Netherlands
The Journal of Medicine

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Editorial office
Academic Medical Centre,
Department of Medicine (F-4)
Meibergdreef 9
1105 AZ Amsterdam
The Netherlands
Tel.: +31 (0)20-566 21 71
Fax: +31 (0)20-691 96 58
E-mail: m.m.levi@amc.uva.nl
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Biosis database; embase/excerpta medica; index medicus (medline) science citation index, science citation index expanded, isi alerting services, medical documentation services, current contents/clinical medicine, PubMed.

Contents

ISSN: 0300-2977

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EDITORIAL

Impact of articles reflected by the journal's impact factor

M. Levi

Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands,
e-mail: m.m.levi@amc.uva.nl

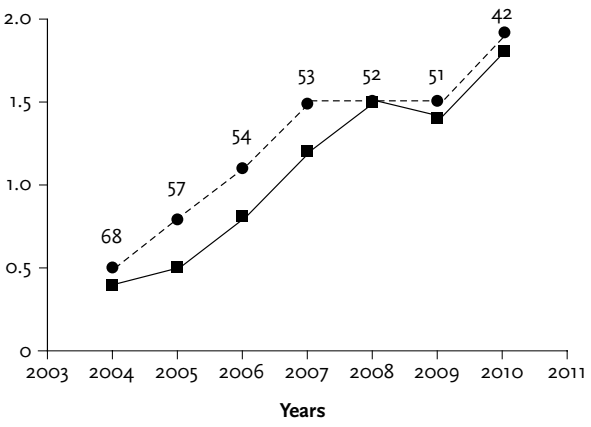
The impact factor of a journal depends on the citations of articles in the first two years after publication. Obviously, this is a debatable criterion, which has generated a lot of discussion in the literature.[1,2] Indeed, citations may not reflect the quality of a paper, may also be used to disqualify previously published articles, or can be subject to (excessive) self-referencing.[3] On the other hand, it has been shown that journal impact factors correlate with Hirsch factors of board members and impact of articles, as judged by experts in the field.[4] Other factors that may determine relevance, such as downloads of full-text articles from a journal's website, seem to correlate well with the impact factor, as has also been shown for the Netherlands Journal of Medicine.[5] Taken together, the journal's impact factor is something that is related to the (perceived) quality of a medical journal and probably an important issue for authors when considering where to submit their paper to.

In view of the above, I am pleased to be able to report that the impact factor of the Netherlands Journal of Medicine increased to 1.89 in 2010. In fact, this is the highest impact factor the journal has achieved in its history. As shown in *figure 1*, the impact factor has steadily risen over the past few years, but the more than 25% jump as compared with 2009 is reassuring that the Journal is doing well. With its 42nd position in the list of clinical journals, the Netherlands Journal of Medicine has entered the top 50. This position fits well with the broad orientation and relatively productive position of clinical science in the Netherlands.[6] However, the goal of the editorial team for 2011 is to achieve an impact factor of more than 2, as this will put the journal in the ranks of the truly discerning scientific journals in clinical medicine.

Impact factors are determined by the mean number of citations that articles in the journal attract. However, this mean number may obscure the fact that some articles generate a relatively high number of citations, whereas others seem to be totally ignored. *Table 1* shows the five highest cited papers from 2009 and 2010, respectively. The authors of these papers are to be commended for their articles, which have attracted so much attention in a relatively short time span.

The editorial team of the Netherlands Journal of Medicine is very grateful to the many authors who submitted their papers to our Journal, to the reviewers who were very helpful in judging the submitted articles, and to the editorial board members who helped to keep the journal in good shape. We are very pleased with the increase of our impact factor and we will do anything we can to further improve the status of our Journal.

Figure 1. *Impact factor of the Netherlands Journal of Medicine*



The official impact factor of the Netherlands Journal of Medicine (based on two-year citations) is shown with the bullets, whereas the less common five-year impact factor is shown with squares. The figure above the symbols represents the relative position in the ranking of clinical journals.

