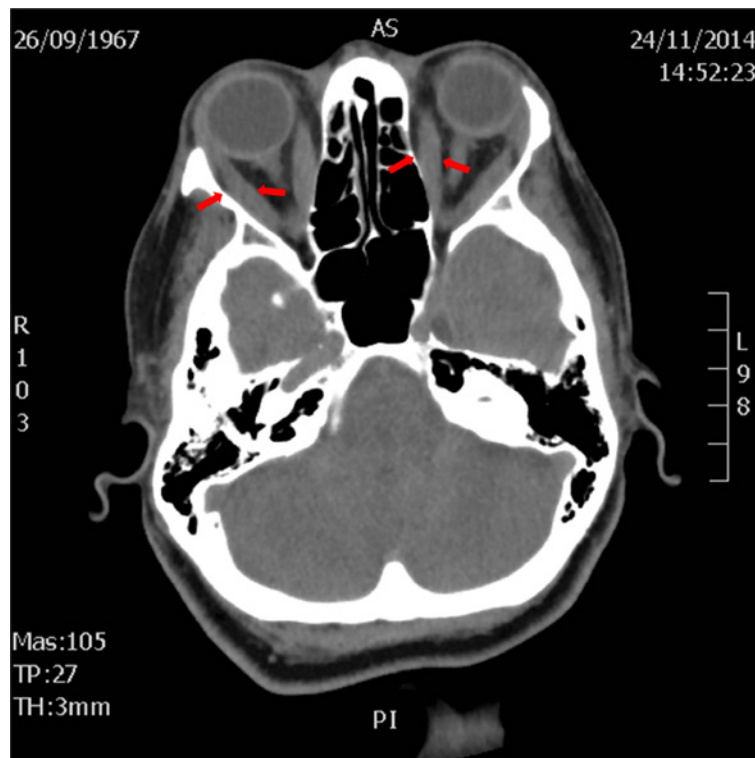


# The Netherlands Journal of Medicine

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*A 47-year-old woman with fever and periorbital oedema; what is your diagnosis?*

RECOMMENDATIONS FOR CALCIUM CHANNEL ANTAGONIST POISONING

DUTCH GUIDELINES CHRONIC LYMPHOCYTIC LEUKAEMIA

CHLAMYDIA PSITTACI PNEUMONIA

DAPSONE HYPERSENSITIVITY SYNDROME

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# Contents

## EDITORIAL

- On calcium channel antagonist poisoning: towards evidence-based decision making in poisoned patients 58

R.A.M. Quax, J. Alisma

## REVIEWS

- Practical recommendations for calcium channel antagonist poisoning 60

S.J. Rietjens, D.W. de Lange, D.W. Donker, J. Meulenbelt

- Dutch guidelines for the diagnosis and treatment of chronic lymphocytic leukaemia 68

Dutch/Belgium HOVON CLL working group: S. Kersting, M-D. Levin, M. Chamuleau, S.M.G.J. Daenen, E.C. Dompeling, J.K. Doorduijn, M. van Gelder, M. Hoogendoorn, J.M. Kerst, M. Nijland, M.R. Nijziel, E.F.M. Posthuma, R.A.P. Raymakers, M.R. Schaafsma, M.H. Silbermann, H.M. van der Straaten, J.H. Veelken, J.M.I. Vos, S. Wittebol, M.H.J. van Oers, A.P. Kater

## ORIGINAL ARTICLES

- Chlamydia psittaci*: a relevant cause of community-acquired pneumonia in two Dutch hospitals 75

S.M.C. Spooenberg, W.J.W. Bos, E.J. van Hannen, F. Dijkstra, E.R. Heddema, H. van Velzen-Blad, R. Heijligenberg, J.C. Grutters, B.M. de Jongh, Ovidius study group

## CASE REPORTS

- Familial focal segmental glomerulosclerosis: mutation in inverted formin 2 mimicking Alport syndrome 82

I.M. Rood, E.M.H.F. Bongers, D. Lugtenberg, I.H.H.T. Klein, E.J. Steenberg, J.F.M. Wetzels, J.K.J. Deegens

- A rare stress cardiomyopathy in a patient with Guillain-Barré syndrome 86

M. Boon, P.J.W. Dennesen, R.F. Veldkamp

- A lethal case of the dapsone hypersensitivity syndrome involving the myocardium 89

R.M. Hoogveen, T. van der Bom, H.H. de Boer, R.M. Thurlings, B.S. Wind, H.J.C. de Vries, A.U. van Lent, U. Beuers, A.C. van der Wal, F.J. Nellen

## PHOTO QUIZZES

- An 88-year-old woman with an ulcerous tumour on the leg 93

N. Atiq, R.E. Kibelaar, F. de Vries, M. Hoogendoorn

- A 47-year-old woman with fever and periorbital oedema 96

P.E.A. Messiaen, K. Peeters, M.J.A. van Esbroeck, E.J.L. Bijmens, K.K. Wouters, I.C. Gyssens, J.C.H. van der Hilst

## LETTER TO THE EDITOR

- Training in acute medicine in a peripheral teaching hospital in the Netherlands 98

G.H. Kluge, J.W. Mulder, D.P.M. Brandjes

# On calcium channel antagonist poisoning: towards evidence-based decision making in poisoned patients

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Almost 500 years ago, Paracelsus already stated: ‘*Sola dosis facit venenum*’. This famous adage underlines a basic principle in the world of toxicology: every substance can be poisonous, as long as the dose is high enough. This fundamental rule still applies in modern times, reflected by the substantial burden of both accidental and intentional poisoning in the US.<sup>1</sup> Also in the Netherlands, an astonishing documented number of 33,700 patients were exposed to toxic substances in 2014, mostly pharmaceutical drugs (56%), followed by household cleaning substances (16%) and food, drinks and stimulants (7%).<sup>2</sup>

In contrast to the current era of evidence-based medicine, many antidotes or treatment strategies in intoxicated patients are solely based on anecdotal reports, case series, animal studies and expert opinions. This is reflected by the absence of clear guidelines and further underlined by the findings of Duineveld and colleagues,<sup>3</sup> who demonstrated considerable variation in the care of intoxicated patients in Dutch hospitals. In a subsequent prospective study, two algorithms to predict the need for treatment in intoxicated patients were implemented in clinical practice, with promising results in terms of good sensitivity and better specificity than routine clinical care.<sup>4</sup> These findings underscore the need for evidence-based practical guidelines, in order to improve clinical care and maximise efficacy.

In the current issue of the Journal, Rietjens and co-workers provide an in-depth overview of the treatment options in calcium channel antagonist (CCA) poisoning, and merge these recommendations into a practical algorithm.<sup>5</sup> Along with ‘classical’ strategies for detoxification and supportive care (e.g. activated charcoal, calcium, atropine, vasopressors), the relatively new approach to the poisoned patient using hyperinsulinaemia/euglycaemia and intravenous lipid emulsion therapy is highlighted and incorporated in their algorithm.

After the first case report on hyperinsulinaemia/euglycaemia therapy as adjunctive treatment in CCA overdose in humans in 1999, accumulating case reports and case series with beneficial (haemodynamic) effects have been gathered,<sup>6</sup> and similar favourable results have been reported in beta-blocker intoxicated patients treated with hyperinsulinaemia/euglycaemia.<sup>6,7</sup> Based on animal models and increasing clinical experience, the modulatory effects of hyperinsulinaemia/euglycaemia in CCA and beta-blocker poisoning are generally ascribed to their positive inotropic properties, possibly (partly) mediated by enhanced intracellular glucose transport in cardiomyocytes and improved perfusion of the coronary (micro)vasculature.<sup>7</sup> Interestingly, a dose-response effect was observed in a pig model of beta-blocker poisoning, where improved outcome in the pigs treated with high-dose insulin (10 IU/kg/h) was accompanied by a higher cardiac output, as compared with the pigs treated with placebo and 5 IU/kg/h insulin.<sup>8</sup> This shows that not only the dose makes the poison, but in this case the dose also makes the antidote.

The first reports on altered drug concentrations following emulsified fat infusion date back to the 1960s and 1970s,<sup>9</sup> where it was used for reversal of local anaesthetic systemic toxicity. Nevertheless, the full therapeutic potential of intravenous lipid emulsion in ameliorating the toxicity profile of drugs was only recognised by Weinberg and his colleagues in the late 1990s, using a rat model.<sup>10</sup> Ever since, beneficial effects of intravenous lipid emulsion have been described in a wide variety of lipophilic drugs,<sup>9</sup> including CCA and beta-blockers, adding intravenous lipid emulsion to the repertoire of treatment options in the severely intoxicated patient. It must be noted that initiation of intravenous lipid emulsion must be carefully considered for each individual poisoning, as effectiveness is likely to primarily depend on the degree of lipid solubility of the

ingested substance, as exemplified by the opposing effects of intravenous lipid emulsion in metoprolol (relatively hydrophilic) and propranolol (lipophilic) poisoning in animal studies.<sup>11,12</sup>

Both hyperinsulinaemia/euglycaemia and intravenous lipid emulsion are now increasingly being recommended by poison control centres. Nevertheless, clinicians do not always follow these recommendations, possibly due to the fact that they are unfamiliar with these treatment regimens.<sup>13</sup> We hope that the explanation of the underlying pharmacological principles and the detailed dosing regimens provided by Rietjens and co-workers will take away the last scepticism and further stimulate the approachable and adequate use of hyperinsulinaemia/euglycaemia and intravenous lipid emulsion in CCA intoxication. As can be extracted from the flowchart, we would like to point out that hyperinsulinaemia/euglycaemia and intravenous lipid emulsion can, and sometimes must, be combined to optimise clinical outcome in severe CCA poisoning. In cases of refractory shock, extracorporeal life support can be considered a bridge to recovery.

Although the abovementioned interventions can be life-saving, especially in patients in whom the ingested drug is known, it must be emphasised that a standardised evaluation following the well-known ABCDE paradigm remains the cornerstone in intoxicated patients. Clinical clues, usually clustered in toxidromes (e.g. confusion, mydriasis, urinary retention/dry mouth, hyperthermia and dry skin in anticholinerg syndrome), might give direction in the possibly intoxicated patient.

In line with the recommendations for the treatment of CCA poisoning by Rietjens and colleagues, we strongly advocate further development of evidence-based national guidelines for other frequently (intentionally) ingested toxic substances. Given the relatively low incidence of (severe) intoxications, we believe that national collaboration is of utmost importance.

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# Practical recommendations for calcium channel antagonist poisoning

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## ABSTRACT

Calcium channel antagonists (CCAs) are widely used for different cardiovascular disorders. At therapeutic doses, CCAs have a favourable side effect profile. However, in overdose, CCAs can cause serious complications, such as severe hypotension and bradycardia. Patients in whom a moderate to severe intoxication is anticipated should be observed in a monitored setting for at least 12 hours if an immediate-release formulation is ingested, and at least 24 hours when a sustained-release formulation (or amlodipine) is involved, even if the patient is asymptomatic. Initial treatment is aimed at gastrointestinal decontamination and general supportive care, i.e., fluid resuscitation and correction of metabolic acidosis and electrolyte disturbances. In moderate to severe CCA poisoning, a combined medical strategy might be indispensable, such as administration of vasopressors, intravenous calcium and hyperinsulinaemia/euglycaemia therapy. Especially hyperinsulinaemia/euglycaemia therapy is an important first-line treatment in CCA-overdosed patients in whom a large ingestion is suspected. High-dose insulin, in combination with glucose, seems to be most effective when used early in the intoxication phase, even when the patient shows hardly any haemodynamic instability. Intravenous lipid emulsion therapy should only be considered in patients with life-threatening cardiovascular toxicity, such as refractory shock, which is unresponsive to conventional therapies. When supportive and specific pharmacological measures fail to adequately reverse refractory conditions in CCA overdose, the use of extracorporeal life support should be considered. The efficacy of these pharmacological and non-pharmacological interventions generally advocated in CCA poisoning needs further in-depth mechanistic foundation, in order to improve individualised treatment of CCA-overdosed patients.

## KEYWORDS

Calcium channel antagonist, calcium channel blocker, hyperinsulinaemia/euglycaemia therapy, intoxication, overdose, poisoning

## INTRODUCTION

Calcium channel antagonists (CCAs) are widely prescribed for a variety of indications, e.g. hypertension, coronary artery disease and specific cardiac arrhythmias.<sup>1,2</sup> In general, CCAs have a favourable adverse effect profile. However, CCA overdose is common and associated with a relatively high degree of morbidity and mortality.<sup>1,3</sup> In the Netherlands, 158 CCA exposures (~50% intentional) were reported to the Dutch Poisons Information Centre in 2014. Adults (18-65 years) were mostly involved (48%), followed by the elderly (> 65 years, 27%) and young children (0-4 years, 20%). Within the group of cardiovascular drugs, CCAs are the leading class of drugs associated with poisoning fatality.<sup>3</sup> In this review, we will discuss the most relevant treatment options for CCA-overdosed patients.

## MECHANISM OF ACTION

Three main classes of CCAs are currently on the market: dihydropyridines (e.g. nifedipine, amlodipine), phenylalkylamines (verapamil) and benzothiazepines (diltiazem).<sup>1,2,4,5</sup> At therapeutic doses, the dihydropyridines predominantly affect vascular smooth muscle cells and show little effect on the myocardium, making this class of CCAs particularly attractive in treating hypertension. In contrast, the non-dihydropyridine CCAs (verapamil, diltiazem) have the strongest affinity to the myocardium<sup>2,4,6</sup> and are used to treat angina or cardiac arrhythmias.

The primary mechanism of action of CCAs is inhibiting calcium influx by antagonism of the L-type voltage-gated calcium channels in vascular smooth muscle cells and cardiomyocytes of the working myocardium as well as the conduction system.<sup>7</sup> Blockage of L-type calcium channels in the myocardium results in decreased myocardial contractility, decreased heart rate, decreased excitability and decreased conductivity within the heart. Antagonism of vascular L-type calcium channels results in relaxation of vascular smooth muscle cells, increasing coronary vascular dilatation and decreasing systemic blood pressure.<sup>2,6,7</sup>

## PHARMACOKINETICS

Most CCAs are rapidly absorbed from the gastrointestinal tract (peak concentration ~1-2 h). Some CCAs (e.g. amlodipine) and also sustained-release preparations show a much slower absorption (peak concentration within 6-12 h). Many CCAs have a low bioavailability due to extensive hepatic first-pass metabolism. In general, CCAs are highly protein bound and have a large volume of distribution.<sup>2,5,8,9</sup> In overdose, peak concentrations can be delayed up to ~24 hours for sustained-release preparations,<sup>8</sup> delaying the onset of intoxication symptoms. Furthermore, the apparent half-life of many CCAs can be increased after overdose, because of saturation of metabolism causing prolonged toxicity.<sup>2,5,8</sup>

## TOXICITY RESULTING FROM CCA OVERDOSE

### Cardiovascular toxicity

The observed cardiovascular toxicity after CCA overdose is largely an extension of the therapeutic effects. In general, overdose of diltiazem (benzothiazepine) and verapamil (phenylalkylamine) can cause serious hypotension and bradycardia, which can be aggravated by a diversity of sinoatrial, atrioventricular (AV) and bundle branch conduction disturbances, including complete AV-blockage.<sup>6,10,11</sup> In contrast, dihydropyridine CCAs tend to create more prominent hypotension accompanied by reflex tachycardia after overdose.<sup>6,10,11</sup> However, in severe poisoning, the selectivity for cardiac versus peripheral vascular effects can be profoundly decreased, making the cardiovascular effects less predictable. Initially, patients may be asymptomatic early after ingestion, but can subsequently deteriorate rapidly towards severe and refractory cardiogenic shock.<sup>5</sup>

### Non-cardiovascular toxicity

Interestingly, various non-cardiovascular effects have been described following CCA overdose, such as confusion,

agitation, impaired consciousness and seizures,<sup>5,8,11-13</sup> which might be caused by hypoperfusion of the central nervous system. Moreover, in overdose, CCAs may inhibit calcium channels outside the cardiovascular system. Non-selective blockage of L-type channels of pancreatic islet cells decreases insulin release resulting in hyperglycaemia.<sup>11,13-17</sup> A metabolic state similar to diabetic ketoacidosis may develop, in addition to lactic acidosis.<sup>2,15,18</sup> Lactic acidosis is a manifestation of poor tissue perfusion and can be aggravated by inhibition of mitochondrial calcium entry leading to decreased pyruvate dehydrogenase activity.<sup>18,19</sup> Other effects are bowel infarction and ileus.<sup>2,20-22</sup> Pulmonary oedema<sup>23-26</sup> (both cardiogenic and non-cardiogenic) might be caused by excessive fluid resuscitation and precapillary vasodilatation, resulting in increased transcapillary pressure.<sup>26,27</sup> Refractory shock and cardiac arrest may ultimately result in death.

Beta-blocker overdose usually causes similar symptoms, although subtle differences may be present. Dihydropyridine CCA overdose generally causes profound hypotension and reflex tachycardia, rather than bradycardia. Nevertheless, distinguishing dihydropyridine overdose from beta-blocker overdose remains difficult. Glucose concentration is not useful for discriminating between CCA and beta-blocker overdose, as both hypoglycaemia and hyperglycaemia are occasionally reported in beta-blocker overdose.<sup>15</sup>

## TREATMENT OF CCA OVERDOSE

As treatment of CCA overdose can be complicated, consultation with a clinical toxicologist, hospital pharmacist and a Poisons Information Centre is strongly recommended. Several factors influence the response to CCA poisoning: drug class, dose, formulation, time of ingestion, co-exposures (e.g. beta-blockers) and pre-existing diseases.<sup>8</sup> Elderly patients and patients with underlying cardiovascular disease are expected to be more vulnerable to the toxic effects of CCAs.<sup>6</sup>

A rational approach to the treatment of CCA overdose is presented in *figure 1*. Severe cases may require several simultaneous interventions. Recommended doses and possible adverse effects are presented in *table 1 and 2*, respectively.

In this review, we will provide diagnostic and treatment recommendations for CCA overdose, which are based upon 'low quality of evidence'. Unfortunately, the current clinical evidence is limited, because it is generally based upon animal studies, case reports and low-quality observational data, which are prone to confounding and biases (see also the review by St-Onge et al.<sup>28</sup>). Often multiple treatments are simultaneously applied in one patient, making it hard to identify the relative advantages of one intervention over another.

