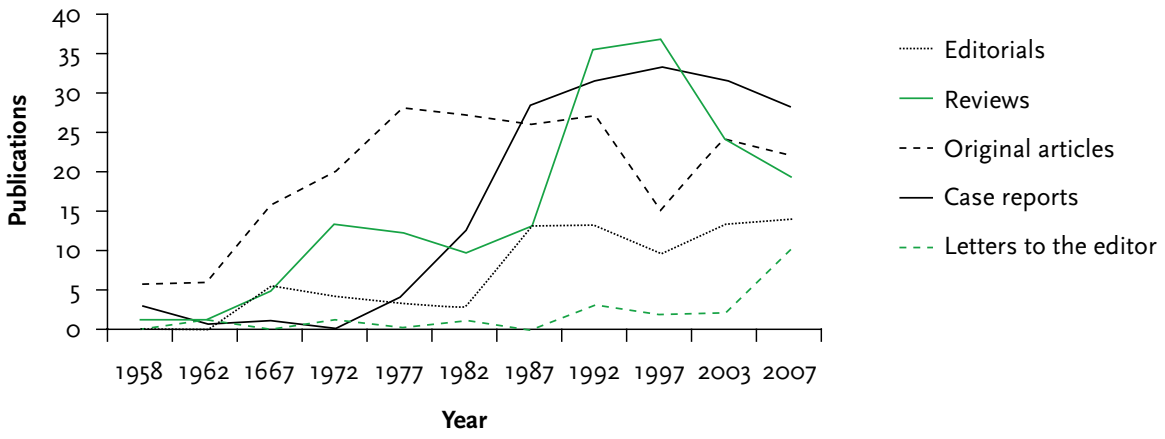



**Figure 2.** *Number of subscriptions to the Netherlands Journal of Medicine*

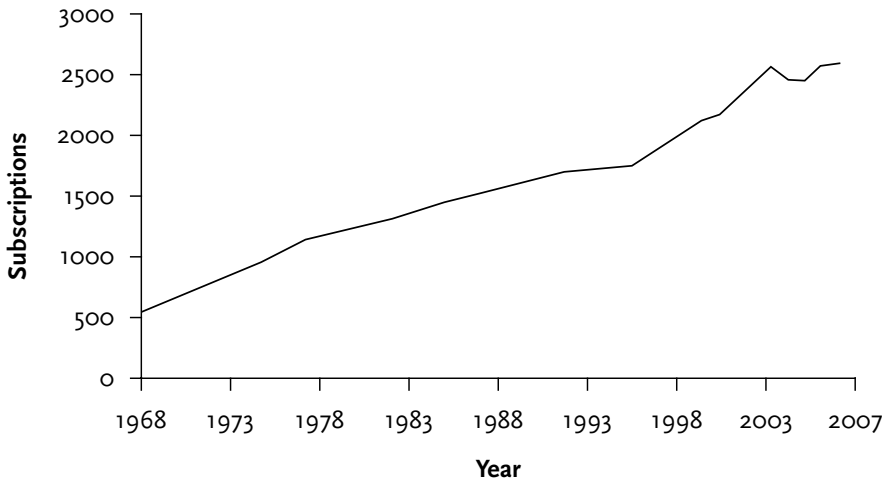


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PHOTO QUIZ

# Reactivation of dormant microorganisms following a trauma

**P. Sendi[1]*, A. Friedl[2], P. Graber[1], W. Zimmerli[1]**

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## CASE REPORT

A 19-year-old man was referred to our centre in July 2005 with a fistula in his left heel. Five months prior to admission he had been involved in a scooter accident and received a chest contusion and abrasions to his left inner heel. Within one week, the skin lesion on the foot developed into a fluid-filled blister, which the patient punctured with a sewing needle. Thereafter, he suffered from persistent secretion of serosanguineous fluid and pus from the puncture area (*figure 1*), but standard culture of the fistula pus had not shown bacterial growth. The medical history he gave was unremarkable. He had been born in the Philippines and immigrated to Switzerland at the age of four years. Physical examination revealed a sternal protrusion, about the size of half a tennis ball (*figure 2*) that had developed gradually since the accident.

## WHAT IS YOUR DIAGNOSIS?

See page 364 for the answer to this photo quiz.

**Figure 1.** *Secretion of serosanguineous fluid and pus from an area which included a fluid-filled blister that was punctured with a sewing needle*