Table 1. *Top 5 cited papers in 2009 and 2010*

2009		2010	
Author / Title	Citations	Author / Title	Citations
Van der Klis *et al.*[7] Second national serum bank for population-based seroprevalence studies in the Netherlands	14	Anas *et al.*[8] Recent insights into the pathogenesis of bacterial sepsis.	8
Kuipers *et al.*[9] Hypomagnesaemia due to use of proton pump inhibitors--a review.	12	Kolesnyk *et al.*[10] Effects of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in patients with chronic kidney disease	7
Van Meerten & Hagenbeek[11] CD20-targeted therapy: a breakthrough in the treatment of non-Hodgkin's lymphoma	9	Levi *et al.*[12] Bleeding in patients using new anticoagulants or antiplatelet agents: risk factors and management	6
Khan[13] Rhabdomyolysis: a review of the literature	8	Lowenberg *et al.*[14] Platelet-vessel wall interaction in health and disease	5
Hoeks *et al.*[15] Adult issues in phenylketonuria	7	Seger[16] Chronic granulomatous disease: recent advances in pathophysiology and treatment	5

REFERENCES

1. Pendlebury DA. The use and misuse of journal metrics and other citation indicators. Arch Immunol Ther Exp (Warsz). 2009;57(1):1-11.
2. Callaham M, Wears RL, Weber E. Journal prestige, publication bias, and other characteristics associated with citation of published studies in peer-reviewed journals. JAMA. 2002;287(21):2847-50.
3. Kulkarni AV, Aziz B, Shams I, Busse JW. Author self-citation in the general medicine literature. PLoS One. 2011;6(6):e20885.
4. Pagel PS, Hudetz JA. Bibliometric analysis of anaesthesia journal editorial board members: correlation between journal impact factor and the median h-index of its board members. Br J Anaesth. 2011; published on line June 23, 2011.
5. Levi M. Submission, acceptance, citation and download of articles in the Netherlands Journal of Medicine. Neth J Med. 2010;68:332-35.
6. Levi M. How academic is internal medicine in the Netherlands? A bibliometric analysis. Neth J Med. 2009;67(10):318-9.
7. van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. Neth J Med. 2009;67(7):301-8.
8. Anas AA, Wiersinga WJ, de Vos AF, van der Poll T. Recent insights into the pathogenesis of bacterial sepsis. Neth J Med. 2010;68(4):147-52.
9. Kuipers MT, Thang HD, Arntzenius AB. Hypomagnesaemia due to use of proton pump inhibitors--a review. Neth J Med. 2009;67(5):169-72.
10. Kolesnyk I, Struijk DG, Dekker FW, Krediet RT. Effects of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in patients with chronic kidney disease. Neth J Med. 2010;68(1):15-23.
11. van Meerten T, Hagenbeek A. CD20-targeted therapy: a breakthrough in the treatment of non-Hodgkin's lymphoma. Neth J Med. 2009;67(7):251-9.
12. Levi MM, Eerenberg E, Lowenberg E, Kamphuisen PW. Bleeding in patients using new anticoagulants or antiplatelet agents: risk factors and management. Neth J Med. 2010;68(2):68-76.
13. Khan FY. Rhabdomyolysis: a review of the literature. Neth J Med. 2009;67(9):272-83.
14. Lowenberg EC, Meijers JC, Levi M. Platelet-vessel wall interaction in health and disease. Neth J Med. 2010;68(6):242-51.
15. Hoeks MP, den Heijer M, Janssen MC. Adult issues in phenylketonuria. Neth J Med. 2009;67(1):2-7.
16. Seger RA. Chronic granulomatous disease: recent advances in pathophysiology and treatment. Neth J Med. 2010;68(11):334-40.

ERRATUM

Unfortunately, in the articles in Neth J Med. 2010 Dec;68(12):424-429 and in Neth J Med. 2011 Jan;69(1):39-40, the name of one of the authors was cited as 'W. Gamal'. This should have been W.G. Ibrahim.
We apologise for any inconvenience.

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REVIEW

Optimal management of Graves orbitopathy: a multidisciplinary approach

M.R. Soeters[1*], C.J.J. van Zeijl[1], A. Boelen[1], R. Kloos[2], P. Saeed[2], T.M. Vriesendorp[1], M.P. Mourits[2]

Departments of [1]Endocrinology and Metabolism, [2]Orbital Center, Department of Ophthalmology, Academic Medical Center, University of Amsterdam, the Netherlands, *corresponding author: tel.: +31 (0)20-56 66 071, e-mail: m.r.soeters@amc.uva.nl

ABSTRACT

Graves' thyroid disease is a relatively common disorder in endocrinology and general internal medicine practice. Graves' hyperthyroidism is mediated by circulating stimulating autoantibodies. Up to 60% of patients with Graves' hyperthyroidism develop some form of Graves' orbitopathy. Immune reactivity to the thyroid-stimulating hormone receptor is also thought to play a role in the immunopathogenesis of Graves' orbitopathy.