### Laboratory testing

Besides routine laboratory testing, an emphasis should be put on blood gas analyses and assessment of lactate, electrolytes, blood glucose and renal function.<sup>5,8</sup> CCA serum concentration analyses are not routinely performed and are not usually available on time to guide therapy. In addition, CCA concentrations do not correlate with clinical presentation,<sup>5,8,29</sup> although CCA detection above the therapeutic level can indicate an overdose. A general toxicological screening in blood or urine provides information on relevant co-exposures.

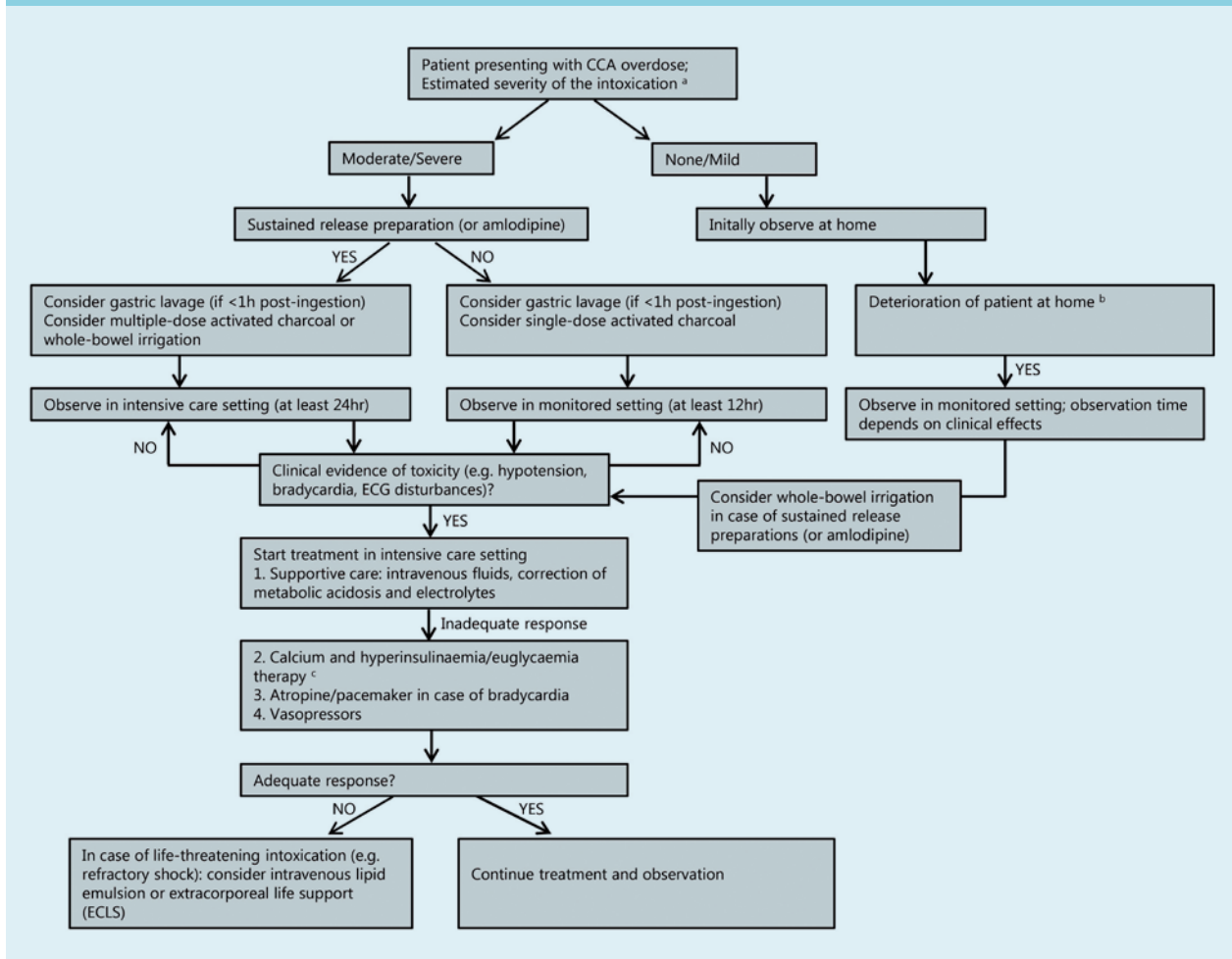
### Monitoring and supportive care

Close monitoring of vital signs (including electrocardiography (ECG)) in an intensive care setting is fundamental for patients in whom a moderate to severe

intoxication is anticipated (based on CCA dose and underlying comorbidities), as rapid changes in clinical condition may occur.<sup>1,8,29</sup> Patients should be observed for at least 12 hours if an immediate-release formulation is ingested and at least 24 hours when a sustained-release formulation (or amlodipine) is involved, even if asymptomatic.<sup>5</sup>

Treatment should first be focused on gastrointestinal decontamination and supportive care, e.g. correction of metabolic disturbances and electrolytes. Furthermore, assuring haemodynamic stability and adequate respiration is essential. Acidosis can worsen myocardial dysfunction and the responsiveness to catecholamines,<sup>30</sup> which might be due to increased drug binding at the calcium channel, and should be treated by administering sodium bicarbonate (target of maintaining blood pH of at least

Figure 1. Algorithm depicting treatment of CCA overdose



Dosing recommendations for the administration of antidotes are discussed in table 1. Please note that in the most critically ill patients, multiple therapies should be administered simultaneously. <sup>a</sup>A Poisons Information Centre or hospital pharmacist can assist in estimating the severity of the intoxication, which is primarily based on CCA dose (mg/kg). Also other variables should be taken into account, such as age and underlying diseases. <sup>b</sup>Patients in whom the intoxication is estimated as none or mild can initially be observed at home. These patients should be closely observed; in case of deterioration (see main text), the patient should be sent to hospital for further observation and treatment. <sup>c</sup>Hyperinsulinaemia/euglycaemia therapy should be initiated in the early stages of the intoxication, especially when a large ingestion of a CCA is suspected, even when the patient shows hardly any haemodynamic instability. CCA = calcium channel antagonist.



**Table 1.** Recommended dosing regimens of the specific treatments for CCA overdose

Treatment	Recommended dose	Remarks
<b>Sodium bicarbonate</b> <sup>5</sup>	Correction of metabolic acidosis: initial IV dose of sodium bicarbonate: 1 mmol/kg (=1 mEq/kg)	Target: maintain blood pH of at least 7.4
<b>Calcium</b> <sup>5,6,8</sup>	Initial IV bolus: adults: 10-20 ml of 10% calcium chloride or 30-60 ml of 10% calcium gluconate <sup>a</sup> ; children: 0.2 ml/kg of 10% calcium chloride or 0.6 ml/kg of 10% calcium gluconate. Repeated boluses: every 15-20 min up to 3 to 4 additional doses or a continuous infusion of 0.2 to 0.4 ml/kg/h of 10% calcium chloride or 0.6 to 1.2 ml/kg/h of 10% calcium gluconate	IV calcium should be administered slowly (e.g. 10 ml of 10% calcium chloride in ~10 min). Keep the plasma ionised calcium concentration in the upper range of normal reference values (or slightly above). Prevent precipitation of calcium (see main text). Avoid calcium in case of co-exposure to digoxin
<b>High-dose insulin / glucose</b> <sup>5,16,37,38</sup>	Initial IV insulin bolus: 1 unit/kg along with 0.5 gram/kg glucose. Glucose bolus can be omitted if serum glucose >300 mg/dl (i.e. >16.7 mmol/l). Infusion of insulin following the bolus: start at 0.5 units/kg/h, titrate up to 2 units/kg/h if no improvement is present after 30 min. Start a continuous glucose infusion, beginning at 0.5 g/kg/h. No improvement observed: increase the insulin infusion rate in a step-wise manner up to 10 units/kg/h	Regular monitoring of glucose levels: initially, at least every 30 min and titrate accordingly to maintain euglycaemia (5.0-8.0 mmol/l) Typical duration of insulin therapy is 1-2 days
<b>Vasopressors</b>	Norepinephrine appears most suitable; in case of hypotension: initial dose of norepinephrine: start with 50-100 nanogram/kg/min	See main text for further information on type of vasopressor
<b>Atropine</b> <sup>5,8</sup>	Start with 0.5-1.0 mg (0.02 mg/kg in children) IV every 2-3 min up to a maximum dose of 3 mg in patients with symptomatic bradycardia	Doses <0.5 mg (adult) or <0.1 mg (child) should be avoided due to potential paradoxical bradycardia
<b>Glucagon</b> <sup>5</sup>	Adult bolus dose: 3-5 mg IV, slowly over 1-2 min. No haemodynamic improvement within 5 min: retreatment with a dose of 4-10 mg. The initial paediatric dose is 50 µg/kg. Repeated doses may be useful. Actually, glucagon is not the most suitable treatment option for CCA poisoning (see main text)	Monitor glucose levels regularly. Maintenance infusion (2-5 mg/h in adults) is suggested once a desired effect is achieved, which can be tapered as the patient improves
<b>IV lipid emulsion (ILE)</b> <sup>40</sup>	Initial IV 1.5 ml/kg bolus (20% ILE solution), to be given over 1 min. Directly followed by a 0.25 ml/kg/min infusion. Evaluate the effect 5 min after the start of the bolus. Two more 1.5 ml/kg boluses (with 5 min between boluses) and/or doubling the infusion rate to 0.5 ml/kg/min can be considered if haemodynamic instability persists. After attaining circulatory stability, the ILE infusion can be continued for at least 10 min	The recommended maximal cumulative dose of ILE to be administered is 10 ml/kg (20% ILE solution)

<sup>a</sup>10 ml calcium chloride 10% contains 27.3 mg Ca<sup>2+</sup> (corresponds with 680 µmol/ml); 10 ml calcium gluconate 10% contains 8.9 mg Ca<sup>2+</sup> (corresponds with 225 µmol/ml); CCA = calcium channel antagonist; ILE = intravenous lipid emulsion; IV = intravenous; min = minutes.

7.4).<sup>15</sup> When extensive resuscitation of fluids is needed, norepinephrine should be added. Prevent fluid overload in order to avoid pulmonary oedema.

### Gastrointestinal decontamination

Early and aggressive gastrointestinal decontamination is of utmost importance in patients with a potentially toxic ingestion of a CCA. However, gastric lavage should only be considered in patients who present within one hour after ingestion of a large dose. Induced emesis is not recommended as patients could rapidly deteriorate,

increasing the risk of aspiration.<sup>1,2,5</sup> Activated charcoal can be considered in patients who have ingested a potentially toxic dose,<sup>1,2,5</sup> preferably within one to two hours after ingestion (adults: 50 g; children: 1 g/kg, max 50 g). Because of the risk of aspiration, gastric lavage and activated charcoal are contraindicated in patients with unprotected airways, e.g. in patients with depressed consciousness without endotracheal intubation.

Whole-bowel irrigation using polyethylene glycol solution, or multiple doses of activated charcoal should be considered in patients who have ingested a high

**Table 2.** Possible adverse effects of the specific treatments for CCA overdose

Treatment	Possible adverse effects
Calcium <sup>5,8,28</sup>	Hypercalcaemia Extravasation of intravenously administered calcium solution Flushing, nausea, vomiting, constipation and confusion
High dose insulin / glucose <sup>1,5,16,28,34</sup>	Hypoglycaemia Clinical signs of hypoglycaemia might be masked, due to the CCA overdose itself or e.g. co-ingestion of sedative drugs Possible hypoglycaemic effects of high-dose insulin therapy can last for a long period, even when the insulin therapy has already been stopped Hypokalaemia
Atropine <sup>5,8</sup>	Anticholinergic effects (decreased gastrointestinal motility might influence gastrointestinal decontamination) Doses <0.5 mg (adult) or <0.1 mg (child) might cause paradoxical bradycardia
Glucagon <sup>1,2,8,28</sup>	Hyperglycaemia (or paradoxical hypoglycaemia) Nausea, vomiting
IV lipid emulsion (ILE) <sup>41,50-52</sup>	Interference with the immune system, pulmonary function, hepatic function and elevation of triglyceride concentrations Large doses of ILE: lipaemia, hypertriglyceridemia, interference with a number of laboratory analyses, pancreatitis ILE could affect the efficacy of lipophilic drugs (when used concurrently)

CCA = calcium channel antagonist; ILE = intravenous lipid emulsion; IV: intravenous.

dose of a sustained-release preparation.<sup>1,5,31</sup> Whole-bowel irrigation should be used cautiously in debilitated patients. The potential advantage of reducing CCA absorption should be weighed against the possible disadvantages, e.g. pulmonary aspiration in patients with unprotected airways.

#### Enhanced elimination

Haemodialysis and haemoperfusion are unlikely to be effective, as CCAs generally have high protein binding and a high volume of distribution.<sup>1,2,8,9</sup> Extracorporeal albumin dialysis, using a molecular adsorbent recirculating system that efficiently removes protein-bound toxins from the circulation, may be considered when conventional symptomatic treatment fails.<sup>32,33</sup> However, this rescue therapy is not widely available and its efficacy remains controversial.

#### Calcium

The aim of administering calcium in CCA overdose is to increase extracellular calcium concentrations, theoretically allowing calcium influx via unblocked L-type calcium channels.<sup>5,6</sup> Animal studies generally show positive results, i.e. reduced mortality as well as haemodynamic improvement.<sup>28</sup> Case series and case reports in humans are inconsistent,<sup>28</sup> probably explained by differences in calcium dose or the severity and the stage of intoxication. Although the positive effects of calcium are often short-lived and more severely intoxicated patients may not improve significantly by administration of calcium alone,<sup>5</sup> we

recommend the use of calcium as a first-line treatment in CCA overdose, which should be started simultaneously with hyperinsulinaemia/euglycaemia therapy in severe cases.

Calcium should not be co-administered with a sodium bicarbonate infusion because a precipitate is formed.<sup>8</sup> Multiple other medications are known to form a precipitate with calcium. ECG monitoring should generally be performed as a safety measure when administering calcium in order to detect episodes of potentially life-threatening ventricular arrhythmias and conduction abnormalities. When co-intoxication with digoxin is suspected, calcium should be avoided, because this may worsen the digoxin toxicity.<sup>5,8</sup>

#### Hyperinsulinaemia/euglycaemia therapy

Hyperinsulinaemia/euglycaemia therapy has become a first-line intervention in CCA-intoxicated patients.<sup>1</sup> Failure of this therapy is predominantly reported when it was introduced too late. It, therefore, seems rational to initiate this therapy in the early stages of the intoxication, especially when a large ingestion of a CCA is suspected<sup>16,34,35</sup> even when the patient shows hardly any haemodynamic instability. The response to insulin is usually delayed for 15 to 60 minutes. This is an additional argument to start hyperinsulinaemia/euglycaemia therapy early, but alongside alternative treatments.

It is thought that high-dose insulin supports cardiac metabolism during shock states. When cardiomyocytes are

'stressed', their metabolism switches from free fatty acids to glucose. In this context, insulin promotes carbohydrate metabolism by increasing myocardial glucose uptake, enhancing inotropic function.<sup>15,34,36</sup> High-dose insulin also affects intracellular calcium handling, which contributes to the inotropic effects.<sup>34</sup> A large variation in applied insulin dosages has been reported in CCA-overdosed patients. High-dose insulin (IV bolus of 1 unit/kg followed by an infusion of 0.5-2.0 units/kg/h) generally demonstrated positive effects on haemodynamic parameters in humans and animals.<sup>28</sup> In the past, lower dosages of insulin were generally used due to concerns about provoking hypoglycaemia. Increased clinical experience and successful treatment of patients using relatively high quantities of insulin resulted in the current dosing strategy typically suggested in the medical literature (*table 1*). Yet, even more aggressive insulin protocols have been described, suggesting to increase the insulin infusion rate in a step-wise manner up to 10 units/kg/h if no clinical improvement is observed.<sup>37</sup> Obvious side effects of high-dose insulin therapy are hypoglycaemia and hypokalaemia (*table 2*).<sup>1,5,16,28,34</sup>

#### Vasopressors

Large doses, as well as the use of multiple vasopressor and inotropic agents, may be required in treating hypotension in severe CCA overdose.<sup>1,10,15</sup> No single adrenergic agent has been shown to be superior to another.<sup>15</sup> However, norepinephrine appears to be the most suitable initial catecholamine to use in hypotensive CCA-overdosed patients, as it has strong  $\alpha_1$ -adrenergic activity (to increase peripheral vascular resistance) and moderate  $\beta_1$ -adrenergic activity (to enhance myocardial function).  $\alpha_1$ -Adrenergic agonists (e.g. phenylephrine) seem rational choices if the hypotension is primarily caused by peripheral vasodilation, typically present in patients with dihydropyridine CCA overdose. Theoretically, the use of pure  $\beta$ -adrenergic receptor agonists, such as isoproterenol, and, to a lesser extent, dobutamine, is controversial as this may worsen hypotension mediated by  $\beta_2$ -adrenergic receptor agonist-induced peripheral vasodilation.<sup>38</sup>

#### Atropine

Atropine may be administered in the setting of bradycardia,<sup>5</sup> although it may not always be effective in increasing heart rate in severely poisoned patients and may even worsen bradycardia in case of conduction disturbances predominantly located distally to the AV node (*table 1* and *2*).

#### Glucagon

Glucagon stimulates adenylyl cyclase, increasing intracellular cyclic adenosine monophosphate and calcium

influx into myocytes.<sup>2</sup> The clinical efficacy of glucagon as an antidote for CCA toxicity is uncertain. In animal models of CCA poisoning, glucagon generally improved heart rate and cardiac output, but human data show variable results.<sup>28,39</sup> Therefore, glucagon may not be the most suitable treatment option for CCA poisoning. However, in case of combined CCA and beta-blocker intoxication, glucagon should be considered.

#### Intravenous lipid emulsion

Intravenous lipid emulsion (ILE) is a relatively new intervention, used in the treatment of cardiovascular instability unresponsive to supportive care during intoxication with lipophilic drugs.<sup>40-45</sup>

The proposed mechanism of action is intravascular sequestration of the toxic drug to a newly formed intravascular lipid phase ('lipid sink'), decreasing the free blood concentration of the lipophilic drug. This in turn leads to redistribution of the lipophilic drug from the target tissues into the blood, lowering drug concentration in the target tissues.<sup>46</sup> Alternatively, ILE might serve as energy source for cardiomyocytes.<sup>46</sup> Moreover, ILE could induce inotropic effects,<sup>47</sup> likely via increasing intracellular calcium levels in cardiomyocytes.