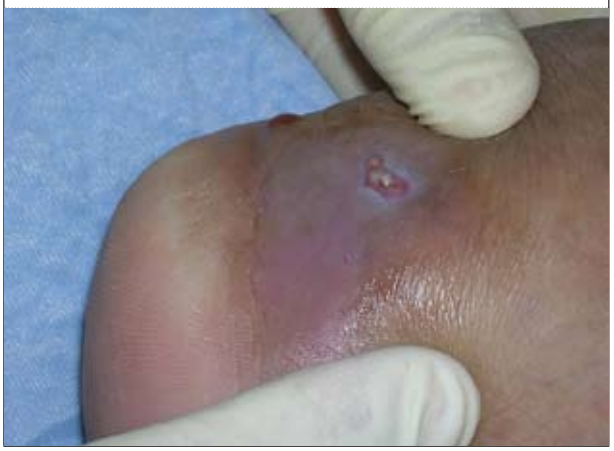
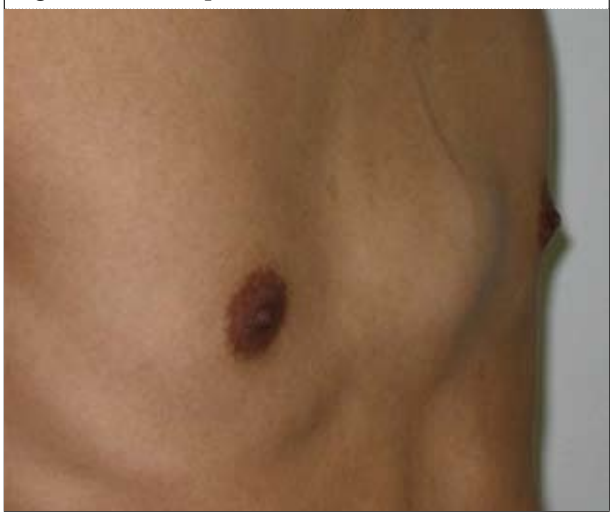


**Figure 2.** *Sternal protrusion*



ANSWER TO PHOTO QUIZ (ON PAGE 363)

REACTIVATION OF DORMANT MICROORGANISMS FOLLOWING A TRAUMA

## DIAGNOSIS

The diagnosis is pneumonia, sternal abscess and calcaneus osteomyelitis due to *Mycobacterium tuberculosis*.

Chest radiography showed a calcified scar in the apical-posterior segment of the right upper lobe. A computerised tomography scan of the thorax demonstrated infiltrates in the left and right upper lobe typical for reactivation tuberculosis and a sternal abscess with an area of osteolysis. Magnetic resonance imaging findings of the left heel were consistent with calcaneus osteomyelitis. Upon further questioning, the patient admitted to a discreet chronic cough, night sweats (which he attributed to the summer heat) and a weight loss of 6 kg over the past five months. He denied travel during the last three years and was not aware of any tuberculosis contacts. Culture from bronchoalveolar lavage fluid, sternal puncture aspirate and of biopsies from the heel fistula showed growth of *Mycobacterium tuberculosis* with the same resistance patterns. HIV serology was negative. A one-year course of antituberculosis therapy was initiated. Surgical treatment comprised resection of the fistula and the infected part of the calcaneus; the soft-tissue defect was covered with a sural flap. One year after completion of therapy, sternal protrusion was completely absent. Currently the patient is in good health.

In North American and Western European countries, immigrants and foreign-born residents are increasingly contributing to the incidence of tuberculosis, and their prevalence in the host country often mirrors that in the country of origin. Tuberculosis control in the Philippines remains unsatisfactory. There are about a quarter of a million new cases and 39,000 deaths due to tuberculosis annually, in a country of approximately 83 million people.[1] The rate of latent tuberculosis infection in children living in households of patients with pulmonary tuberculosis is almost 70%.[2] Contrary to North American countries, Filipinos represent a minority of the immigrants in certain Western European countries, such as France, Austria, the Netherlands or Switzerland. Thus, their prevalence of latent and active tuberculosis might be underappreciated in clinical practice. It is very likely that this patient had latent tuberculosis when he moved to Switzerland 15 years ago. Since our patient was not suffering from HIV infection or malnutrition and was not using any immunosuppressive medication, reactivation of latent tuberculosis was likely triggered by the chest contusion during the scooter accident. Trauma as a trigger of reactivation has been reported previously,[3] although this relationship is difficult to demonstrate conclusively. The interval between immigration and onset of symptoms should also be noted. In a Canadian study, the mean interval between immigration and diagnosis was 11 years, and extrapulmonary infection accounted for 60% of the tuberculosis cases in Asian immigrants.[4] The detailed pathogenesis of the calcaneus osteomyelitis remains unclear, in part due to the long history prior to presentation, but local reactivation, haematogenous seeding or autoinoculation are possible. Filipino immigrants are highly likely to have a latent tuberculosis infection and remain at a lifetime risk for reactivation, not rarely at an extrapulmonary site. Thus, microbiological investigations in this population should include active search for *Mycobacterium tuberculosis*.

## REFERENCES

1. Dye C, Scheele S, Dolin P, Panthania V, Raviglione MC. Global burden of tuberculosis. Estimated incidence, prevalence, and mortality by country. JAMA 1999;282:6778-6.
2. WHO. Country profiles on tuberculosis, Philippines. In: WHO report 2007 global tuberculosis. Geneva: WHO, 2007:129.
3. DuBrow EL, Landis FB. Reactivation of pulmonary tuberculosis due to trauma. Chest 1975;68:596-8.
4. Salazar-Vergara RM, Sia IG, Tupasi TE, et al. Tuberculosis infection and disease in children living in households of Filipino patients with tuberculosis: a preliminary report. Int J Tuberc Lung Dis 2003;7:S494-500.

**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 g.derksen@aig.umcn.nl, tel.: +31 (0)24-361 04 59 or fax: +31 (0) 24-354 17 34.

**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)24-354 17 34).

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.

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.

**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.

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The editorial board will consider articles reviewing books.

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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.

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