Graves' orbitopathy is characterised by a wide open eye appearance, caused by upper eyelid retraction and soft-tissue swelling that causes exophthalmus. Symptoms include photophobia, sandy feeling in the eye, painful eye movements and diplopia. Visual acuity may be reduced. In some cases emergency treatment is necessary to prevent irreversible vision loss. Smoking should be stopped. Mild to moderate Graves' orbitopathy may be an indication for corticosteroid treatment or radiotherapy. Once inflammatory signs and symptoms have waned, surgery can be performed to correct residual diplopia, exophthalmus or lid retraction. The presence of Graves' orbitopathy has consequences for the management of Graves' hyperthyroidism. Adequately controlled Graves' thyroid dysfunction is likely to improve Graves' orbitopathy, while radioactive iodine treatment can worsen the condition. Due to the wide variety in clinical presentation and the possible interference between treatment of thyroid disease and eye disease, the management of more complicated patients with Graves' orbitopathy can best be performed in combined thyroid-eye clinics, in which the patient is seen simultaneously by the ophthalmologist and the endocrinologist.

KEYWORDS

Graves, hyperthyroidism, orbitopathy, ophthalmopathy, endocrinology

INTRODUCTION

Graves' orbitopathy (also known as Graves' ophthalmopathy, thyroid eye disease and thyroid-associated ophthalmopathy) is a potentially sight-threatening chronic autoimmune disorder characterised by an inflammation of retrobulbar tissues, leading to accumulation of hydrophilic glycosaminoglycans (GAGs) and/or an increase in orbital adipose tissue and thereby in increased volumes of orbital connective tissue and extra-ocular muscles. The annual incidence rate is low: 16 women and 3 men per 100,000. In up to 90% of adults with Graves' hyperthyroidism, extraocular muscle and/or fat enlargement is detected by computed tomography (CT) or magnetic resonance (MRI) scans of the orbit and clinical features are evident in 25 to 50% of patients with Graves' hyperthyroidism.[1,2] Of all patients with Graves orbitopathy, 5 to 10% have no abnormalities in thyroid function, and up to 10% of patients with Graves' orbitopathy initially present with hypothyroidism.

Graves' hyperthyroidism is a relatively common disorder, but the diagnosis and treatment of Graves' orbitopathy may be problematic because of considerable variety in disease presentation and interactions between thyroid and eye disease management. Common clinical features of Graves' orbitopathy are eyelid retraction, proptosis, extraocular muscle dysfunction, periorbital oedema and conjunctivitis.[1] Approximately 5% of patients have severe Graves' orbitopathy with sight-threatening corneal ulceration or compressive optic neuropathy.

Here we discuss clinical features of Graves' orbitopathy, imaging modalities and treatment options. The combined approach of an endocrinologist and ophthalmologist will render the best care for a patient with Graves' orbitopathy.

PATHOPHYSIOLOGY

The close association between Graves' hyperthyroidism and Graves' orbitopathy suggests a shared underlying

pathophysiological mechanism. Thyroid-stimulating hormone (TSH) receptor-stimulating antibodies bind the TSH receptor on the thyroid gland causing increased thyroid growth and excessive thyroid hormone production known as Graves' hyperthyroidism.[13,14] Immune reactivity to the TSH receptor is currently also thought to play an important role in the immunopathogenesis of Graves' orbitopathy, as the TSH receptor can be detected in orbital adipose tissue of patients with Graves' orbitopathy.[1,14] Indeed, elevated TSH receptor expression has been demonstrated in orbital tissues from patients with active Graves' orbitopathy compared with patients with inactive disease.[15] The receptor autoantibodies titre and the clinical activity of Graves' orbitopathy correlate, making TSH receptor antibody levels of prognostic value for the severity and the outcome of Graves' orbitopathy.[16-18] However, the precise role for the TSH receptor has not been established. There is increasing evidence that orbital fibroblasts, which express the TSH receptor, are major participants in the immunopathogenesis of Graves' orbitopathy.[19] Orbital fibroblasts can produce excessive amounts of the highly hydrophilic GAG hyaluronan upon stimulation with various cytokines. In addition, a subpopulation, termed preadipocytes, can differentiate into mature adipocytes under strict culture conditions *in vitro* and may contribute to the increase in adipose tissue.[20-22] Histology shows accumulation of hydrophilic GAG, predominantly hyaluronan, and an increase in orbital adipose/connective tissue.[1] The different clinical features of active Graves' orbitopathy are caused by inflammatory processes of retro-ocular connective tissue with infiltration of predominantly type 1 helper T (Th1) cells but also mast cells, B cells, plasma cells and macrophages. The presence of primarily Th1 cells and associated cytokines (interleukin-2, interferon-γ, tumour necrosis factor (TNF)) in early disease indicates that cell-mediated immunity prevails in early disease.[19,23,24] In later stages of Graves' orbitopathy, Th2 cells and cytokines (interleukin-4, interleukin-5, interleukin-10 and interleukin-13) predominate stimulating B-cells to produce autoantibodies.