Several case reports show beneficial effects of ILE, although ILE was not successful in all CCA-overdosed patients.<sup>28</sup> Nevertheless, ILE should be considered in patients with life-threatening cardiovascular toxicity, who show an insufficient response to aforementioned conventional therapies. Notably, ILE has been used as parenteral nutrition for many years, with an acceptable safety profile, and is available in most hospitals.

#### Extracorporeal life support

When supportive and pharmacological interventions are not sufficiently effective, extracorporeal life support (ECLS) should be considered.<sup>48</sup> ECLS was shown to increase survival in patients with severe shock or cardiac arrest following poisoning due to drug overdose.<sup>28,48,49</sup> Due to potential adverse events of ECLS, i.e. thromboembolism, haemorrhage, infection and limb ischaemia, ECLS should only be applied in experienced centres.

#### Other therapies

The use of a pacemaker should be considered when pharmacological interventions fail to improve haemodynamically significant bradycardia. Treatment options also proposed for CCA overdose are phosphodiesterase III inhibitors, levosimendan, L-carnitine, methylene blue and intra-aortic balloon pump.<sup>1,2,28</sup> Information on the efficacy and safety of these options is sparse. Further studies should be conducted before any of these therapies are implemented in CCA overdose.

## SUMMARY AND CONCLUSION

CCA overdose can cause life-threatening intoxications with predominant cardiovascular instability. Patients in whom a moderate to severe intoxication is anticipated should be observed in a high-care setting and treatment should first be aimed at gastrointestinal decontamination and supportive care, i.e. the administration of intravenous fluids, and correction of metabolic acidosis and electrolytes. Moderately/severely intoxicated patients may require a well-tailored combination of interventions, e.g. the administration of calcium, high-dose insulin-glucose therapy and vasopressors. Especially hyperinsulinaemia/euglycaemia therapy is promising for patients in whom a large ingestion of a CCA is suspected, which should be initiated early in the stage of intoxication even when the patient shows hardly any haemodynamic instability. ILE may be considered in patients with life-threatening cardiovascular toxicity, with an insufficient response to conventional therapies. If clinical stabilisation cannot be achieved after applying conventional pharmacological measures, the initiation of ECLS should be considered. The efficacy and safety of the treatments currently available for CCA poisoning, including optimal dosing strategies, should be further explored in controlled clinical trials, which would optimise evidence-based decision making in managing CCA-overdosed patients.

## DISCLOSURES

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# Dutch guidelines for the diagnosis and treatment of chronic lymphocytic leukaemia

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## ABSTRACT

In recent years, considerable progress has been made in the treatment of patients with chronic lymphocytic leukaemia (CLL). Therefore, the CLL working group of the Dutch/Belgium Haemato-Oncology Foundation for Adults in the Netherlands (HOVON) framework revised the Dutch guidelines based on new studies and expert opinion of members of the working group.

group is to facilitate access to new treatments and contribute to the development of new treatment options. Besides conducting six trials in recent years, the HOVON CLL working group also formulated the Dutch guideline on diagnosis and treatment of CLL in 2011.<sup>1</sup> Recently, this guideline was revised based on new studies and the expert opinion of members of the working group and published in the *Nederlands Tijdschrift voor Hematologie*.<sup>2</sup> This journal has been given permission to publish this current manuscript.

## KEYWORDS

Chronic lymphocytic leukaemia, CLL, guidelines, HOVON

## INTRODUCTION

In recent years, considerable progress has been made in the treatment of patients with chronic lymphocytic leukaemia (CLL). Within the Dutch/Belgium Haemato-Oncology Foundation for Adults in the Netherlands (HOVON) framework, the purpose of the HOVON CLL working

## DIAGNOSIS

The diagnosis of CLL can be suspected when peripheral blood lymphocytosis is present and morphology shows smudge cells and small lymphocytes with dense nuclei and partially aggregated chromatin. The diagnosis should be confirmed by flow cytometry. The amount of circulating monoclonal B cells should be  $\geq 5 \times 10^9/l$  with an immunophenotype characteristic for CLL, with expression of CD19, CD5 and CD23 and weak expression of CD20. Additional expression of CD200 and CD43 can

be seen. Expression of CD79b should be weak or absent and FMC7 should be absent. Bone marrow examination is not necessary, but may be indicated for differentiation of anaemia or thrombocytopenia due to bone marrow suppression or autoimmune destruction.

The diagnosis of small lymphocytic lymphoma (SLL) can be made if either palpable or radiographic lymphadenopathy is present, but when the number of circulating monoclonal B cells is  $< 5 \times 10^9/l$ . Histopathological evaluation of the lymph node will show lymphoid cells with dense nuclei with partially aggregated chromatin in combination with a CLL / SLL appropriate immunophenotype.<sup>3</sup>

### History and physical examination

In early stages the disease presents with few or no symptoms. History should include World Health Organisation (WHO) performance score, fever, weight loss, night sweats and infections. Physical examination should include palpation of all lymph node areas, spleen and liver.

### Additional examination

Additional examinations are needed for staging the disease, to detect complications of the disease (haemolysis, autoimmune thrombocytopenia, hypogammaglobulinaemia) and to diagnose active or chronic infection which may be aggravated by treatment with monoclonal antibodies (hepatitis B, C). Prognostic markers (cytogenetics, especially del(17p) and p53 mutation) are required in studies and are strongly recommended in daily practice if treatment is indicated. IgVH mutational status is required in studies and optional in daily practice.

In daily practice, imaging studies are of little value. An abdominal ultrasound is not necessary if all lymph node areas and the liver and spleen are easily palpable on physical examination. A chest X-ray is recommended to diagnose hilar lymphadenopathy and pre-existing lung abnormalities or infections.

### Clinical stage and indications for starting treatment

The staging systems according to Rai and Binet are still used for indicating the start of treatment (*table 1*).<sup>4,5</sup> In an advanced stage of the disease, there is always a treatment indication. In early stage disease, treatment is only indicated when active disease is present. The criteria for active disease consist of disease-related symptoms, bone marrow failure, refractory anaemia or autoimmune thrombocytopenia, and the presence and progression of splenomegaly, lymphadenopathy and lymphocytosis (*table 2*). Marked hypogammaglobulinaemia, a monoclonal protein or a high leukocyte count in the absence of any of the above criteria are not indications for treatment. Autoimmune phenomena, especially autoimmune haemolytic anaemia and immune thrombocytopenia, can

occur and should be distinguished from cytopenias due to bone marrow infiltration. This can be done by blood testing (signs of haemolysis) but does require bone marrow examination in certain cases. Autoimmune cytopenias without signs of infiltration should be treated similarly to patients without CLL with prednisone 1 mg/kg, with tapering after response. If, in general, no response is seen after one month of treatment, response is less likely to occur and treatment of underlying CLL warranted.<sup>6</sup>

### Response evaluation after start of treatment

In daily practice, history, physical examination and blood cell counts are sufficient for the evaluation of response. Bone marrow examination is only indicated to resolve unexplained cytopenia. If there is an indication to follow stricter response criteria (as in studies), imaging (CT neck, thorax and abdomen) and bone marrow examination are generally required. The response can then be classified according to response criteria in complete remission, partial remission or progressive disease (*table 3*). Moreover, the value of minimal residual disease (MRD) monitoring by either multi-colour flow cytometry or polymerase-chain reaction based methods is currently being tested as surrogate endpoint. So far, MRD showed improved correlation with progression-free survival as compared with response rates based on International Workshop on CLL criteria.<sup>7</sup> At this moment, MRD guidance should still be considered experimental.<sup>8</sup>

## T R E A T M E N T

### Choice of treatment

It is important to take a number of aspects into consideration when there is a treatment indication. Intensity of treatment should be weighed against age, WHO performance score, comorbidity and toxicity of previous therapy. It can help to classify patients on clinical grounds into three groups: fit (patients without comorbidity), unfit (patients with any comorbidity; WHO 0-2) or frail (patients with severe comorbidities; WHO 3-4). An alternative is to calculate a Cumulative Illness Rating Scale (CIRS) score. (<http://farmacologiaclinica.info/scales/CIRS-G/>) A CIRS  $\leq 6$  is considered fit.<sup>9</sup>

If, however, the performance score is reduced by disease activity (cytopenia or lymphadenopathy), this is not a reason to refrain from intensive immunochemotherapy.

The risk profile of CLL (especially del(17p) / p53 mutation) and the associated expected response to standard therapy are important factors in guiding treatment choices. The frequency of del(17p) and p53 mutation increases with successive relapses and fluorescence in situ hybridisation (FISH) or p53 mutation analysis at relapse is therefore important. Finally, the choice of treatment can be based

**Table 1.** Clinical stage according to Rai and Binet

Rai	Binet	Indications for starting treatment
0 Lymphocytosis	A ≤ 2 lymph node regions	No
I Lymphadenopathy	B ≥ 3 lymph node regions	Optional (if active disease, see table 2)
II Splenomegaly and/or hepatomegaly		Optional (if active disease, see table 2)
III Anaemia <6.9 mmol/l*	C Anaemia <6.2 mmol/l* / thrombocytopenia <100x10 <sup>9</sup> /l	Yes, regardless of progression
IV Thrombocytopenia <100x10 <sup>9</sup> /l *		Yes, regardless of progression

\*If anaemia and thrombocytopenia is not caused by autoantibodies.

**Table 2.** Criteria for active disease

	At least one of the following six criteria must be present
1.	At least one of the following disease-related symptoms: a. Weight loss ≥10% within the previous 6 months b. Extreme fatigue (WHO performance status ≥2) c. Fever ≥38.6°C for ≥2 weeks, without evidence infections d. Night sweats without evidence infections
2.	Evidence of progressive marrow failure as manifested by the development of, or worsening of anaemia and/or thrombocytopenia
3.	Autoimmune anaemia and/or thrombocytopenia poorly responsive to corticosteroid therapy
4.	Massive (i.e., >6 cm below the left costal margin) or progressive splenomegaly
5.	Massive nodes or clusters (i.e., >10 cm in longest diameter) or progressive lymphadenopathy
6.	Progressive lymphocytosis with an increase of >50% over a 2-month period, or an anticipated doubling time of less than 6 months

**Table 3.** Response determination in studies.

	Parameter	Complete remission	Partial remission	Progressive disease
	Response definition	All of the following criteria	At least 2 criteria of 1,2,3 plus 1 criterion of 5a-c (minimal duration of 2 months)	At least 1 criterion
1	Peripheral blood lymphocytes	<4.0x10 <sup>9</sup> /l	≥50% decrease from baseline	≥50 increase (≥5.0x10 <sup>9</sup> /l)
2	Lymphadenopathy	Absent (none >1.5 cm)	≥50% decrease from baseline, no increase of new lymph nodes	≥50% increase or new (>1.5 cm)
3	Hepatomegaly / splenomegaly	Absent	≥ 50% decrease from baseline	≥50% increase or new (>1.5 cm)
4	B-symptoms	Absent	Not applicable	Not applicable
5a	Neutrophils	>1.5x10 <sup>9</sup> /l	>1.5x10 <sup>9</sup> /l	Not applicable
5b	Platelets	>100x10 <sup>9</sup> /l	>100x10 <sup>9</sup> /l or ≥ 50% improvement over baseline	≥50% decrease from baseline or to <100x10 <sup>9</sup> /l attributable to CLL
5c	Haemoglobin	>6.8 mmol/l	>6.8 μmol/l or ≥ 50% improvement over baseline	Decrease of >1.3 μmol/l from baseline or to <6.2 μmol/l attributable to CLL
6	Bone marrow	Normocellular, no lymphoid nodi, <30% lymphocytes	Not applicable	Not applicable
7	Other	Not applicable	Not applicable	CLL transformation



on whether the patient prefers decreased toxicity over increased progression-free survival.

## TREATMENT IN STUDIES

### Considerations

The purpose of the HOVON CLL working group is to facilitate access to new treatments and to contribute to the development of new treatment options. To make this possible, the aim is to have studies open for both fit and unfit patients with an indication for first-line or relapse treatment, so that many patients can participate. An overview of the studies of the HOVON CLL working group can be found on <http://www.hovon.nl/studies/studies-per-ziektebeeld/cll.html>

## TREATMENT OUTSIDE STUDY

### Front-line treatment in fit patients

- Fludarabine-cyclophosphamide-rituximab (FCR), target six cycles:

Fludarabine 40 mg/m<sup>2</sup> orally every four weeks on day 1-3; cyclophosphamide 250 mg/m<sup>2</sup> orally every four weeks on day 1-3; first infusion of rituximab at a dose of 375 mg/m<sup>2</sup>, then 500 mg/m<sup>2</sup> every four weeks

Patients treated with fludarabine should receive prophylaxis for *Pneumocystis jiroveci* pneumonia and herpes for six months after the last dose. Irradiated blood products should be given until one year after the last dose.

In fit patients, the goal of treatment is to obtain long-term progression-free survival with the improvement of overall survival. In late 2010, the results of a large randomised German study were published, where fludarabine-cyclophosphamide (FC) was compared with FCR. FCR was clearly superior with a response rate of 90% (including 44% complete responses), an almost 20-month longer duration of response and improved survival (after 3 years: 87% versus 82%; HR 0.664,  $p = 0.012$ ).<sup>10</sup> FCR is the first treatment that has proven improved survival. This was confirmed in the long-term follow-up of this study with a median follow-up of 5.9 years, with a median overall survival that was not reached for the FCR group and 86 months for the FC group.<sup>11</sup> A limitation of the study is that the patients were not representative of the 'normal' CLL population: they were fit (CIRS score  $\leq 6$ ; creatinine clearance  $> 70$  ml / min) and relatively young. Only 10% were older than 70 years. Nevertheless, FCR is now the standard first-line treatment for fit patients regardless of age. The average response time is almost five years.

### Front-line treatment in unfit patients

- Chlorambucil in combination with a CD20 monoclonal antibody, target 12 cycles or one year:

Chlorambucil 10 mg/m<sup>2</sup> orally every four weeks on day 1-7 OR 10 mg orally every four weeks on day 1-14 OR 0.1-0.15 mg/kg/day orally, continuously OR 0.4 mg/kg orally every four weeks on day 1-14, OR 20 mg orally every four weeks on day 1-5

Rituximab: First infusion 375 mg/m<sup>2</sup>, then 500 mg/m<sup>2</sup>, every four weeks

Obinutuzumab, cycle 1: first infusion 100 mg + 900 mg on day 1, then 1000 mg on day 8 and 15; cycles 2-6: 1000 mg every four weeks

Ofatumumab: first infusion 300 mg, then 2000 mg, every week (8 times), then 2000 mg every four weeks (4 times)

- Bendamustine-rituximab, target six cycles:

Bendamustine 90 mg/m<sup>2</sup>, IV every four weeks on day 1-2

Rituximab: First infusion 375 mg/m<sup>2</sup>, then 500 mg/m<sup>2</sup>, every four weeks

Patients treated with bendamustine may receive *Pneumocystis jiroveci* pneumonia and herpes prophylaxis for six months after the last dose. Irradiated blood products are recommended until one year after the last dose.

In unfit patients, the goal of treatment is to improve symptoms and prolong progression-free survival, with acceptable toxicity. The combination of chlorambucil with the recommended dosage of the CD20 monoclonal antibodies obinutuzumab and ofatumumab seems superior to the combination with standard-dose rituximab. In the three-arm phase 3 study of the German CLL group (CLL11 study), a relatively low dose of single-agent chlorambucil was compared with either a combination with standard-dose rituximab or with the novel monoclonal CD20 antibody obinutuzumab at a higher dose.<sup>12</sup> Both combination arms showed improvement in response rate and response duration compared with chlorambucil monotherapy, with a progression-free survival of 26.7 months with chlorambucil-obinutuzumab versus 11.1 months with chlorambucil alone and 16.3 months with chlorambucil-rituximab. Combination with obinutuzumab was more effective than combination with rituximab (hazard ratio 0.39; 95% CI 0.31 to 0.49;  $p < 0.001$ ). Overall survival was significantly better in the obinutuzumab combination versus monotherapy. A similar concept was tested by the English CLL study group, where the chlorambucil-ofatumumab combination was compared with chlorambucil monotherapy.<sup>13</sup> Also in this study, the combination arm was superior, both in terms of response rates (overall response of chlorambucil-ofatumumab 82%

versus chlorambucil alone 69%,  $p = 0.001$ ) and duration of response (progression-free survival of chlorambucil-ofatumumab 22.4 months versus chlorambucil alone 13.1 months).

The CLL 10 study (in fit CLL patients) showed that in patients older than 60 years bendamustine in combination with rituximab was as effective as FCR. Bendamustine has both biochemical overlap with purine analogues and with alkylators, and has a more favourable toxicity profile as compared with fludarabine.<sup>14</sup> The average response time for bendamustine-rituximab is 3.75 years.

Bendamustine has been registered for first-line treatment of patients who cannot tolerate therapy containing fludarabine because of toxicity, specifically neutropenic fever.

In frail patients, the goal of treatment is symptom control. The mean response duration of single-agent chlorambucil is one year, in combination with rituximab this increases to about 16 months, in combination with obinutuzumab and ofatumumab to more than two years.<sup>12,13</sup>

#### **Treatment at relapse in fit patients without del(17p) / p53 mutation and response duration $\geq 2$ years**

- Repeat previous immunochemotherapy or introduce monoclonal CD20 antibody in case of previous treatment without immunotherapy
- Bendamustine-Rituximab

In a study with previously treated CLL patients, FCR significantly improved progression-free survival compared with FC, with a median duration of 30.6 months for FCR versus 20.6 months for FC.<sup>15</sup> The combination bendamustine and rituximab in relapsed CLL showed a response rate of almost 60%, mostly partial remissions. The median event-free survival was 14.7 months.<sup>16</sup> In the Netherlands, bendamustine has not yet received an add-on status for this indication and is therefore not reimbursed.

#### **Treatment at relapse in fit patients with relapse < 2 years after FCR or refractory disease**

- R-DHAP, target six cycles (followed by non-myeloablative allogeneic stem cell transplantation):  
Dexamethasone 40 mg orally or IV every four weeks on day 1-4, cisplatin 100 mg/m<sup>2</sup> IV on day 1, cytarabine 2000 mg/m<sup>2</sup> IV every 12 hours on day 2, rituximab: first infusion 375 mg/m<sup>2</sup>, then 500 mg/m<sup>2</sup>.  
Antibacterial and antifungal prophylaxis should be administered during R-DHAP. The prescribed medication is according to local policy.

- Kinase inhibitors (ibrutinib 420 mg (3 capsules) orally 1 x daily, continuously until progression:

Idelalisib 150 mg orally 2 x daily, continuously until progression, rituximab (max 8 times), 375 mg/m<sup>2</sup> on day 1, 500 mg/m<sup>2</sup> in week 3, 5, 7, 9, 13, 17, 21)

- Alemtuzumab (week 1: 3 mg SC on day 1, 10 mg SC on day 2, 30 mg SC on day 3; week 2-12: 30 mg SC x 3 a week (Mon-Wed-Fri).

Patients treated with alemtuzumab should receive prophylaxis for *Pneumocystis jiroveci* pneumonia and herpes for six months after the last dose. Irradiated blood products should be given until one year after the last dose. Moreover, monitoring of cytomegalovirus (weekly) and Epstein-Barr virus (monthly) should be performed at least one year after last alemtuzumab dose.

In patients with a relapse < 2 years after FCR or refractory disease, allogeneic stem cell transplantation should be considered.<sup>17</sup> Allogeneic stem cell transplantation in patients with refractory and high-risk CLL results in responses to 70% and a five-year survival of about 50%.<sup>18,19</sup> A graft versus leukaemia effect may be responsible for this possibly curative response. The non-relapse mortality is approximately 20%, and is mainly caused by infections and graft-versus-host disease. This highly effective treatment is therefore only available to a very limited (fit, relatively young) patient population. The better disease control before transplantation, the greater the chance of a long-term response. In particular, node size is an important predictor of treatment success.

HOVON 88 showed the potency of R-DHAP to achieve clinical responses in these high-risk patients who were consolidated by a non-myeloablative stem cell transplantation.<sup>20</sup> It is important to give antibiotic prophylaxis with R-DHAP, because of a high incidence of infectious complications in this study before prophylaxis was advised.

If allogeneic stem cell transplantation is not considered, treatment with the CD52 antibody alemtuzumab is a possibility, especially in disease with little lymphadenopathy. In patients with lymph nodes of > 5 cm less response to alemtuzumab is seen and alternative treatment is warranted.<sup>21</sup> It is important to monitor opportunistic infections, particularly cytomegalovirus. Alemtuzumab is no longer registered for CLL, but still available (free of charge) until at least 2017.

#### **Treatment for relapse in unfit patients**

- Repeat previous immunochemotherapy
- Bendamustine-rituximab
- Kinase inhibitors
- Ofatumumab: first infusion 300 mg, then 2000 mg, every week (8 times), then 2000 mg every four weeks (4 times)

If a relapse occurs more than six months after the previous immunochemotherapy and there is no del(17p) / p53 mutation, repeating this treatment can be considered. With a shorter duration of response or del(17p) / p53 mutation, such repetition is not expected to be effective. For unfit patients, bendamustine in combination with rituximab could be considered. Ofatumumab, the fully human monoclonal CD20 antibody, has proven effectiveness not only in patients who are refractory to fludarabine as well as alemtuzumab (OR 58%), but also in patients who are refractory to fludarabine, and have greatly enlarged nodes (OR 47%). The median duration of response in both groups was about six months.<sup>22</sup> Based on these findings, ofatumumab has been registered and is reimbursed as an add-on for CLL patients who are refractory to fludarabine and alemtuzumab.

#### Treatment at relapse with del(17p) / p53 mutation

- R-DHAP, followed by non-myeloablative allogeneic stem cell transplantation
- Kinase inhibitors
- Alemtuzumab

Del(17p) or p53 mutation is associated with resistance to most immunochemotherapy. The frequency of del(17p) and p53 mutation increases with successive relapses. FISH or p53 mutation analysis at relapse is therefore important if clinical consequences are present. Responses in allogeneic stem cell transplantation occur independently of del(17p) / p53 mutation. R-DHAP treatment is an effective induction regimen before non-myeloablative stem cell transplantation in this patient population. Alemtuzumab has proven effectiveness in patients with del(17p)/ p53 mutation.<sup>23</sup>

#### Indications for kinase inhibitors

- Presence of del(17p) / p53 mutation
- Need for treatment < 2 years after FCR or bendamustine-rituximab
- Need for treatment < 6 months after chlorambucil in combination with a CD20 monoclonal antibody

Kinase inhibitors interrupt crucial signalling pathways from the membrane to the cell nucleus. The most effective kinase inhibitors block the B-cell receptor signalling pathway, by inhibiting the kinases Btk (ibrutinib = Imbruvica®) and PI3K- $\delta$  (idelalisib, = Zydelig®). These inhibitors can be given orally and in case of idelalisib in combination with rituximab. Two recently published studies, both in high-risk patients, showed impressive response duration.<sup>24-26</sup> Rapid relapses occur after treatment cessation, and therefore these drugs should be continued until relapse or progression. These new drugs have specific side effects such as gastrointestinal side effects and skin toxicity with idelalisib<sup>27</sup> and thrombocyte

aggregation inhibition and atrial fibrillation with ibrutinib. Ibrutinib is therefore used with caution in patients with atrial fibrillation and/or treatment with therapeutic anticoagulation. Only limited bone marrow suppression occurs. A specific phenomenon of treatment with kinase inhibitors is a temporary lymphocytosis in combination with a rapid decrease of lymphadenopathy in the first weeks after initiation of therapy.

Ibrutinib and idelalisib are registered for the treatment of CLL patients after at least one prior therapy, or as front-line therapy in the presence of del(17p) or p53 mutation in patients not eligible for immunochemotherapy. These expensive drugs are reimbursed as add-on.

The average response time is still unclear. After 1.5 years more than half of the patients are still in remission.

Given the lack of literature about side effects with prolonged use, and the question whether successful reinduction is possible for patients who progress on a kinase inhibitor, it is advised to treat only those patients with kinase inhibitors that have active disease during or shortly after optimal immuno-chemotherapy.

#### DISCLOSURES

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S. Kersting: advisory boards of Novartis, Gilead.

M-D. Levin: Advisory boards of Roche and Amgen.

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M. Chamuleau: advisory board of Roche; congress speaker Roche; research support of Celgene.

M. van Gelder: advisory boards of Janssen, Roche; consulting honorarium Mundipharma; congress speaker Janssen, Roche; research support of Celgene.

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# *Chlamydia psittaci*: a relevant cause of community-acquired pneumonia in two Dutch hospitals

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## ABSTRACT

**Background:** Of all hospitalised community-acquired pneumonias (CAPs) only a few are known to be caused by *Chlamydia psittaci*. Most likely the reported incidence, ranging from 0% to 2.1%, is an underestimation of the real incidence, since detection of psittacosis is frequently not incorporated in the routine microbiological diagnostics in CAP or serological methods are used.

**Methods:** *C. psittaci* real-time polymerase chain reaction (PCR) was routinely performed on the sputum of 147 patients hospitalised with CAP, who participated in a clinical trial conducted in two Dutch hospitals. In 119/147 patients the paired complement fixation test (CFT) was also performed for the presence of *Chlamydia* antibodies. Positive CFTs were investigated by micro-immunofluorescence for psittacosis specificity. Case criteria for psittacosis were a positive PCR or a fourfold rise of antibody titre in CFT confirmed by micro-immunofluorescence. Furthermore, we searched for parameters that could discriminate psittacosis from CAPs with other aetiology.

**Results:** 7/147 (4.8%) patients were diagnosed with psittacosis: six with PCR and one patient with a negative PCR, but with CFT confirmed by micro-immunofluorescence. Psittacosis patients had had a higher temperature

(median 39.6 vs. 38.2 °C;) but lower white blood cell count (median 7.4 vs. 13.7 x10<sup>9</sup>/l) on admission compared with other CAP patients.

**Conclusion:** In this study, *C. psittaci* as CAP-causing pathogen was much higher than previously reported. To detect psittacosis, PCR was performed on all CAP patients for whom a sputum sample was available. For clinical use, PCR is a fast method and sputum availability allows genotyping; additional serology can optimise epidemiological investigations.

## KEYWORDS

*Chlamydia*, *Chlamydophila*, pneumonia, polymerase chain reaction, psittacosis

## INTRODUCTION

*Chlamydia psittaci* is an intra-cellular Gram-negative bacterium which may cause zoonotic pneumonia in humans.<sup>1</sup> Usually, transmission occurs through inhalation of aerosols from contaminated bird substances such as droppings, plumage, or tissue.<sup>2</sup> Few studies have evaluated the incidence of *C. psittaci* in hospitalised community-

acquired pneumonia (CAP); reported incidence rates range from 0% in Korea in the years 1999-2000,<sup>3</sup> to 0.002% in Australia in 2004-2006,<sup>4</sup> 0.004% in Canada in 1996-1997,<sup>5</sup> and 0.36% in Spain in 1996-1997.<sup>6</sup> These rates are probably an underestimation of the true incidence since even brief exposure can lead to systemic infection<sup>7</sup> and testing for *C. psittaci* is often only included in the diagnostic algorithm if the clinician is aware of contact with birds in the patient's history. Although the disease is notifiable to the public health authorities in the Netherlands, detection of psittacosis is frequently not incorporated in routine microbiological diagnostic panels for pneumonia. Since standard  $\beta$ -lactam based antibiotic therapy is not adequate for the treatment of *C. psittaci*, this might lead to progression of infection.

Psittacosis can be diagnosed by culture, but *C. psittaci* is difficult to grow. Furthermore, biosafety level three facilities and cell cultures are required which are not available in most medical microbiology laboratories. Currently, polymerase chain reaction (PCR) of sputum or bronchial secretions and serology are the cornerstones of microbiological diagnosis. All aforementioned studies on psittacosis incidence used only serological diagnostic methods such as complement fixation test (CFT) and micro-immunofluorescence. Due to the retrospective aspect and cross-reactivity with other *Chlamydia* species, detection by serology is not optimal. If sputum is available, PCR is a method with fast results and lacking genus broad cross-reactivity.<sup>8</sup> The availability of sputum enables genotyping of the strain. A recent study from Germany reported a *C. psittaci* incidence of 2.1% in CAP, based on *C. psittaci* PCR on pharyngeal swabs in all CAP patients who presented to the emergency department.<sup>9</sup>

We analysed a cohort of 304 patients hospitalised with CAP in which PCR and CFT for psittacosis diagnosis were incorporated in the diagnostic algorithm. We also analysed whether specific clinical parameters were associated with CAP caused by *C. psittaci*. Furthermore, we evaluated the contribution of serology to the diagnosis of psittacosis by PCR.

## MATERIALS AND METHODS

### Study design and patients

Our analyses were performed in all patients in whom a sputum or bronchoalveolar lavage sample was available in a cohort of 304 hospitalised patients with CAP who participated in a randomised clinical trial conducted in the Netherlands from November 2007 until September 2010 (NCT00471640). The original trial investigated the adjunctive treatment of dexamethasone 5 mg given intravenously. Detailed inclusion and exclusion criteria are described elsewhere.<sup>10</sup> In summary, patients with

CAP admitted to the St. Antonius Hospital (Nieuwegein, the Netherlands) or the Gelderse Vallei Hospital (Ede, the Netherlands) were included. Pneumonia was defined as a new pulmonary infiltrate on chest radiograph, in combination with at least two of the following criteria: cough, sputum production, temperature  $> 38.0$  °C or  $< 35.0$  °C, auscultatory findings consistent with pneumonia, C-reactive protein concentration  $> 15$  mg/l, and white blood cell count  $> 10 \times 10^9$  cells/l or  $< 4 \times 10^9$  cells/l or  $> 10\%$  of rods in leukocyte differentiation. Patients who were immunocompromised, who were admitted to the intensive care unit immediately, or who received immunosuppressive therapy were excluded. The study was approved by the local Medical Ethics Committees of both hospitals and all patients gave written informed consent.

### Psittacosis diagnosis

Sputum or bronchoalveolar lavage was analysed with *C. psittaci* specific real-time PCR.<sup>11</sup> Furthermore, acute-phase serum and convalescent serum samples were analysed for the presence of antibodies against *Chlamydia* species, with CFT using a *Chlamydia*-specific lipopolysaccharide (Virion-Serion, Rueschlikon, Switzerland). The interval between the first (day of admission) and second serum sample was at least ten days with a maximum of 120 days. A fourfold or greater rise of the antibody titre in the convalescent versus the acute-phase serum was considered indicative for a recent *Chlamydia* infection (sequence 1:  $< 4$ , 4, 8, 16, etc. or 1:  $< 10$ , 10, 20, 40, etc.). Sera of patients with a positive psittacosis PCR or CFT result were tested with micro-immunofluorescence (Focus Diagnostics, United States of America), using the same criteria for a rise in antibody titre that were used for CFT (sequence 1:  $< 16$ , 16, 32, 64, etc.). The case criteria for a diagnosis of psittacosis was a positive PCR or a fourfold rise of antibody titre in CFT confirmed by micro-immunofluorescence. Stored sputum samples were genotyped by partial ompA gene sequencing.<sup>12</sup>

### Diagnosis of other respiratory pathogens

Two sets of separate blood samples and sputum samples were cultured. Urine antigen tests were performed for the detection of *Streptococcus pneumoniae* and *Legionella pneumophila* serogroup 1. Real-time PCRs on sputum were also performed to detect *Legionella* species, *Mycoplasma pneumoniae* and *Coxiella burnetii*. Moreover, St. Antonius Hospital samples were also tested for *C. pneumoniae* until November 2008. Acute versus convalescent serological testing was performed for antibodies to *M. pneumoniae*, *C. burnetii* and respiratory viruses (adenovirus, influenza virus A and B, parainfluenza virus 1, 2 and 3, and the respiratory syncytial virus). Pharyngeal swabs were taken for PCR to detect (para)-influenza virus, adenovirus and respiratory syncytial virus.

**Clinical parameters and outcome**

Clinical and laboratory parameters (table 1) were documented. For patients in whom information on bird contact was not available in the admission documents, the Dutch notification database of the Centre for Infectious Disease Control of reported psittacosis patients was checked for information on this subject.

**Statistical analyses**

Overall, descriptives were stated as number (%) or median (interquartile range (IQR)). Patient characteristics between psittacosis patients and other aetiology were compared using Chi-square test, Fisher's exact test, or Mann-Whitney U test, where appropriate. For this analysis, a p-value < 0.002 was considered significant, using

**Table 1.** Baseline characteristics of 147 patients hospitalised with community-acquired pneumonia categorised in psittacosis versus non-psittacosis pneumonia

Characteristics	Psittacosis (n = 7)	Other aetiology (n = 140)	Odds ratio (95% CI)
Age in years (IQR)	54 (46-58)	64 (51-76)	0.96 (0.92-1.00)
Male sex (%)	5 (71.4)	81 (57.9)	1.82 (0.34-9.71)
Smoking (%) <sup>∞</sup>	2 (28.6)	47 (33.6)	1.21 (0.20-7.47)
Nursing home resident (%)	0	3 (2.1)	NA
Season of admission (%)			
Summer	1 (14.3)	22 (15.7)	
Winter	1 (14.3)	46 (32.9)	0.48 (0.03-8.01)
Spring	4 (57.1)	50 (35.7)	1.76 (0.19-16.66)
Autumn	1 (14.3)	22 (15.7)	1.00 (0.06-17.02)
Comorbidities (%)			
COPD	0	21 (15.0)	NA
Congestive heart failure	0	21 (15.0)	NA
Renal disease	0	11 (7.9)	NA
Diabetes mellitus	0	21 (15.0)	NA
Liver disease	0	1 (0.7)	NA
Days of illness (IQR)*	5.0 (3.0-5.0)	5.0 (3.0-7.0)	0.88 (0.36-2.16)
Physical examination (IQR)			
Systolic BP in mmHg	133 (125-149)	130 (116-145)	1.00 (0.97-1.04)
Diastolic BP in mmHg	9 (65-82)	75 (64-83)	1.01 (0.95-1.08)
Pulse rate in beats/min	112 (90-120)	98 (84-111)	1.03 (0.99-1.08)
Temperature in °C	39.6 (38.8-40.0)	38.2 (37.4-38.9)	4.25 (1.46-12.35)
Laboratory parameters			
CRP in mg/l (IQR)*	335 (221-359)	244 (115-357)	2.66 (0.65-10.86)
WBC count $\times 10^9/l$ (IQR)	7.4 (6.9-10.4)	13.7 (10.3-18.7)	0.72 (0.56-0.92)
Creatinine in $\mu\text{mol/l}$ (IQR)*	95 (106-70)	88 (72-110)	1.02 (0.14-7.26)
BUN in mg/dl (IQR)*	5.3 (4.0-6.9)	6.7 (4.7-9.8)	0.34 (0.07-1.68)
Sodium in mmol/l (IQR)	127 (122-132)	134 (131-136)	0.83 (0.73-0.94)
ASAT in U/l (IQR)*	46 (24-154)	35 (23-51)	1.25 (0.75-6.76)
Glucose in mmol/l (IQR)*	8.3 (6.5-9.7)	7.1 (6.0-8.5)	3.47 (0.46-26.18)
IL-6 in pg/ml (IQR)*	26.3 (5.6-47.8)	71.3 (18.0-263.2)	0.73 (0.53-1.02)
Arterial blood gas:			
pCO <sub>2</sub> in kPa (IQR)*	3.7 (3.3-4.1)	4.4 (4.0-4.8)	0.00 (0.00-0.21)
pO <sub>2</sub> in kPa (IQR)*	8.0 (5.0-9.5)	8.7 (7.6-11.0)	0.07 (0.00-1.16)
PSI class (%):			
PSI class I-III	5 (71.4)	83 (59.3)	
PSI class IV-V	2 (28.6)	57 (40.7)	0.58 (0.11-3.11)

Data are presented as number (%) or median (IQR). ASAT = aspartate aminotransferase; BP = blood pressure; BUN = blood urea nitrogen; CI = confidence interval; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; ICU = intensive care unit; IQR = interquartile range; NA = not applicable; PSI = pneumonia severity index; WBC count = white blood cell count; <sup>∞</sup>missing data of 9 patients; \*variables were log normally transformed for regression analysis.

the Bonferroni correction for multiple testing with 26 variables. To identify variables predicting psittacosis, logistic regression analysis was conducted in a univariate model. Variables which were not normally distributed were log-normally transformed to improve distribution. A multivariate analysis was not possible due to the small number of psittacosis patients. Data were analysed with SPSS statistical software for Windows, version 22.0.