CLINICAL FINDINGS

Symptoms of Graves' orbitopathy

In 40% of patients with Graves' orbitopathy, the signs of the eye disease occur simultaneously with the first symptoms of Graves' hyperthyroidism.[3] However, Graves' orbitopathy can occur several years after the diagnosis of Graves' hyperthyroidism. Approximately 5 to 10% of Graves' orbitopathy patients are euthyroid at presentation and some of them may not have a history of thyroid dysfunction, which explains why the diagnosis of Graves' orbitopathy may be delayed. Not surprisingly, these patients are at considerable risk for developing thyroid disease.

As detailed below, the severity of Graves' orbitopathy can vary between no complaints at all (i.e. just appearance of Graves' orbitopathy) or (near) blindness. Often, patients only present with a 'changed appearance'. In case of extensive swelling of the extraocular muscles, compression of the optic nerve may occur in 3 to 5% of patients, leading to loss of vision (dysthyroid optic neuropathy, DON).[4,5] This is an indication for acute treatment (*see treatment of severe Graves' orbitopathy*).

Upper eyelid retraction and proptosis

Many patients with Graves' hyperthyroidism have subtle signs of Graves' orbitopathy at physical examination.[5,6] The 'wide open eye appearance' is caused by upper eyelid retraction (UER) and bulging of the eye out of its socket due to increased intraorbital pressure (proptosis or exophthalmus).

UER is far more common than increased orbital volume.[5,6] It is often mistaken for exophthalmus. It can be diagnosed by observing the upper eye lid during downward gaze: the upper eyelid follows the bulb with some delay (lidlag or Von Graefe's sign). Next to increased sympathetic tone, fibrotic attachments around the levator palpabrae muscle cause UER.

Oedema and fibrosis in the external eye muscles and increased intraorbital fat lead to increased intraorbital volume. Since the orbit is bounded by bony walls, except for the anterior side, increased retrobulbar content will move the eye out of the orbit causing exophthalmus.

The degree of exophthalmus can be quantified with exophthalmometry, for instance a Hertel exophthalmometer.[7] One should realise that racial, gender, and age-related differences in orbital volume exist.[8] Exophthalmometry is specifically important for longitudinal comparison within patients.

Table 1. *Clinical activity of Graves ophtalmopathy according to Mourits et al.* [10,11]

I	Painful, oppressive feeling on or behind the globe
II	Pain on attempted up-, side-, or down-gaze
III	Redness of the eyelids
IV	Redness of the conjunctiva
V	Chemosis (conjunctival oedema)
VI	Inflammatory eyelid swelling
VII	Swelling of caruncle or plica
VIII	Increase of proptosis of more then 2 mm in one to three months
IX	Decrease in visual acuity of one or more lines in one to three months
X	Decrease in eye movement of more then 5° in any direction in one to three months

Table 2. *NO SPECS score*[10]

Class 0	–	No symptoms or signs
Class I	–	Only signs, no symptoms (lid retraction, stare, lid lag)
Class II	–	Soft tissue involvement (absent, mild, moderate, marked)
Class III	–	Proptosis (< 23, 23-24, 25-27, ≥ 28 mm)
Class IV	–	Extraocular muscle involvement (absent, limited motion, evident restriction, fixation)
Class V	–	Corneal involvement (absent, stippling, ulceration, clouding/necrosis/ perforation)
Class VI	–	Sight loss (optic nerve involvement) (>0.67, 0.67-0.33, 0.33-0.10, <0.10, expressed as decimals)

Regensburg *et al*. showed that in Graves' orbitopathy three subgroups can be distinguished based on the orbital content: 1) no fat or muscle volume increase; 2) only fat *or* muscle volume increase, and 3) both fat and muscle volume increase.[9] Although the clinical activity score (CAS; *see below*) between these groups was not different, muscle swelling only was associated with an increased risk of developing DON.

Activity and severity in Graves' orbitopathy

The activity and severity are important determinations in Graves' orbitopathy and have implications for treatment. Active disease is characterised by inflammatory symptoms and signs such as redness, oedema and pain. Severity of disease is defined by the functional or cosmetic impairment.

Characteristically, the course of Graves' orbitopathy can be divided into four phases. In the first phase there is an increase of signs and symptoms of Graves' orbitopathy. The second phase is a plateau phase, during which signs and symptoms are the most severe. In the third phase, signs and symptoms regress, leading to a stable fourth phase in which abnormalities in appearance and functional impairments remain.