## RESULTS

### Psittacosis diagnosis

In total, 304 CAP patients were enrolled in the original trial. Sputum was available in 154/304 patients for analysis with *C. psittaci* PCR. In seven patients the PCR reaction of sputum was inhibited. Therefore, 147/304 (48.4%) patients were eligible for further analysis, of which 77 patients received dexamethasone and 70 patients placebo in the original study. Of the 147 patients, six (4.1%) patients were diagnosed with psittacosis based on a positive PCR result. In 119/147 (81.0%) patients serological analysis for the presence of *Chlamydia* antibodies by CFT was performed as well. In nine patients, serum was positive on CFT. A confirmation with micro-immunofluorescence was positive in five out of nine patients, and four of these five were also positive on PCR. One PCR-positive patient was seronegative and one was sero-inconclusive. Based on our

diagnostic criteria, we considered the one patient who was PCR negative but CFT and micro-immunofluorescence positive to be positive for psittacosis. In total, 7/147 (4.8%) patients were diagnosed with psittacosis based on PCR and serology results. Table 2 shows an overview of the test results. No other pathogens were detected in these seven patients. None of these patients were known to be involved in an outbreak. In three of six patients, sputum was available in storage for genotyping; two patients had genotype A, one patient genotype B.

In our cohort, most CAPs were caused by *S. pneumoniae* (38/147, 25.9%), followed by *C. burnetii* (12/147, 8.2%).<sup>10</sup> The latter was high due to a national outbreak of Q fever.<sup>13</sup> *Haemophilus influenzae* was detected in six patients and *Legionella* species in five patients. In the 47 patients in whom *Chlamydia pneumoniae* PCR was performed, no positive results were found.

Of the 157/304 patients in whom no sputum was available for PCR, paired CFT was performed in 93 (59.2%) patients. Of these patients, three were positive on CFT, but specificity for *C. psittaci* was not confirmed by micro-immunofluorescence.

### Description of psittacosis patients

Median age of the seven patients with a *C. psittaci* CAP was 54 years (IQR 46-58) and 71.4% were male. The median interval between onset of disease and admission to hospital in patients with psittacosis was 5.0 days (IQR 3.0-5.0 days).

**Table 2.** Test results for detection of *Chlamydia psittaci* by polymerase chain reaction, complement fixation test, and micro-immunofluorescence test

Patient	PCR	Complement fixation test			Micro-immunofluorescence test			Psittacosis
		First sample	Second sample	Result	First sample	Second sample	Result	
1	+	<1:4	>1:128	+	<1:16	1:64	+	+
2	+	<1:4	>1:128	+	1:32	1:128	+	+
3	+	<1:4	1:256	+	<1:16	1:64	+	+
4	+	<1:10	1:80	+	<1:16	1:256	+	+
5	+	>1:160	>1:160	NC	1:128	Missing	NC	+
6	+	<1:4	<1:4	-	<1:16	1:16	-	+
7	-	<1:4	1:80	+	<1:16	1:64	+	+
8	-	<1:4	1:16	+	<1:16	<1:16	-	-
9	-	<1:4	1:128	+	<1:16	Missing	NC	NC
10	-	<1:10	1:80	+	<1:16	Missing	NC	NC
11	-	<1:4	1:64	+	<1:16	Missing	NC	NC

NC = non conclusive; PCR = polymerase chain reaction. Interval between first (day of admission) and second serum sample was at least 10 days; a fourfold or greater rise of the antibody titre in the convalescent versus the acute-phase serum was considered indicative for a recent *C. psittaci* infection (sequence 1: <4, 4, 8, 16, etc., or 1: <10, 10, 20, 40, etc. for CFT and 1: <16, 16, 32, 64, etc. for MIF).



**Table 3.** Clinical and demographical characteristics of seven hospitalised patients with community-acquired pneumonia caused by *Chlamydia psittaci*

Pt	Age	Gender	Bird contact	Symptoms on admission	Days of symptoms	Pulmonary medical history	Treatment	LOS in days	PCR
1	46	Male	Unknown CIDC database: deceased bird in garden	Dyspnoea, Productive coughing, Abdominal and thoracic pain	2	No	β-lactam, later cephalosporin and macrolide	13.5	Positive Strain B
2	26	Female	Parrot at home	Dyspnoea, coughing, vomiting, thoracic and low lumbar pain, fainting, dizziness	5	No	β-lactam	3.5	Positive Strain A
3	55	Male	Two birds at home	Coughing, diarrhoea, headache	7	No	β-lactam and macrolide	6.5	Positive
4	50	Male	Bird contact mentioned	Fever, productive coughing, headache, nausea, vomiting, abdominal pain	5	No	Tetracycline	17.0	Positive Strain A
5	54	Male	Pigeons	Fever, coughing, dyspnoea, confusion	5	Aspergillus pneumonia, yearly pneumonia	Cephalosporin and quinolone, later erythromycin (on ICU)	12.5 (ICU day after admission)	Positive
6	65	Male	>100 different birds at home	Headache, muscle ache, loss of appetite	3	History of psittacosis	β-lactam and quinolone	4.5	Positive
7	58	Female	No	Fever, muscle ache, loss of appetite, headache	4	No	cephalosporin and tetracycline, afterwards doxycycline and amoxicillin-clavulanic acid	8.5	Negative

CIDC = Centre for Infectious Disease Control; LOS = length of hospital stay; PCR = polymerase chain reaction.

Of the seven patients, five patients initially reported a positive history of bird contact, one a negative history, and for one patient history was not documented in the medical record. For this patient information on bird contact could be found in the Dutch notification database: a dead bird in the garden was reported. *Table 3* shows an overview of clinical parameters of the seven psittacosis patients.

#### Characteristics of *C. psittaci* versus other pathogens

Baseline characteristics of the seven psittacosis patients were compared with the 140 CAP patients with other or unknown aetiology (*table 1*). There were no significant clinical or demographical differences between these two aetiological groups. Psittacosis patients had a higher

median temperature (39.6 °C, IQR 38.8-40.0 vs. 38.2 °C, IQR 37.4-38.9) but lower white blood cell count (7.4 ×10<sup>9</sup>/l, IQR 6.9-10.4 vs. 13.7 ×10<sup>9</sup>/l, IQR 10.3-18.7) on admission, with ORs of 4.25 (95% CI 1.46-12.35) and 0.72 (95% CI 0.56-0.92), respectively. All odds ratios with 95% confidence intervals can be found in *table 1*.

#### Therapy

In five patients antibiotics adequate for psittacosis treatment were started in the emergency department (tetracycline, macrolide or quinolone antibiotics). In one patient adequate treatment was started later during admission based on a positive *C. psittaci* PCR. One patient was treated with β-lactam only. Length of stay did not

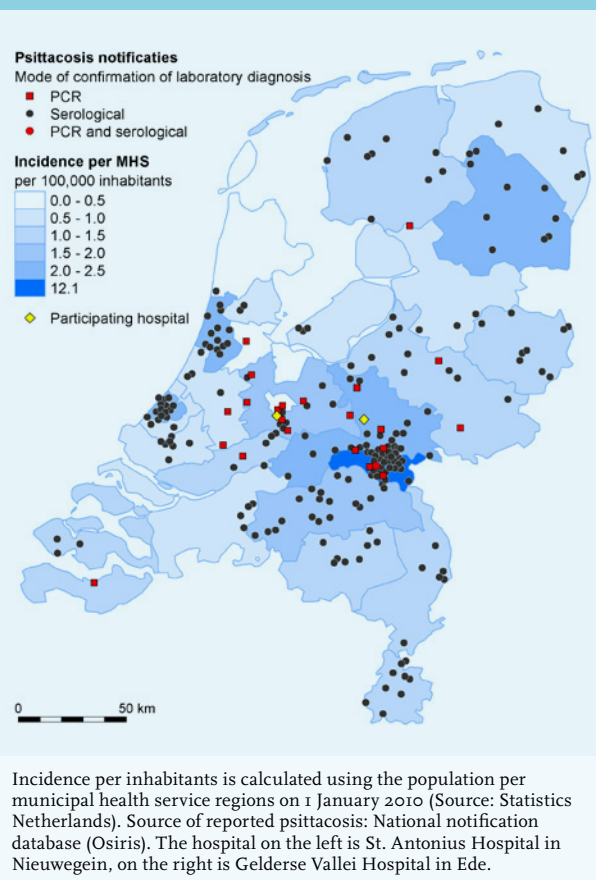
differ between these two patients and the five patients who immediately received antibiotics regarded as adequate for the treatment for psittacosis. No patients died during the hospital stay.

## DISCUSSION

In this study, PCR to detect *C. psittaci* was performed in all CAP patients for whom sputum was available. We found a much higher incidence of psittacosis (4.8%) than previously reported.

In most studies on CAP, psittacosis diagnostics are performed with serological tests only and no PCR.<sup>14</sup> A recent study on aetiology in patients hospitalised with CAP in the United States did not perform *C. psittaci* diagnostics at all.<sup>15</sup> A recent study from Germany did perform *Chlamydia* species PCR on pharyngeal swab specimens in all CAP patients presenting to the emergency department, and found an incidence of 2.1% for *C. psittaci*.<sup>9</sup> PCR leads to a quick diagnosis and lacks broad genus cross-reactivity.<sup>16</sup> Furthermore, *C. psittaci* sputum strains can be genotyped, which is relevant for public health notification and source detection and control. However, sputum or bronchial alveolar lavage to perform a PCR are not always available. In these cases, serology can be informative. Moreover, in our study, one patient with a negative PCR had a positive CFT confirmed by micro-immunofluorescence. Including only patients in whom both PCR and serology were performed, the incidence of *C. psittaci* would be even higher in this study: 7/119 (5.8%). In this small population, we cannot explain why some patients were PCR positive and serology negative and vice versa. Serology does have the disadvantage of cross-reactivity with other *Chlamydia* strains. Furthermore, to confirm psittacosis two serum samples have to be drawn with an interval of at least ten days to detect a fourfold increase in antibody titre. Therefore, serology can only be used to diagnose psittacosis in retrospect and is mainly of additional value to optimise epidemiological investigations. *Figure 1* shows reported psittacosis in the Netherlands in the period of study inclusion, based on the National Notification Database of the Centre for Infectious Disease Control. Nationally, this figure shows more psittacosis diagnosed serologically than by PCR. Regionally, in the areas where the study was conducted (in the centre of the Netherlands) and consequently psittacosis PCR was performed more routinely, the number of cases of PCR-confirmed psittacosis was higher compared with other areas. However, information on the number of PCRs and serology tests performed per region is unknown. The high number of reported psittacosis in the south-east area was mainly due to an outbreak of psittacosis after a bird fair in November 2007.<sup>17</sup>

**Figure 1.** Reported psittacosis in the Netherlands in the period November 2007 until September 2010



We addressed the question whether we could identify clinical parameters that discriminate psittacosis from CAPs caused by other pathogens. Due to the small sample size, the power of the statistical analysis was limited. The white blood cell count was almost normal in psittacosis patients, a finding consistent with a study on 135 serology-confirmed psittacosis cases.<sup>18</sup> Since elevated white blood cell count was one of the inclusion criteria of the original clinical trial, this could mean that psittacosis patients were less likely to be included and the real psittacosis incidence is even higher. In accordance with a study on the differences between *C. psittaci* and *L. pneumophila* in patients admitted to the ICU, patients with psittacosis seemed to have less comorbidities compared with those with CAP with other aetiologies, although this difference was not significant in our study.<sup>19</sup> Further research with larger groups of psittacosis patients is needed to establish whether psittacosis can be identified as a separate clinical entity. This lack of specific alerting symptoms implies that doctors should be aware of the possibility of psittacosis in any case of CAP. In our study, one patient was treated with a  $\beta$ -lactam antibiotic only. This antibiotic is not recommended for the treatment of psittacosis, because *C. psittaci* lacks peptidoglycan. Either  $\beta$ -lactam antibiotics

are also somewhat effective in psittacosis or the patient recovered due to the natural course of the disease.

Some limitations of the study must be mentioned. First, as mentioned previously, the number of psittacosis cases is small which limits statistical analysis. Second, since only two hospitals participated in this study it is unknown whether the results can be generalised. Third, CFT detects antibodies to *Chlamydia* species, but is not specific for *C. psittaci* due to cross-reactivity between the different *Chlamydia* species. Therefore, we performed micro-immunofluorescence to confirm the specificity of CFT results. It is assumed that micro-immunofluorescence does have species specificity. Finally, we included only patients in whom sputum was available for PCR diagnostics, which was 48.4% of the total CAP cohort. In the non-sputum group in whom serological analysis was performed, we found no evidence of an excess of psittacosis. On the contrary, there were less CFT/micro-immunofluorescence serologically positive patients in the non-sputum group than expected: 0/94 in the non-sputum group compared with 5/119 in the sputum group ( $p=0.07$ ). In the literature, there is no evidence that patients with psittacosis produce more sputum compared with CAPs with other aetiology.

In conclusion, in these two Dutch hospitals, where psittacosis PCR was performed if sputum samples were available, *C. psittaci* was more common as CAP-causing pathogen than previously reported. *C. psittaci* PCR is a fast method and sputum availability allows genotyping. Serology can be added to optimise epidemiological investigations.

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## DISCLOSURES

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# Familial focal segmental glomerulosclerosis: mutation in inverted formin 2 mimicking Alport syndrome

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## ABSTRACT

Focal segmental glomerulosclerosis (FSGS) is one of the most common patterns of glomerular injury. FSGS can be caused by mutations in genes encoding proteins that play key roles in the function of the podocyte and glomerular basement membrane. In this case report we present a family with FSGS initially suspected to be Alport syndrome. Genetic analysis according to the Dutch guidelines of FSGS revealed a mutation in *INF2*.

### What was known on this topic?

Familial focal segmental glomerulosclerosis (FSGS) is caused by mutations in genes which have an important role in the function of podocytes. Several genes have been identified; however, in many families the cause remains unknown. Research on gene mutations and comprehensive investigation of phenotypes will teach us more about the disease and this will result in early diagnosis, better prediction of prognosis for the individual patient, genetic counselling and hopefully this will contribute to directed personalised therapy in the future.

### What does this add?

We present a family with a mutation in the gene encoding inverted formin 2 (*INF2*), a known cause of familial FSGS. However, the initial suspected diagnosis was Alport syndrome. This case report creates awareness of the large variability in phenotypic expression of FSGS caused by a mutation in one of the genes affecting the podocyte. Namely, familial FSGS can present with nephrotic or non-nephrotic range proteinuria, with or without microscopic haematuria, and with or without neurological symptoms. Awareness of the variability in presentation of FSGS caused by a genetic defect in

combination with a thorough family history taking is important to diagnose these patients and emphasise the importance of genetic testing. Moreover, this is the first report with a comprehensive phenotypic description of a family with a mutation in *INF2* and a short overview of the knowledge about other patients described in literature with a mutation in the *INF2* gene.

## INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) is one of the most common patterns of glomerular injury. FSGS is a very heterogeneous disease with many underlying genetic and environmental causes. Here we report on a family who presented with haematuria and proteinuria, clinically resembling Alport syndrome. Further analysis revealed FSGS due to a mutation in the gene encoding inverted formin 2 (*INF2*).

## CASE REPORT

Patient 1, a 31-year-old Caucasian female, presented with oedema of the lower extremities. Additional work-up revealed microscopic haematuria and proteinuria of 2.7 g/24 hours, a normal serum albumin (44 g/l) and renal insufficiency (endogenous creatinine clearance 40 ml/min). Based on renal biopsy, a diagnosis of focal segmental extracapillary glomerulonephritis was established. The patient did not receive any immunosuppressive drugs. Within three years after diagnosis she progressed to end-stage kidney disease (ESKD) and haemodialysis was started. One year later, a postmortal kidney transplantation was performed and the graft functioned well until the

patient died at the age of 64. The family history revealed that the patient's mother died at the age of 40 due to an unknown renal disease. Her brother had developed ESKD at the age of 26 and was treated with haemodialysis.

Patient 2, the son of patient 1, tested negative for proteinuria and haematuria at the age of 10. However, at the age of 18, he was referred to the outpatient clinic because of microscopic haematuria and asymptomatic proteinuria (5 g/24 hours). Serum albumin (38 g/l) and renal function (endogenous creatinine clearance 115 ml/min) were normal. Physical examination, including neurological examination, was unremarkable. The renal biopsy report described ten glomeruli of which two with complete sclerosis. Open glomeruli showed increased mesangial matrix, focal reduplication of capillary loops, synechiae and sclerosis. Immunofluorescence showed depositions of IgM along the capillary wall and within the mesangium. Immunofluorescence for type IV collagen was not performed. Electron microscopic examination was described as sporadic splitting of the lamina densa with augmented mesangial matrix. Based on the renal biopsy, the microscopic haematuria and the pattern of inheritance, an X-linked inherited disease (Alport syndrome) was

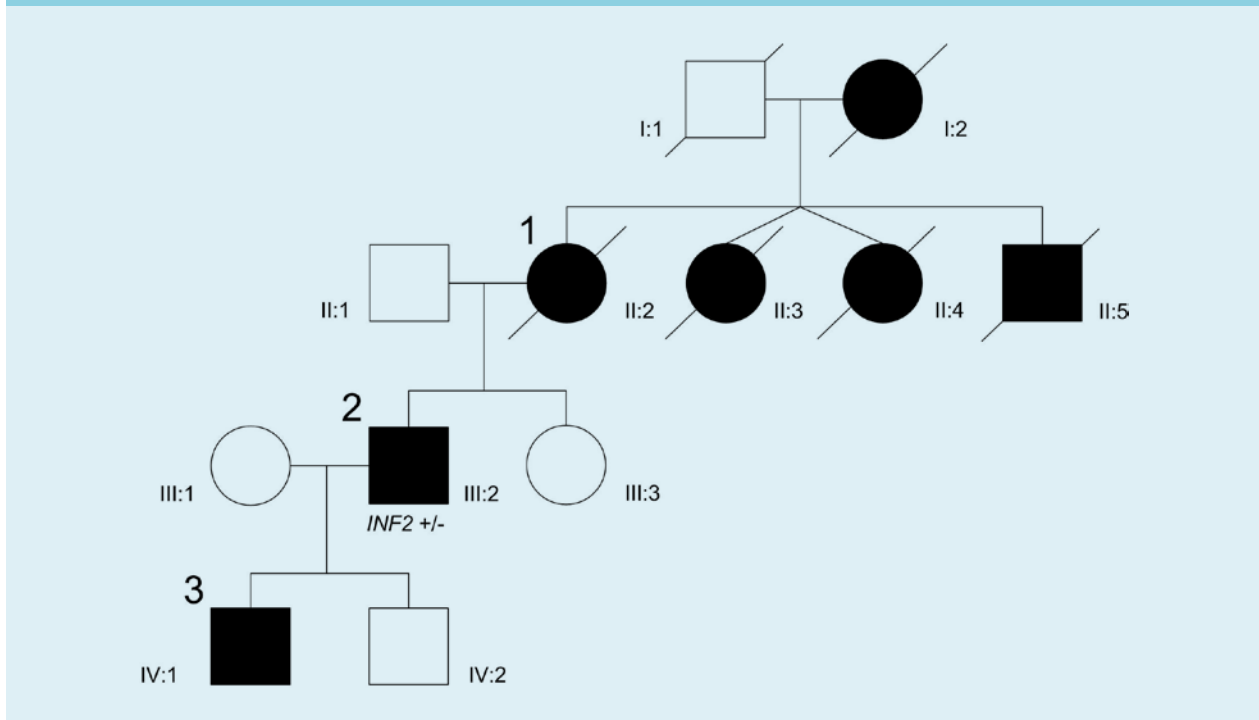
suspected. However, an audiogram was negative for hearing loss at age 23 years and Sanger sequencing showed no mutation in the *COL4A5* gene. Five years after presentation, ESKD developed. Two years later a postmortal kidney transplantation was performed. After 23 years, the graft is still functioning well.

Patient 3, the son of patient 2, was referred to our outpatient clinic at the age of 17 because of asymptomatic proteinuria (1.8 g/24 hours) without haematuria. His past medical history was negative. Physical examination was unremarkable. Serum albumin (44 g/l) and renal function (endogenous creatinine clearance 150 ml/min) were normal.

After the referral of patient 3, an X-linked disease was excluded and an autosomal dominant pattern of inheritance was suspected (figure 1). Subsequently, we reviewed the renal biopsy specimens from patient 2; slides from patient 1 were no longer available. Light microscopic examination showed primarily focal segmental sclerotic lesions (perihilar and tip lesions; figure 2a), review of the electron microscopic images revealed a sporadic irregular lamina densa with alternating thickening and thinning of portions of the glomerular basement membrane. Because

**Figure 1.** Pedigree of family with focal segmental glomerulosclerosis

The pedigree suggests an autosomal dominant pattern of inheritance. Patient 1 (III:2) was available for genetic analysis. In this patient a heterozygous missense mutation *c.529C>T* (*p.(Arg177Cys)*) was detected, denoted as *INF2 +/-*. Informed consent could not be obtained for further segregation analysis in this family. Squares represent males, circles females and diagonal lines deceased individuals. Affected individuals (FSGS/ renal disease) are indicated by solid black symbols



the lesions were sporadic, stored renal biopsy material was used for additional electron microscopic examination. These images all showed partial foot process effacement and some variation in glomerular basement membrane thickness as often seen in podocytopathies (figure 2). Based on this information, a diagnosis of familial FSGS was made. Additional diagnostic work-up comprised genetic screening for mutations causing autosomal dominant FSGS, according to the Dutch guideline for FSGS.<sup>1,2</sup> Analysis of the inverted formin 2 (*INF2*) gene revealed a heterozygous missense mutation c.529C>T (p.(Arg177Cys)) within the diaphanous inhibitory domain of the protein. This mutation is not reported in our in-house variant database containing exome sequencing variants detected in 5031 individuals, nor in ExAC, a large database collecting variants in over 60,000 exomes as proxy for variant allele frequencies in the general population.<sup>3</sup> The mutation has only been described once in the literature in a patient with FSGS.<sup>4</sup>

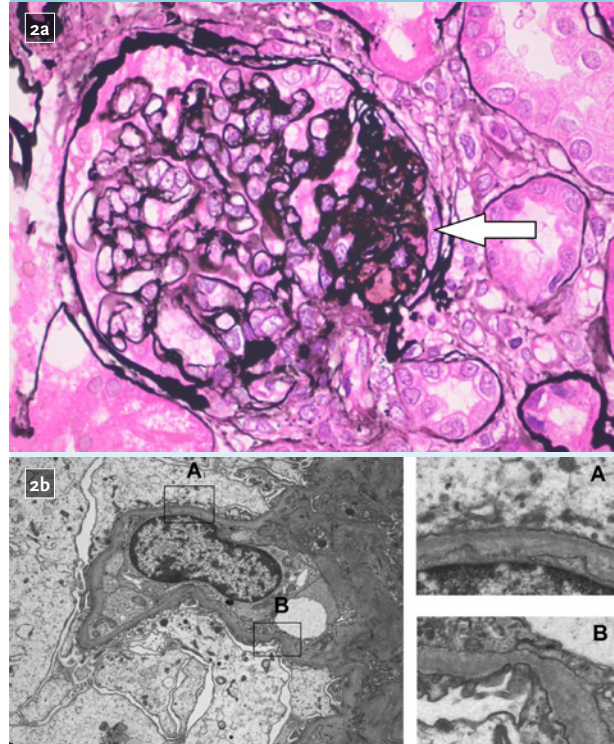
## DISCUSSION

Injury to podocytes plays a central role in the pathogenesis of FSGS and the aetiology is diverse, including mutations in genes encoding proteins that are crucial for the function of podocytes and the glomerular basement membrane.<sup>5</sup> The prevalence of gene mutations is especially high in children with sporadic steroid-resistant FSGS and in patients with a family history of FSGS.<sup>1</sup> Genetic testing should be considered in these patients, especially because results can affect the care of the patient and their family. In this case report we describe a family with a missense mutation in the *INF2* gene, initially thought to have Alport syndrome.

In 2010, the *INF2* gene was identified as a novel gene causing autosomal dominant FSGS.<sup>6</sup> *INF2* mutations are currently one of the most common causes of autosomal dominant FSGS (12-17%), but are rarely found in sporadic cases of FSGS.<sup>6-8</sup> Until now, up to 44 different mutations in *INF2* have been reported.<sup>4,7,9,16</sup> Patients with a mutation in the *INF2* gene typically present during adolescence or early adulthood with moderate to nephrotic range proteinuria, frequently progressing to ESKD in the third or fourth decade of life. Some patients present with microscopic haematuria or hypertension.<sup>6,8</sup> The clinical expression of *INF2* mutations is highly variable, even in individuals carrying the same genetic mutation.<sup>4,6,8,9,11-13</sup> It is hypothesised that mutations in other (FSGS) genes or environmental factors contribute to this variability.<sup>8</sup> *INF2* belongs to the formin family, a group of actin-binding proteins that regulate a variety of cytoskeleton-dependent cellular processes. *INF2* is widely expressed in podocytes and mutations in the *INF2* gene are

**Figure 2.** Renal biopsy findings patient 2 at age 18 years

2a. Light microscopy showing focal and segmental glomerulosclerosis, perihilar lesion (white arrow). (Magnification 400x). 2b. Electron microscopic examination showing prominent podocyte swelling and partial effacement of foot processes (A). The glomerular basement membrane (GBM) shows segmental irregularity of the lamina densa (B), but no splitting as seen in Alport disease. There is some variation in GBM thickness as often seen in podocytopathies (B) (Magnification 4000x)



thought to cause FSGS by dysregulation of the podocyte skeleton.<sup>17</sup> *INF2* proteins are also present in Schwann cells and play a role in myelination and myelin maintenance. In approximately 75% of patients with Charcot-Marie-Tooth (CMT) disease associated with FSGS, a mutation in *INF2* was identified.<sup>9</sup> CMT is a disorder affecting peripheral motor and sensory neurons which can cause progressive distal muscle weakness and atrophy, reduced tendon reflexes, sensorineural hearing loss, and deformities in feet and hands.<sup>18</sup> However, presenting symptoms can be discrete: some patients have only pes cavus as a clinical sign.<sup>9</sup> Therefore a careful clinical neurological examination and screening for hearing loss should be considered in all patients with FSGS, including patients with a negative family history, since *de novo* *INF2* mutations appear to be more common in patients with CMT disease with FSGS in comparison with isolated FSGS.<sup>9,14</sup> The reason why *INF2* mutations do not always lead to CMT may be related to the

location of the mutation. All mutations are located in the diaphanous inhibitory domain of the protein. In patients with FSGS and CMT, *INF2* mutations are mainly located in exon 2 and 3. However, there are also patients described in the literature with a mutation in exon 2 or 3 with isolated FSGS. Whether this is a phenotypic variation or lack of thorough phenotypic description is unclear. In contrast, mutations in exons 4 and 6 resulted in isolated FSGS.<sup>4,7-14</sup> This information is based on single publications reporting a mutation in *INF2*. However, there are no studies available with a clear genotype-phenotype correlation.

Initially, Alport syndrome was considered in this family. Alport-like lesions have been described previously in *INF2* mutations.<sup>12</sup> Lee et al. presented a 9-year-old Korean boy with a heterozygous c.658G>A (p.(Glu220Lys) mutation of the *INF2* gene. Renal biopsy revealed FSGS lesions with irregular podocyte foot processes and focal thinning and lamellation of the glomerular basement membrane, mimicking Alport syndrome. However, expression of  $\alpha 3$  and  $\alpha 5$  chains of type IV collagen was normal in immunofluorescence microscopy.

In conclusion, we present a family with a mutation in *INF2* initially thought to have Alport syndrome. Awareness of the heterogeneous phenotypic expression of a mutation in *INF2* is important and this case illustrates the importance of the history taking of the family, thorough examination of kidney biopsy, and importantly the combination with a molecular diagnosis.

## DISCLOSURES

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The authors declare no conflicts of interest.

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# A rare stress cardiomyopathy in a patient with Guillain-Barré syndrome

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## ABSTRACT

We report on a 39-year-old woman who was intubated because of progressive respiratory failure due to muscle weakness and mucous plugging because of Guillain-Barré syndrome. Shortly after uncomplicated intubation she developed hypotension and a profound tachycardia. The electrocardiogram showed sinus tachycardia with nonspecific ST-T segment changes. Echocardiography showed akinesia of the apex, septum and inferior left ventricular wall with an estimated left ventricular ejection fraction of 10%. It was concluded that the patient was suffering from takotsubo cardiomyopathy. Following treatment, she experienced a complete recovery. Takotsubo cardiomyopathy is a rare complication in Guillain-Barré syndrome; eight other cases have been reported in the literature.

## KEYWORDS

Guillain-Barré syndrome, Takotsubo cardiomyopathy, negative T waves, echocardiography, enoximone

## INTRODUCTION

Guillain-Barré syndrome (GBS) is an autoimmune disorder in which autoantibodies affect the peripheral nervous system. Autonomic dysfunction is a common and severe complication of GBS. Various clinical cardiovascular complications in these patients can occur, such as arrhythmias, wide blood pressure fluctuations and even cardiac arrest. Takotsubo cardiomyopathy is a rare complication in GBS. We present a patient with GBS who developed takotsubo cardiomyopathy shortly after admission to the ICU because of respiratory deterioration.

Clinicians should be aware of this potentially lethal complication and the therapeutic options.

## CASE REPORT

A 39-year-old female was admitted to the ICU with high fever and respiratory deterioration. She was recently diagnosed with GBS and suffered progressive muscle weakness despite a five-day course of immunoglobulins. Otherwise, she was healthy and was not taking any prescription drugs. On admission to the ICU her heart rate was 90 beats per minute, blood pressure 140/80 mmHg and a temperature of 39 °C.

One hour later intubation was necessary because of progressive respiratory failure due to muscle weakness and mucous plugging. To induce anaesthesia etomidate and rocuronium were used. Shortly after an uncomplicated intubation, a marked increase in heart rate (170-180 beats/min) was noticed and her blood pressure dropped to as low as 85/45 mmHg. Despite fluid loading her blood pressure stayed low. Because of recent immobilisation, the ICU physician on call decided to perform a computed tomography (CT) scan to rule out pulmonary embolism. It only showed atelectasis of the left lower lobe but no signs of pulmonary embolism. Pneumosepsis with fever, tachycardia and hypotension was then regarded as the most likely diagnosis. In the following hours antibiotics, additional fluids, norepinephrine and hydrocortisone were given. Blood pressure stayed low, tachycardia persisted and urine output ceased. The electrocardiogram revealed a sinus tachycardia with nonspecific ST-T segment changes. Because of the lack of improvement, concomitant heart failure was suspected. Laboratory results showed a normal creatine kinase (56 U/l, normal < 145 U/l), elevated troponin (0.198 µg/l, normal < 0.014 µg/l) and NT-pro-BNP (1391 pmol/l, normal < 15 pmol/l). Thyroid



function was normal. Echocardiography showed akinesia of the septum and inferior left ventricular wall, and apical akinesia which could not be explained by regional coronary hypoperfusion. The estimated left ventricular ejection fraction was only 10%. Revision of the CT scan showed no coronary calcification. In a young non-smoking female with no other risk factors for coronary artery disease and no coronary calcifications on the CT scan, coronary angiography was deemed to be an unnecessary risk with a low probability of finding coronary artery disease. Based on the typical findings on echocardiography it was concluded that the patient was suffering from takotsubo cardiomyopathy. Enoximone infusion was started to assist left ventricular contractility and to reduce afterload. This resulted in a gradual increase in blood pressure and return of diuresis. The next day, additional furosemide was given because of a positive fluid balance and signs of pulmonary congestion on the chest X-ray. In the next 48 hours, the enoximone was tapered off. Because her sinus tachycardia persisted metoprolol was introduced stepwise over three days to reduce the sympathetic tone and improve myocardial work/oxygen consumption ratio; the maintenance dose was 50 mg twice a day. Repeat follow-up echocardiography showed gradual normalisation of left ventricular performance in the next four months.

## DISCUSSION

We present a patient diagnosed with GBS who developed takotsubo cardiomyopathy. Massive catecholamine release due to the stressful event of rapid respiratory deterioration and haemodynamic instability after induction of anaesthesia most likely caused the development of takotsubo cardiomyopathy. In addition, norepinephrine infusion could have aggravated catecholamine excess which might have contributed to her myocardial dysfunction.

Takotsubo cardiomyopathy can be difficult to distinguish from the more common cardiovascular complications in GBS due to autonomic dysfunction such as tachyarrhythmias and bradyarrhythmias, blood pressure fluctuations, acute coronary syndromes and myocarditis.<sup>1</sup> The name Takotsubo refers to a Japanese jar used by fishermen to catch octopuses. The round bottom but tight neck resemble a picture often seen at echocardiography of the left ventricular wall, called apical ballooning. As more reports were published, it became clear that wall movement disorders were not restricted to the apex but could involve multiple segments of the left ventricular wall.<sup>2</sup> The Mayo Clinic proposed criteria for takotsubo cardiomyopathy: (A) transient hypokinesis, akinesia, or dyskinesia in the mid segments of the left ventricular wall with or without apical involvement; regional wall motion abnormalities

not related to one coronary supply region; frequently, but not always, a stressful trigger is present; (B) absence of obstructive coronary artery disease or angiographic evidence of plaque rupture; (C) new ECG abnormalities (ST elevation or T wave inversion) and (D) absence of myocarditis or pheochromocytoma.<sup>3</sup> Recently, Madias argued that these criteria have been outpaced by the rapidly accumulating clinical experience, and thus need to be replaced by more realistic sets of diagnostic rules.<sup>4</sup> The exact pathogenic mechanism of takotsubo cardiomyopathy is still controversial. The catecholamine hypothesis, commonly induced by physical and/or emotional stress, seems the best explanation.<sup>5</sup> Sympathetic excitation of the brain triggers the release of the catecholamines norepinephrine and epinephrine, resulting in hyperdynamic basal contraction and apical systolic dysfunction. Takotsubo cardiomyopathy is a specific type of a broad spectrum of reversible cardiomyopathies,<sup>6,7</sup> often stress related. The association of GBS with stress cardiomyopathy is not well understood. Dysregulation of autonomic tone with excessive sympathetic activation in GBS with elevated catecholamines levels has been reported.<sup>8,9</sup> Also in subarachnoid haemorrhage, traumatic brain injury and other neurological emergencies, excessive catecholamine release secondary to the primary insult has been reported, causing what is known as neurogenic stress cardiomyopathy.<sup>9</sup>

To our knowledge eight case reports associating GBS and takotsubo cardiomyopathy have been reported in the literature.<sup>10-17</sup> In our review of the literature ECG changes (negative T waves) were present in all but one patient. Takotsubo cardiomyopathy presented within the first week of hospital admission and three patients required mechanical ventilation. Treatment consisted of angiotensin-converting enzyme inhibitors (ACE),  $\beta$ -blockers, digoxin and diuretics (*table 1*). One patient needed dopamine infusion for cardiac support. Our patient was successfully treated with enoximone, a phosphodiesterase inhibitor which has positive inotropic as well as vasodilating properties and therefore reduces afterload. Later, she was given a  $\beta$ -blocker. Although the mode of action of  $\beta$ -blockers in heart failure is still incompletely understood, they are believed to reduce sympathetic tone and improve the myocardial work/oxygen consumption ratio, among other things.<sup>18</sup>

Treatment must be individualised for each patient. The use of norepinephrine in this light may be counterproductive. We chose to use enoximone instead of dopamine or dobutamine because of her tachycardia.

Beta-blockade seems sensible in case of dynamic mid-ventricular obstruction, represented by a high intraventricular pressure gradient on echocardiography.<sup>19</sup> ACE inhibition may be used to reduce afterload in haemodynamically stable patients.