Clinical activity can be scored by using the CAS ten-point scoring system, which was developed by Mourits *et al*. (*table 1*).[10,11] The first seven items are easy to score by the internist, and are used to guide diagnostic and treatment strategies (*figure 1*). The colour atlas of Graves' orbitopathy facilitates uniform use of the CAS.[4]

The NOSPECS mnemonic is a useful alternative reminder of what should be assessed in patients with Graves' orbitopathy regarding severity, but it is of lesser practical value (*table 2*).[12] First, NOSPECS is more of an ophthalmological tool that is not easy to perform in the endocrinologist's office. In addition, NOSPECS assumes a rank in the various clinical features that is not always present.[4]

ORTHOPTIC EVALUATION AND IMAGING

Assessment of eye motility involves the squint angle, the range of fusion, unilateral eye excursions (ductions) and field of binocular single vision. The motility pattern depends on the involved muscles and can be extremely deceptive. A patient with mild unilateral involvement of the inferior rectus muscle may experience constant diplopia, whereas a patient with severe bilateral involvement of the inferior rectus muscle may have single vision (at the cost of elevation impairment and ocular torticollis).

Although specific muscle involvement is often suspected when there is gaze limitation in certain directions, imaging (CT, MRI or ultrasound) can be very helpful. Muscle enlargement, and not retrobulbar fat accumulation, is associated with an increased risk of developing DON, as shown by CT and MRI,[9] show swelling of extra-ocular muscles and disappearance of adipose tissue in the apex of the orbit (apical crowding). The latter is suggestive of developing DON. Also, a stretched optic nerve is associated with an increased risk for visual loss.

TREATMENT OF GRAVES' ORBITOPATHY

The natural course of Graves' orbitopathy is usually self-limiting as the activity will improve spontaneously.[25] Artificial tears can be of help in reducing symptoms of tearing and burning due to ocular surface exposure.

The strongest modifiable risk factor in Graves' orbitopathy is smoking. Smoking not only increases the chance of developing Graves' orbitopathy 7-8 fold,[26] it also increases the severity and progression of Graves' orbitopathy with a less favourable response to treatment. Patients must be encouraged to stop smoking.[27]

Whether or not a patient should be actively treated is based on the level of functional and cosmetic impairment (severity) and on the level of inflammation (activity). The type of treatment is based on the phase of the disease. Active disease is usually treated with immunosuppressive treatment and functional and cosmetic impairment usually by surgery.

Mild Graves' orbitopathy: wait and see

In mild Graves' orbitopathy the side effects of immunosuppressive treatment or radiation do not weigh against the expected beneficial effects. Progression from mild to moderate to severe Graves' orbitopathy occurs in about 15%. The best way to predict progression is an increase in the CAS score.[28] Patients who smoke and with high TSH receptor autoantibody titres may be at increased risk.[29] Selenium (a naturally abundant antioxidant) improves the outcome in mild Graves' orbitopathy.

Moderate to severe Graves' orbitopathy: corticosteroid treatment

Moderate to severe Graves' orbitopathy is defined as: no threat to vision but sufficient impact on daily life to justify the risks of immunosuppression.[30] The use of immunomodulatory treatment is especially indicated in very active disease.[31]

For a long time corticosteroids have been used in patients with moderate to severe Graves' orbitopathy, with response rates up to 80%.[32,33] Intravenous prednisolone treatment is recommended because it has been convincingly shown that it has better results compared with high-dose oral therapy.[34] In addition, intravenous therapy is accompanied by less side effects such as diabetes or weight gain. Preferably, an internist or endocrinologist and the ophthalmologist decide together which patients should be treated with prednisolone (see also *the combined thyroid-eye clinic*).[30] An internist or endocrinologist should assess possible contraindications for high-dose prednisone treatment, such as gastrointestinal ulcer disease, severe osteoporosis, latent tuberculosis or hepatitis B or C positivity. Blood pressure and plasma glucose levels should be checked frequently and dietary advice should be given in case of weight gain. Fluid retention can be a problem. Additionally, patients must receive osteoporosis prophylaxis (i.e. bisphosphonate, calcium, and vitamin D)[35] and proton pump inhibition.

The cumulative dose of prednisolone should not exceed 8 grams in one course of therapy. However, the exact dose of prednisolone that yields satisfactory therapeutic effect without adverse events is not exactly known.[30] Recently, the inclusion of patients in a large multicentre randomised clinical trial initiated by the European Group on Graves Ophthalmopathy (EUGOGO) was completed. This study compared the effectiveness of three dosage regimens of prednisolone (cumulative dosage of 2.5 g, 5.0 g or 7.5 g). The results will be available at the end of 2011. Whether selenium on top of prednisolone during moderate to severe Graves' orbitopathy has additive effects remains to be investigated.