**Table 1.** Clinical data on the eight cases reported in the literature

Reference	Sex	Diagnosis on day	Mechanical ventilation	Negative T-waves ECG	Therapy
Bernstein <sup>11</sup>	Female	3	Yes	Yes	None
Finkelstein <sup>10</sup>	Male	6	No	Yes	β-blocker, ACE
Yoshii <sup>12</sup>	Female	1	Yes	Yes	Dopamine
Palazzuoli <sup>13</sup>	Male	3	No	Yes	ACE, furosemide, digoxin
Agarwal <sup>14</sup>	Female	7	No	Yes	Diuretics
Martins <sup>16</sup>	Female	5	Yes	Yes	β-blocker, ACE
Rousseff <sup>15</sup>	Female	1	No	Yes	Nitroglycerin, ACE, diuretics
Quick <sup>17</sup>	Female	?	No	ST elevations	?

Takotsubo cardiomyopathy should be considered in GBS when ECG abnormalities are present. The easiest way to do this is to perform echocardiography. According to the Mayo Clinic guidelines, acute coronary disease and myocarditis should be ruled out when suspected. In our case coronary artery disease was regarded as unlikely because of her young age, absence of risk factors for coronary artery disease and normal creatine kinase. Furthermore, electrocardiography and cardiac ultrasound showed abnormalities that could not be explained by regional coronary hypoperfusion/ischaemia while retrospective analysis of a CT scan showed no coronary calcifications.

## CONCLUSION

We report a case of severe takotsubo cardiomyopathy, which required prompt management in terms of diagnostics and treatment. Takotsubo cardiomyopathy is a rare complication during the acute phase of GBS and must be distinguished from autonomic dysfunction. Especially ECG abnormalities such as negative T waves should alert the clinician to the presence of takotsubo cardiomyopathy.

## DISCLOSURES

The authors declare no conflict of interest. No funding or financial support was received.

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# A lethal case of the dapsonе hypersensitivity syndrome involving the myocardium

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## ABSTRACT

In the Netherlands dapsonе is used for the treatment of dermatitis herpetiformis, leprosy and *Pneumocystis jiroveci* pneumonia and prophylaxis in case of cotrimoxazole allergy. An idiosyncratic drug reaction, known as the dapsonе hypersensitivity syndrome (DHS), appears in about 0.5-3.6% of persons treated with dapsonе. DHS can be associated with fever, rash and systemic involvement. We present a 35-year-old woman who developed severe DHS seven weeks after starting dapsonе. Six weeks after being discharged in a good clinical condition she died from fulminant myocarditis, 11 weeks after the first DHS symptoms and the discontinuation of dapsonе.

## KEYWORDS

Dapsonе, hypersensitivity, HLA-B\*13:01, myocarditis, eosinophilic

## INTRODUCTION

In the Netherlands, dapsonе (4,4'-diaminodiphenylsulfone) is used for the treatment of dermatitis herpetiformis, leprosy and *Pneumocystis jiroveci* pneumonia and prophylaxis in case of cotrimoxazole allergy. Dapsonе has both antibiotic and anti-inflammatory effects. Common side effects of dapsonе are dose-related methaemoglobinemia and haemolytic anaemia.<sup>1</sup> Moreover, an idiosyncratic drug reaction, known as the dapsonе hypersensitivity syndrome (DHS), appears in about 0.5-3.6% of persons treated with dapsonе,<sup>2</sup> ranging from

### What was known on this topic?

Dapsonе hypersensitivity syndrome is an idiosyncratic drug reaction, which occurs in 0.5-3.6% of patients treated with dapsonе. A clinical triad of fever, rash and systemic involvement, mostly of the liver and the haematological system, characterises this syndrome. The mortality is about 9.9%, which can be prevented by HLA testing for HLA-B\*13:01 and withholding dapsonе in patients who test positive.

### What does this add?

Although dapsonе hypersensitivity syndrome hepatitis, cholangitis, pneumonitis, colitis and related thyroiditis have been previously reported, to our knowledge the present report is the first to describe a lethal case of dapsonе hypersensitivity syndrome due to myocarditis with proven HLA B\*13:01 positivity.

mild cutaneous symptoms to severe life-threatening organ failure.<sup>3</sup> The diagnosis of DHS requires at least two of the following: fever, lymphadenopathy, generalised rash and hepatitis.<sup>2</sup> DHS usually occurs four to six weeks after the start of intake of dapsonе.<sup>4</sup> The mortality of DHS is approximately 9.9%.<sup>2</sup> A genome-wide association study found an association between HLA-B\*13:01 and the development of DHS (OR 20.53).<sup>4</sup>

We present a 35-year-old woman with leprosy, who developed severe DHS. At first, she recovered and left the hospital in a good condition. Nonetheless, she died six weeks later from fulminant myocarditis, 11 weeks after the first DHS symptoms appeared.

## CASE REPORT

A 35-year-old female from Thailand was diagnosed with leprosy and treated with multidrug therapy (dapsone 100 mg/day, clofazimine 50 mg/day and rifampicin 600 mg monthly) after excluding G6PD deficiency. She was not using any other medications.

Seven weeks after starting therapy she was admitted via the emergency ward for epigastric and skin pain, vomiting, myalgia and dark coloured urine. Body temperature was 38 °C, blood pressure 104/51 mmHg and heart rate 94 beats/min. On admission she rapidly developed a livid papular skin rash, icteric sclerae and generalised lymphadenopathy (*figure 1*). Dapsone and rifampicin were stopped immediately. Laboratory findings showed, among other things, COOMBS-negative haemolytic anaemia and aberrant serum liver tests compatible with fulminant hepatitis, prolonged prothrombin time, and a low albumin. There was no eosinophilia.

Serological tests for hepatitis A, B, C and E and tests for Epstein-Barr virus, cytomegalovirus, herpes-simplex virus, human immunodeficiency virus, lues, the antistreptolysin titre, blood cultures, repeated ANA, ANCA and anti-double stranded DNA were negative, making an autoimmune disease such as systemic lupus erythematosus unlikely. The diagnosis of DHS was made and HLA-typing disclosed HLA-B\*13:01 positivity in our patient. Initially, prednisolone 60 mg/day was started,<sup>5</sup> but due to her clinical deterioration this was increased to 1000 mg/day, with good response. After three days the prednisolone was lowered to 100 mg/day, and gradually diminished to 60 mg/day five weeks later, when she was discharged. Besides the prednisolone our patient was only using insulin at discharge. In the outpatient clinic she showed further improvement.

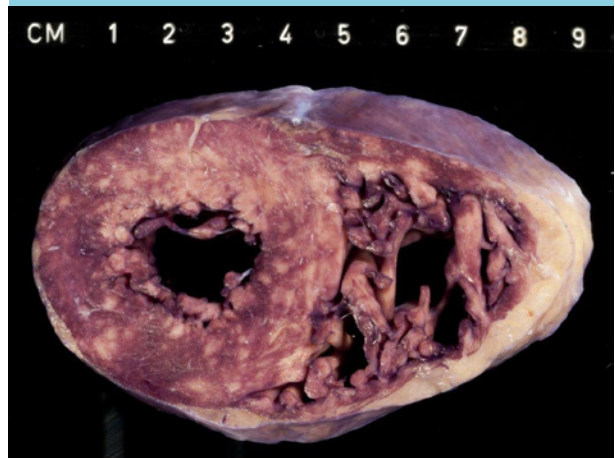
Six weeks after discharge, with a current prednisone use of 45 mg/ day, she suddenly became nauseous. The next morning she developed dyspnoea and lost consciousness. Despite CPR she died on arrival to the hospital. During her initial admission no electrocardiograms were taken because there were no signs of cardiac disease.

Autopsy showed signs of acute bilateral cardiac decompensation. The heart was moderately dilated. The staining of a biventricular slice for lactate dehydrogenase showed a mottled pattern of necrosis, suggestive of myocarditis (*figure 2*). Microscopy showed massive biventricular myocarditis with extensive myocyte necrosis. The inflammatory infiltrate consisted of lymphocytes, macrophages and large numbers of eosinophilic granulocytes admixed with several multinucleated giant cells of the Langerhans type (CD68+, HHF 35-). No granuloma formation was seen (*figure 3*). These findings

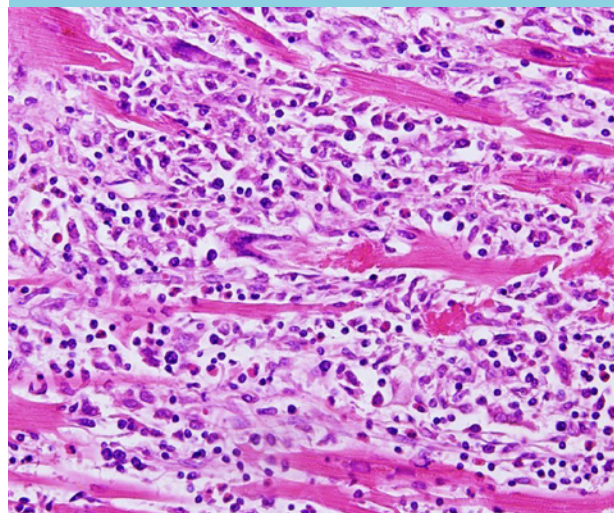
**Figure 1.** Clinical presentation (pictures published with permission from the family of the deceased patient)



**Figure 2.** Biventricular slice of the heart, stained for lactate dehydrogenase. Intact myocytes are purple, damaged cardiac tissue remains pale. The mottled pattern is suggestive of myocarditis



**Figure 3.** High-magnification histological slide of the left ventricle showing a heterogeneous inflammatory infiltrate with lymphocytes, plasma cells and admixed giant cells and eosinophilic granulocytes. In the centre of the image there is obvious necrosis of a cardiomyocyte



were compatible with severe myocarditis (see discussion) and did not fit other infectious causes of her death. Leprosy could not be confirmed during autopsy.

## DISCUSSION

Here we describe an HLA-B\*13:01 positive patient who most likely died of DHS-related eosinophilic myocarditis after apparent recovery. Although discontinued 11 weeks before her death, dapsone is known to cause eosinophilia and eosinophilic myocarditis.<sup>2,6,7</sup>

Zhang et al. reported a strong association of HLA-B\*13:01 with DHS (OR 20.5).<sup>4</sup> With a sensitivity of 85.5% and a specificity of 85.7%, HLA-B\*13:01 is a powerful predictor for DHS.<sup>4</sup> The association between DHS and HLA-B\*13:01 was published during the admission of our patient. These findings, together with the common finding of lymphadenopathy, suggest a central role for dysregulated hyper-activated T cells in the pathogenesis of DHS.

Discontinuation of the offensive drug is the most crucial action in a patient with dapsone hypersensitivity syndrome.<sup>5</sup> If required, supportive therapy and glucocorticoids, at a dose of 1 mg/kg body weight, is recommended.<sup>5</sup> However, this treatment schedule did not decrease the symptoms in our patient nor did it improve the laboratory tests. Therefore, high-dose intravenous prednisone (1000 mg) treatment was given for three days, followed by high-dose oral prednisone, a treatment algorithm that is commonly used for patients with a major flare of systemic autoimmune disease. Then our patient gradually recovered and could be discharged from hospital, five weeks after her admission. Her sudden death, six weeks later, was in this sense unexpected. Although DHS myocarditis has been previously reported,<sup>2,6</sup> to our knowledge, no reports have been published on sudden death due to myocarditis in a patient with apparent improvement of DHS. We would suggest to take an echocardiogram when the diagnosis of dapsone hypersensitivity syndrome is suspected. Although clinical signs may be absent, echocardiogram might show signs which can also be used for follow-up.

Myocarditis can be subdivided into several histopathological entities, depending on the clinical course or the constitution of the inflammatory infiltrate. In this patient, the inflammatory infiltrate showed numerous eosinophilic granulocytes and presence of giant cells of the Langhans type, prompting a diagnosis of either hypersensitivity/eosinophilic or giant cell myocarditis.

Hypersensitivity/eosinophilic myocarditis is characterised by tissue eosinophilia in response to an allergen, typically medication.<sup>8</sup> Although often asymptomatic, a specific subset (necrotising eosinophilic myocarditis) may

follow a fulminant course and progression to sudden unexpected death.<sup>9</sup> Necrotising eosinophilic myocarditis is histologically characterised by a diffuse inflammatory infiltrate with predominant eosinophils, extensive necrosis of the myocardium and sometimes giant cells, as was the case in our patient.

Different drugs have been connected with eosinophilic myocarditis, including sulphonamides.<sup>7</sup> We found no reports for rifampicin, clofazimine or prednisolone (used by our patient) as offensive drugs in relation to eosinophilic myocarditis. Prednisolone is used as effective therapeutic modality for eosinophilic myocarditis.<sup>10</sup> Eosinophilic myocarditis is also found in patients who do not use any medication.<sup>9</sup>

The diagnosis of leprosy is made mainly on clinical grounds. In our patient it was based on the presence of anaesthetic skin lesions, thickening of peripheral nerves and acid-fast bacilli in slit-skin smears. However, correct measurement of peripheral nerve thickness is hard and the quality of skin smears is often poor.<sup>11</sup> Ziehl-Neelsen staining has high specificity and low sensitivity.<sup>12</sup> Antimycobacterial therapy can affect the outcome of Ziehl-Neelsen staining. The acid-fast bacilli became phagocytised by the macrophages and fragments of the bacilli are left in the lesions, which cannot be identified by a Ziehl-Neelsen stain.<sup>12</sup> This possibly accounted for the unconfirmed diagnosis of leprosy during autopsy.

In the study by Zhang et al. the absence of HLA-B\*13:01 was associated with a reduction in the risk of developing DHS from 1.4 to 0.2%.<sup>4</sup> In the Netherlands testing for HLA-B\*13:01 costs € 125.10. Since HLA-B\*13:01 is mostly present in Chinese, Japanese, Indians and Southeast Asian patients we suggest to test high-risk groups for both G6PD deficiency and HLA-B\*13:01 before starting dapsone to prevent the potentially life-threatening condition of DHS.<sup>4</sup>

## DISCLOSURES

No conflicts of interest for all authors. No funding. No prior abstract publication/presentation.

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# An 88-year-old woman with an ulcerous tumour on the leg

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## CASE REPORT

An 88-year-old woman was referred because of rapidly growing swellings on both legs including one large ulcerous tumour on the right leg. Two weeks prior to admission, the swellings and ulcer were growing rapidly, resulting in oedematous and painful legs. The patient's medical history was uneventful. She only used hydrochlorothiazide for hypertension.

Physical examination evidenced multiple red to bluish tumours on both legs with one large ulcerated tumour with a diameter of 6 cm medially on the right lower leg (*figure 1a* and *1b*). No additional abnormalities were found except one enlarged inguinal node on the right side. Laboratory results revealed: haemoglobin 7.6 g/dl, thrombocytes  $475 \times 10^9/l$ , lactate dehydrogenase 1080 U/l, gamma-glutamyltransferase 84 U/l, erythrocyte sedimentation rate 46 mm/h. The other results were normal. Computed tomography of the thorax and abdomen only showed inguinal lymphadenopathy on the right side.

## WHAT IS YOUR DIAGNOSIS?

See page 94 for the answer to this photo quiz.

**Figure 1.** 1a: Ulcerated lesion on the right lower leg. 1b: Red to bluish tumour on the right upper leg



## ANSWER TO PHOTO QUIZ (PAGE 93)

## AN 88-YEAR-OLD WOMAN WITH AN ULCEROUS TUMOUR ON THE LEG

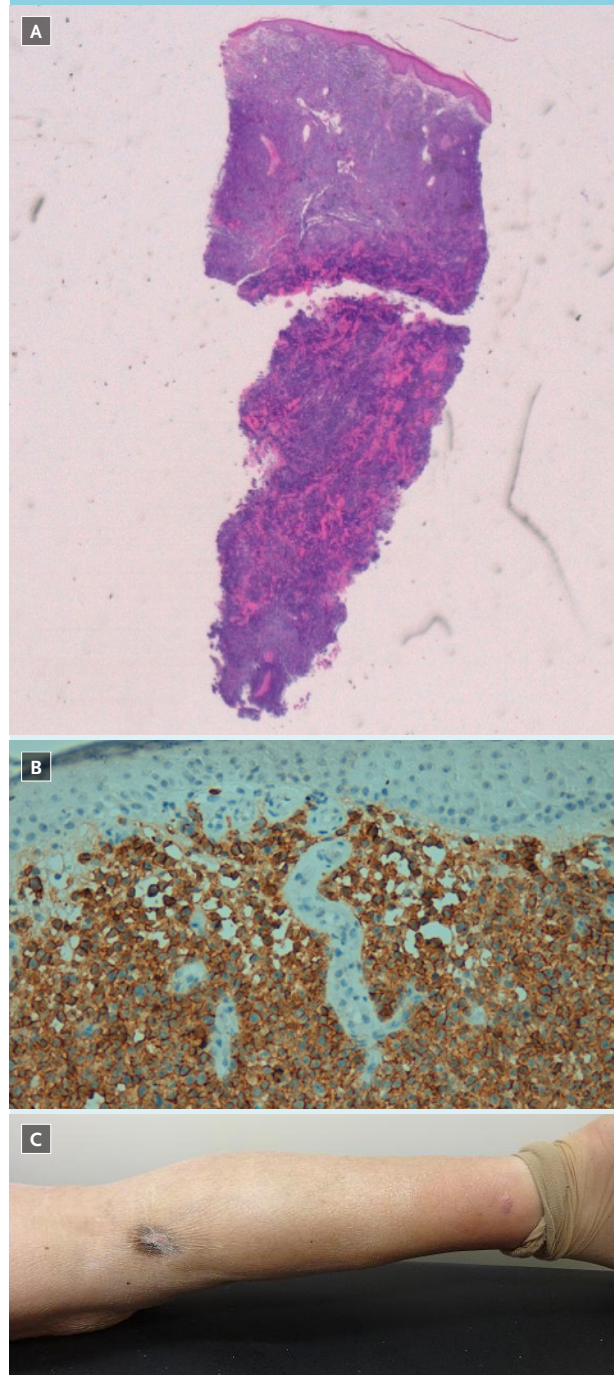
## DIAGNOSIS

Histopathological examination showed a diffuse proliferation of polymorphic centroblasts infiltrating the whole dermis. There was no infiltration of the epidermis, and no angiocentric or intravascular growth pattern. On immunohistochemistry, the cells were positive for CD20, CD79a, CD5, MUM-1, BCL2 and BCL6 and negative for CD3 and CD10. The diagnosis of primary cutaneous diffuse large-B-cell lymphoma, leg type (PCDLBCL-LT), *T<sub>1b</sub>N<sub>1</sub>M<sub>0</sub>*,<sup>1</sup> was clinically, histologically, and immunohistochemistry confirmed (*figure 2a* and *2b*). Additional bone marrow aspiration and biopsy were normal. This patient was in a good physical condition (Karnofsky score 90%), therefore full-dose rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone were given, once every 21 days for six cycles (R-CHOP21). No complications were observed. The skin lesions on both legs of our patient disappeared rapidly after initiation of the R-CHOP treatment (*figure 2c*). After one year of follow-up the patient is in complete remission and no recurrence of the skin lesions has appeared.

PCDLBCL-LT is one of the three main types of primary cutaneous B-cell lymphomas (PCBCL). PCBCLs are lymphomas with a B-cell phenotype that present in the skin without any evidence of systemic or extracutaneous disease at first appearance. In a study of 1905 patients with primary cutaneous lymphoma, PCDLBCL-LT corresponds to 4.5% of all cutaneous lymphomas. PCDLBCL-LT accounted for 20% of all PCBCLs.<sup>2</sup>

As the name suggests, PCDLBCL-LT mainly involves the lower legs of predominantly older women (female:male ratio is 2-4:1) with a median age of 70-75 years. The rest of the body is involved in 10-20% of the cases. Patients characteristically present with one or multiple rapidly growing bluish-reddish nodules or tumours on one or both legs. Some of these nodules/tumours can be ulcerated. By definition, these lymphomas are limited to the skin at initial presentation; however, they can spread to extracutaneous sites too.<sup>3</sup> These sites can include the lymph nodes, bone marrow, and the central nervous system. Several studies report a five-year survival rate of 20-60% with a median of 50%. Presence of multiple skin lesions at first presentation is a negative prognostic factor. A recent retrospective multicentre study in France reported an improvement in the survival of PCDLBCL-LT with three-year survival of 74% and five-year survival of 66%. These rates were 80% and 74% respectively in

**Figure 2.** *2a:* Skin biopsy (HE stain, low power view): on top an intact epidermis, the dermis is massively infiltrated by a diffuse growing tumour proliferation. *2b:* CD20 immunohistochemical stain (high power view): large tumour cells (centroblasts) positive for CD20. There is no infiltration of the epidermis which is typical for primary cutaneous B-cell tumours. *2c:* Disappearance of the ulcerated lesion on the right lower leg (*figure 1a*) after the R-CHOP treatment





those patients who received immunochemotherapy with rituximab.<sup>4</sup>

PCBCLs with predominantly large cells are either PCDLBCL-LT or primary cutaneous follicle centre lymphomas (PCFCL). It is important to distinguish these two types of B-cell cutaneous lymphomas since epidemiology, histopathology, treatment, and prognosis are completely different. Histologically PCDLBCL-LT consists mainly of centroblast, large B-cells and immunoblasts while the follicle centre lymphoma consists mainly of centrocyte cells. In PCDLBCL-LT, BCL-2, FOXP1, IgM and MUM-1 are specifically positive whereas they are negative in the primary cutaneous follicle centre lymphomas. In PCFCL there are networks of follicular dendritic cells; these are lacking in PCDLBCL-LT.<sup>3,5</sup>

It is important to recognise and systemically treat PCDLBCL-LT since in general these types of diffuse large-B-cell lymphoma will have an aggressive biological behaviour in contrast to the PCFCL. Guidelines recommend to treat PCDLBCL-LT with systemic immunochemotherapy, consisting of six cycles of R-CHOP

with or without radiation therapy.<sup>3,5</sup> As there are no randomised controlled trials available in the literature, treatment recommendations for PCDLBCL-LT are mainly based on retrospective and anecdotal studies and on institutional experience.

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# A 47-year-old woman with fever and periorbital oedema

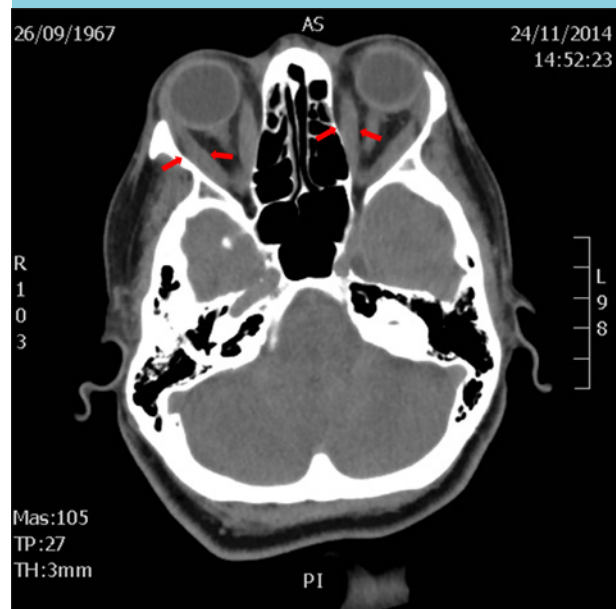
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## CASE REPORT

A 47-year-old Caucasian woman with a history of migraine was referred to the hospital with fever, headache and bilateral periorbital oedema. The symptoms started nine days earlier. Physical examination revealed a temperature of 38.5 °C, heart rate of 101 beats/min and a normal blood pressure. On neurological examination, there were signs of photophobia, normal light reactive pupil reflexes and neck stiffness. Kernig and Brudzinski signs were negative. There were no signs of muscle weakness in the upper or lower limbs. She had bilateral proptosis with chemosis and eyelid oedema without scleral injection. Laboratory investigations showed leukocytes of  $10.9 \times 10^9/l$  with 18.1% eosinophils (absolute eosinophil count  $1.98 \times 10^9/l$ ), C-reactive protein 34 mg/l, creatine kinase levels of 239 U/l (normal < 145 U/l) and mildly elevated transaminases. Cerebral spinal fluid examination yielded 3 leukocytes/ $\mu$ l and normal protein and glucose levels. Contrast-enhanced computed tomography of the brain confirmed the absence of central neurological lesions but showed bilateral oedema of the ocular muscles, most pronounced in the lateral and medial rectus muscle with diameters of 7 to 8 mm (figure 1, arrows). Four days later, her 22-year-old son and 19-year-old daughter were hospitalised as well with similar complaints and findings.

**Figure 1.** CT scan of the brain showing bilateral oedema of ocular muscles (arrows)



## WHAT IS YOUR DIAGNOSIS?

See page 97 for the answer to this photo quiz.

## ANSWER TO PHOTO QUIZ (PAGE 96)

## A 47-YEAR-OLD WOMAN WITH FEVER AND PERIORBITAL OEDEMA

## DIAGNOSIS

The clustering of cases in one family led to a detailed re-examination, including food history. Nine days before the onset of symptoms, the patient consumed slowly roasted wild boar fillet at a local restaurant, together with her siblings. The combination of eosinophilia, elevated creatinine kinase levels and periorbital oedema led to the suspicion of trichinellosis. This was confirmed by biopsy of the quadriceps muscle in the index patient, revealing larvae with morphological features of *Trichinella* species, subsequently identified as *T. spiralis* by polymerase chain reaction (figure 2).

Trichinellosis is a rare parasitic zoonosis caused by the ingestion of raw or undercooked meat containing *Trichinella* larvae. The parasite infects both domestic animals, such as pigs, and wild animals including bears, moose and wild boar and has a worldwide distribution.<sup>1</sup> Humans become infected when eating undercooked meat from domestic pigs or game contaminated with encysted *Trichinella* larvae.<sup>2</sup> Sexually matured adult worms produce larvae in the small intestine, which subsequently invade muscle tissue. Two clinical stages are described: an initial intestinal stage with nausea or diarrhoea and a later muscular stage with periorbital oedema, myalgia or muscle weakness as major symptoms. The disease is mostly self-limiting: the adult worms live on average four weeks and the muscular phase is the end-stage of the infection. However, major complications may arise during invasion including myocarditis, encephalitis and pulmonary superinfection.

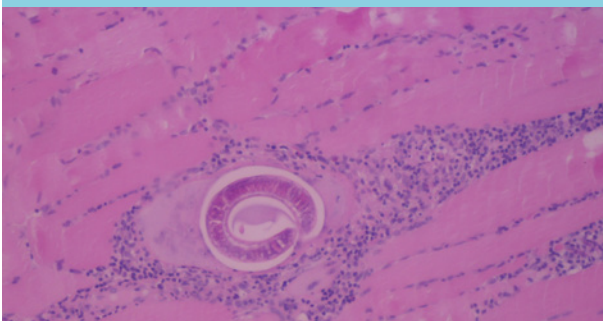
Our patient was diagnosed with trichinellosis in the second clinical stage. Photophobia and periorbital oedema are related to ocular muscle larval invasion rather than neurological pathology. The diagnosis was confirmed by muscle biopsy and by serology at the National Reference Laboratory for Infectious and Tropical Diseases at the Antwerp Institute of Tropical Medicine. The treatment

consisted of oral mebendazole 900 mg for three days, followed by 1500 mg for ten days, with co-administration of methylprednisolone 32 mg once daily. The patient recovered quickly with complete normalisation of the creatine kinase and eosinophil count after two weeks. The need for treatment, choice and dosage of anti-parasitic drugs after the first disease stage is, however, not firmly established.<sup>2,3</sup>

The incidence rate of trichinellosis has been decreasing in the European Union (EU) during the last decade as a consequence of strict implementation of EU regulations in slaughterhouses.<sup>4</sup> The prevalence is higher in Eastern European countries mainly related to consumption of domestic pork. In Western Europe the infection is rare and mostly a consequence of consumption of game meat, hunted locally or imported from countries with a high trichinellosis prevalence in wild animals. As a result of this outbreak in which eventually 16 patients were diagnosed with trichinellosis, the Belgian Federal Agency for the Safety of the Food Chain conducted an investigation focusing on the supply chain of the imported wild boar meat, leading to one certified supplier in North-Eastern Spain. The recalled batches of meat from this supplier were examined as well but none of these samples contained *Trichinella* larvae, probably because of a delay of several weeks between the consumption of contaminated meat and the start of the investigation. No irregularities were reported in the suspected slaughterhouse. No other outbreaks were reported in the EU at the same time.

This case illustrates an unusual diagnosis in a patient presenting with inflammation and neurological symptoms. It may help to increase the clinician's awareness of the features suggestive of trichinellosis, particularly in clustered cases. A detailed food history is essential to determine the source of an outbreak. Despite EU measures, in 2015, trichinellosis remains a threat to food chain safety in Western Europe, mainly due to consumption of contaminated game meat.

**Figure 2.** Muscle biopsy from the patient showing lymphohistiocytosis and a *Trichinella* larva



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# Training in acute medicine in a peripheral teaching hospital in the Netherlands

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Some years ago, there was a lot of discussion about a shortage of emergency physicians in the Accident & Emergency (A&E) departments and the poor training given to house doctors fulfilling this function in the evening and night hours.<sup>1</sup>

The report 'Spoedeisende hulp: vanuit een stevige basis' ('A&E Department: On a firm basis') of the workgroup 'Kwaliteitsindeling SEH' ('Quality Classification in the A&E Department') from 2009 describes the minimal mandatory quality requirements to run an A&E department. The Ministry of Health then concluded that no single Dutch A&E department completely fulfilled these criteria. It was agreed that the Inspection for Health Care should introduce more stringent control on the presence of properly trained personnel. Training in acute medicine in accordance with the ABCDE (Airway-Breathing-Circulation-Disability-Exposure) systematics became mandatory. Such a training program has been described for the interns of the department of internal medicine in the Academic Medical Centre Amsterdam<sup>2</sup> and a comparable course was set up in the Leiden University Medical Centre by the A&E department.<sup>3</sup>

## Training in acute medicine, a task for the ICU

In a recent article in Intensive Care Medicine<sup>4</sup> it was shown that, in the Netherlands, contrary to earlier non-evidence based reports, care on smaller ICUs was as good as care on medium sized and larger ICUs; this includes the sickest and ventilated patients. The 'smaller' ICUs (with a median size of seven ventilation beds, which is not small at all with respect to the rest of the world) have a number of other important tasks next to their core business, intensive care treatment. They run the Medical Emergency Team (MET) thus decreasing the number of in-hospital resuscitations, ICU admissions, ICU length of stay and hospital mortality. Furthermore, they manage A&E admissions in patients who are unstable or critically ill and finally, they organise

the training of nurses in emergency medicine and intensive care medicine. Consequently, it seemed logical to let the ICU department organise the training in acute medicine for all interns being engaged at any moment on the A&E department. In the Slotervaart Hospital we developed an introductory course which was mandatory for all starting interns. A similar course in the Amsterdam region, organised by the ICU team, is being offered by the Spaarne Hospital in Hoofddorp.

Our course was implemented by the intensivists in 2010, and by now has been given 21 times (four times per year), in cooperation with different hospital consultants. Each participant receives the course book 'Medische spoedsituaties' ('Medical Emergencies') (T.J. Olgers, M. Oosterloo, J.C. ter Maaten) and a pre-test with 50 corresponding multiple choice questions six weeks before the start of the course. A literature study and pre-test have to be done at home before the start of the course and pre-tests are collected and corrected at the beginning of the course. The course itself consists of three days of training, with three hours of theoretical lessons per half-day session. At the end of each session two patient cases are managed and discussed with an intensivist in small groups of three to four participants to put the acquired knowledge into practice. At the end of the third day each participant concludes with a practical exam by managing one virtual critically ill A&E patient case on his own. In week two all participants follow a resuscitation training on an Advanced Life Support simulator under supervision of a certified trainer. After seven days of self-study a final theoretical exam, consisting of 50 multiple choice questions, is completed. This exam is evaluated and discussed with all participants in week three. The total number of lessons comprises 31 hours and passing both (practical and theoretical) exams is mandatory to obtain the course certificate. *Table 1* shows the course program with average rating by the participants.

**Table 1:** Course program 'Introduction Acute Medicine' with average rating by participants (out of last five introduction courses)

Course program 'Introduction Acute Medicine'	Duration	Instructor	Rating*
<b>Day 1</b>			
Introduction course	30 min	Intensivist	4.10
Admission policy & treatment restrictions	30 min	Intensivist	3.92
Resuscitation	45 min	Intensivist	3.97
Sepsis and multi organ failure	60 min	Intensivist	4.25
Blood gas analysis	30 min	Intensivist	4.42
Interactive patient cases 1+2	60 min	Intensivist	4.46
Electronic prescription system	30 min	Pharmacologist	3.69
(Auto)intoxications	45 min	Intensivist	4.52
Report code abuse of the elderly	30 min	P.A. Geriatrics	3.70
Management of massive blood loss	45 min	Intensivist	4.38
Acute abdomen	60 min	Surgeon	4.29
Interactive patient cases 3+4	60 min	Intensivists	4.49
<b>Day 2</b>			
Introduction			
Electronic patient data system	30 min	IT-specialist	3.25
Medical emergency team (MET)	30 min	Intensivist	3.85
Report code child abuse	30 min	Paediatrician	3.86
Delirium and approach of the geriatric patient	30 min	Geriatrician	3.86
Introduction medical library	15 min	Librarian	3.40
Acute respiratory insufficiency	45 min	Intensivist	4.16
Interactive patient cases 5+6	60 min	Intensivists	4.52
Pain therapy	30 min	Intensivist	4.42
Pre-analysis and point of care	30 min	Biochemist	3.56
Haemodynamics & shock	45 min	Intensivist	4.27
Advanced Paediatric Life Support	90 min	Paediatrician	4.30
Interactive patient cases 7+8	60 min	Intensivists	4.57
<b>Day 3</b>			
Donation procedure	30 min	Donation coordinator	3.82
Acute coronary syndrome & rhythm disturbances	60 min	Cardiologist	3.62
Acute neurology	90 min	Neurologist	4.04
Interactive patient cases 9+10	60 min	Intensivists	4.45
Antibiotics	90 min	Microbiologist	3.95
Electrolyte disturbances	90 min	Intensivist	4.29
Practical exam (Interactive patient cases 11-15)	90 min	Intensivists	4.39

Week 2			
Introduction advanced life support (practical training)	90 min	ALS instructor	#
Advanced life support (practical training)	90 min	ALS instructor	#
Written exam	90 min	Intensivist	#
Week 3			
Evaluation written exam	60 min	Intensivist	#
<b>Course total</b>	<b>31 h</b>		

\*(average): unsatisfactory = 1; moderate = 2; satisfactory = 3; good = 4; excellent = 5; # not rated.

### Aim of the course

This course aims at making the starting interns confident with all facets of acute medicine and to teach them the ABCDE principle, internalise it and put it into practice.

In our view, the advantage of this custom-made course, contrary to a national (ATLS) two-day course, is to familiarise the interns with the local consultants and to make them proficient in the hospital guidelines. This, in our opinion, lowers the threshold to consult consultants and reduces the need for calling in the Medical Emergency Team (MET). This increases effectivity and safety of the emergency patient admission process. More specifically, the course is longer than the ATLS course and includes non-trauma cases, thus offering a broader and more in depth knowledge to the participants.

Each course is being evaluated and the participants are enthusiastic about the small study groups, the low threshold atmosphere and the practical design of the course. Since 2013 evaluation is done via an online system (SurveyMonkey). The course corresponds well with the theoretical knowledge acquainted during the study, being consistently rated as 'precisely corresponding with present knowledge' of the participants.

In the future, interactive patient cases, rated as the most valuable part of the course, will be simulated in a real-life A&E environment, with a simulation mannequin and full monitoring. We will evaluate the effect of this measure after one year.

Naturally a four-day course does not make a starting intern fully competent in acute medicine. For this reason it is important that the acquired theoretical knowledge is put into practice and the intern is supervised when dealing with acutely ill patients. This supervision in our hospital is offered by the admitting consultant, and in critically ill patients by the intensivist who is readily available for consultation.

The intensivist organises the course and indeed carries out most of the lectures, which corresponds well with his central role in caring for the acutely ill patient. This creates

uniformity within training and patient treatment. It gives the ICU department a central role in the hospital structure.

### CONCLUSION

The four-day introductory course Acute Medicine is being received very positively by the participants. By involving the hospital consultants in this course, an effective and safe approach to treating the acutely ill patient is being created. In this way, this course fulfils the requirements of the Ministry of Health.

### REFERENCES

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4. Kluge GH, Brinkman S, van Berkel G, et al. The association between ICU level of care and mortality in the Netherlands. *Intensive Care Med*. 2015;41:304-11.