Very severe Graves' orbitopathy or DON

The treatment of DON is difficult because of the absence of randomised trial data and the lack of distinction between possible and definite DON.[36] Data from a small study favour treatment with high-dose intravenous steroids.[37] In this study patients received 1 g methylprednisolone iv daily for three consecutive days, repeated after one week, followed by an oral tapering dose.[37] Usually the iv dose of prednisolone is given in a clinical setting in order to permit regular checks of visual acuity. When there is clinical deterioration, urgent orbital decompression should be considered.

Other immunosuppressive therapy

Whereas corticosteroids are established in the treatment of moderate to severe Graves' orbitopathy and DON, this is less the case for other immunosuppressive therapies, the only exception being cyclosporine.[30] Prummel *et al*. compared single cyclosporine *vs* prednisolone monotherapy use and showed that cyclosporine alone has much lower efficacy.[38] However, cyclosporine in *addition* to prednisolone has an additive beneficial effect and should be used when steroids alone fail in moderate to severe orbitopathy.[39] Normally, cyclosporine is continued after the tapering down of steroids. The recommended cyclosporine dose is 3 mg/kg/day up to a maximal dose of 5 mg/kg/day.

Intravenous administration of immunoglobulin seems equally effective to prednisolone, but costs, iv administration and the chance of transmitting infections negate the administration of immunoglobulin.[40-42]

Tumour necrosis factor-α blockers may have therapeutic value in Graves' orbitopathy. Etanercept showed 60% patient-reported improvement and 60% CAS reduction although almost 30% of patients showed Graves' orbitopathy flare up after cessation of the study drug.[43] The same (decrease in CAS) is true for anti-CD20 monoclonal antibodies (rituximab) although the studies that have been performed are quite small.[44,45] The value of both etanercept and rituximab needs to be confirmed in larger randomised clinical trials.

Radiotherapy

Retrobulbar radiotherapy may be a good alternative to treat moderate to severe Graves' orbitopathy since intraorbital lymphocytes are particularly sensitive to radiotherapy.[46] Radiotherapy (10 fractions of 2 Gray (Gy)) compared with sham radiotherapy decreased the NOSPECS score in 63% compared with 31% respectively.[47] A head-to-head comparison between prednisolone and radiotherapy showed equal effectiveness of the therapies.[33] Prednisolone showed a quicker recovery with better effect on soft tissue whereas radiotherapy showed better outcome for muscle motility. The beneficial effect of radiotherapy on muscle motility and diplopia has been established by other studies as well.[47,48]

Usually, the dosage of Rx can be low:[46] 1 Gy per week (compared with 1 or 2 Gy daily) showed to be equally effective in patients with moderate to severe Graves' orbitopathy.[49] Patients with diabetes (and hypertension) have a relative contraindication for radiotherapy as they have higher risk for developing post-radiotherapy retinopathy.[50]

Surgical treatment

Surgical treatment is usually performed in phase 4, when the activity of the disease has waned. It is only in patients with active disease who have refractory or progressing

DON or corneal ulcer that urgent orbital decompression surgery may be needed. Indications for orbital decompressions include a stretched optic nerve, prevention of further corneal damage, alleviating complaints of tearing and grittiness, but also cosmetic complaints. During an orbital decompression, part of the bony walls is removed in order to provide more space for the extraocular muscles and orbital fat. Diplopia usually warrants surgery of the extraocular muscles. If orbital decompression is done, extraocular muscle surgery is generally postponed for several months to be able to first evaluate the effect of decompression on the diplopia. Finally, eyelid surgery such as lengthening (in case of upper eye lid retraction) may be a final step in the rehabilitation of the patient with Graves' orbitopathy.

TREATMENT OF GRAVES' ORBITOPATHY WITH CONCOMITANT GRAVES' HYPERTHYROIDISM

Apart from the treatment modalities for Graves' orbitopathy as outlined above, concomitant Graves' hyperthyroidism may influence the course of the eye disease. Restoration of hyperthyroidism with block and replacement treatment has a neutral effect on the course of Graves' orbitopathy.[29] Stopping block and replacement therapy is associated with a significant risk of recurrence of hyperthyroidism. Some fear a simultaneous flare of Graves' orbitopathy because of increasing antibody titres. Thyroidectomy also seems to have a neutral effect on Graves' orbitopathy.[51] This may be different for hypothyroidism, which usually shows deteriorating of Graves' orbitopathy.[52,53]

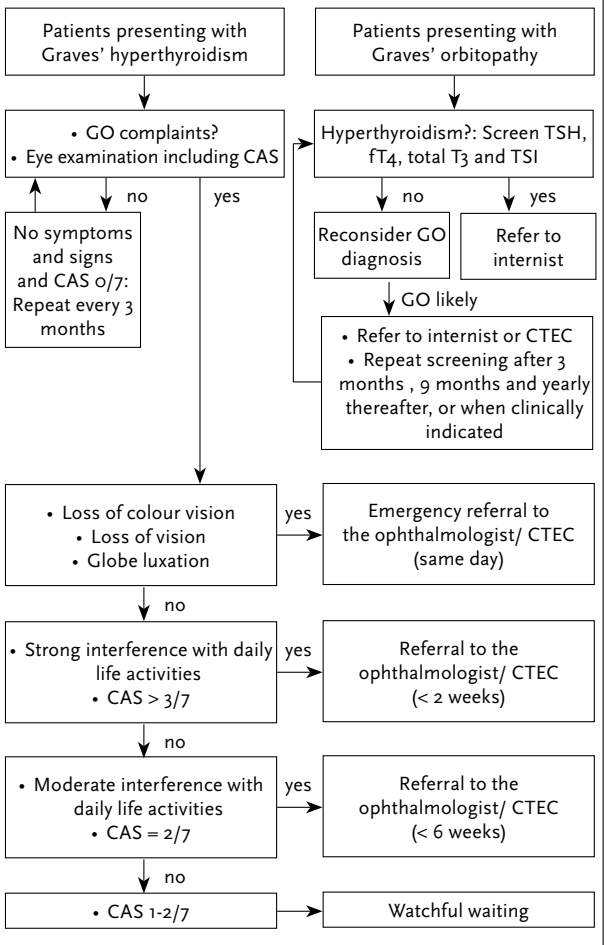
^{131}Iodine (^{131}I) may induce progression or relapse of Graves' orbitopathy that is explained by leakage of antigens from the thyroid gland, again eliciting an autoimmune response.[1] Progression of Graves' orbitopathy after ^{131}I treatment can be prevented by corticosteroid treatment.[32,54] Also, the occurrence of hypothyroidism after ^{131}I therapy is associated with Graves' orbitopathy (*de novo* and pre-existing). Risk factors for progression of Graves' orbitopathy due to ^{131}I therapy include cigarette smoking, TSH receptor antibody levels, severity of Graves' hyperthyroidism and pre-existing Graves' orbitopathy.[16,26,52,54,55] If these risk factors are present (smoking in particular) treatment with corticosteroids is warranted to prevent ^{131}I induced Graves' orbitopathy.[29] A study by Bartalena *et al.* showed that prednisolone not only prevents worsening of Graves' orbitopathy after ^{131}I, but actually also improves Graves' orbitopathy in some patients.[54] In these studies prednisolone 0.5 mg per kg was used for four weeks starting two-three days after ^{131}I therapy. Steroids were tapered down in two months.[32,56]

RATIONALE OF COMBINED THYROID-EYE CLINICS

When patients are assessed in combined thyroid-eye clinics (CTECs), they have a favourable outcome compared with patients who are not managed in such clinics.[57] A survey study showed participation in a multidisciplinary setting for management of Graves' orbitopathy in approximately half of the responders, but this was not the case in more than one third. Moreover, a lack of 'best practice' was shown in a significant number of responders for everyday clinical issues such as the referral to an ophthalmologist in case of a possible DON. Therefore, the EUGOGO recommends the implementation of CTECs.

The wide variety in clinical presentation and the interactions between the endocrinological and ophthalmological treatment (see '*treatment of Graves' orbitopathy with concomitant Graves' hyperthyroidism*') that

Figure 1. *Flowchart for patients presenting with Graves' hyperthyroidism or Graves' orbitopathy*



GO = Graves' orbitopathy; CAS = clinical activity score; CTEC = combined thyroid-eye clinic; TSH = thyroid-stimulating hormone; TSI = thyroid-simulating immunoglobulin.

require Graves' orbitopathy to be treated by a multidisciplinary team that includes at least an ophthalmologist and an internist or endocrinologist. In case of endangered vision, emergency referral to a combined thyroid-eye clinic may be necessary, in mild cases 'watchful waiting' is justified (*figure 1*). Patients with Graves' orbitopathy may have complicating circumstances that hinder the decision making and subsequent treatment of Graves' orbitopathy and Graves' hyperthyroidism (e.g. toxicity of thyreostatics or pregnancy).

CONCLUSION

Much progress has been made in the diagnosis and treatment of Graves' orbitopathy. Each patient with Graves' hyperthyroidism should be meticulously scrutinised for Graves' orbitopathy.

A workup in so-called combined thyroid-eye clinics improves the management of Graves' orbitopathy.

REFERENCES

1. Bahn RS. Graves' ophthalmopathy. N Engl J Med. 2010;362:726-38.
2. Forbes G, Gorman CA, Brennan MD, Gehring DG, Ilstrup DM, Earnest F. Ophthalmopathy of Graves' disease: computerized volume measurements of the orbital fat and muscle. Am J Neuroradiol. 1986;7(4):651-6.
3. Wiersinga WM, Smit T, van der Gaag R, Koornneef L. Temporal relationship between onset of Graves' ophthalmopathy and onset of thyroidal Graves' disease. J Endocrinol Invest. 1988;11(8):615-9.
4. Dickinson AJ, Perros P. Controversies in the clinical evaluation of active thyroid-associated orbitopathy: use of a detailed protocol with comparative photographs for objective assessment. Clin Endocrinol (Oxf). 2001;55(3):283-303.
5. Bartley GB, Fatourechi V, Kadrmas EF, Jacobsen SJ, Ilstrup DM, Garrity JA, et al. Clinical features of Graves' ophthalmopathy in an incidence cohort. Am J Ophthalmol. 1996;121(3):284-90.
6. Saks ND, Burnstine MA, Putterman AM. Glabellar rhytids in thyroid-associated orbitopathy. Ophthal Plast Reconstr Surg. 2001;17(2):91-5.
7. Vardizer Y, Berendschot TT, Mourits MP. Effect of exophthalmometer design on its accuracy. Ophthal Plast Reconstr Surg. 2005;21(6):427-30.
8. Regensburg NI, Wiersinga WM, van Velthoven ME, Berendschot TT, Zonneveld FW, Baldeschi L, et al. Age and gender-specific reference values of orbital fat and muscle volumes in Caucasians. Br J Ophthalmol. 2010;7: ahead of print.
9. Regensburg NI, Wiersinga WM, Berendschot TT, Potgieser P, Mourits MP. Do subtypes of graves' orbitopathy exist? Ophthalmology. 2011;118(1):191-6.
10. Mourits MP, Prummel MF, Wiersinga WM, Koornneef L. Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol (Oxf). 1997;47(1):9-14.
11. Mourits MP, Koornneef L, Wiersinga WM, Prummel MF, Berghout A, van der Gaag R. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. Br J Ophthalmol. 1989;73(8):639-44.
12. Werner SC. Modification of the classification of the eye changes of Graves' disease. Am J Ophthalmol. 1977;83(5):725-7.
13. Weetman AP. Grave's disease 1835-2002. Horm Res. 2003;59(Suppl 1):114-8.
14. Rapoport B, McLachlan SM. The thyrotropin receptor in Graves' disease. Thyroid. 2007;17(10):911-22.
15. Wakelkamp IM, Bakker O, Baldeschi L, Wiersinga WM, Prummel MF. TSH-R expression and cytokine profile in orbital tissue of active vs. inactive Graves' ophthalmopathy patients. Clin Endocrinol (Oxf). 2003;58(3):280-7.
16. Eckstein AK, Plicht M, Lax H, Neuhauser M, Mann K, Lederbogen S, et al. Thyrotropin receptor autoantibodies are independent risk factors for Graves' ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab. 2006;91(9):3464-70.
17. Gerding MN, van der Meer JW, Broenink M, Bakker O, Wiersinga WM, Prummel MF. Association of thyrotrophin receptor antibodies with the clinical features of Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2000;52(3):267-71.
18. Lipman LM, Green DE, Snyder NJ, Nelson JC, Solomon DH. Relationship of long-acting thyroid stimulator to the clinical features and course of Graves' disease. Am J Med. 1967;43(4):486-98.
19. Garrity JA, Bahn RS. Pathogenesis of graves ophthalmopathy: implications for prediction, prevention, and treatment. Am J Ophthalmol. 2006;142(1):147-53.
20. Feldon SE, O'loughlin CW, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. Am J Pathol. 2006;169(4):1183-93.
21. Sorisky A, Pardasani D, Gagnon A, Smith TJ. Evidence of adipocyte differentiation in human orbital fibroblasts in primary culture. J Clin Endocrinol Metab. 1996;81(9):3428-31.
22. Valyasevi RW, Erickson DZ, Harteneck DA, Dutton CM, Heufelder AE, Jyonouchi SC, et al. Differentiation of human orbital preadipocyte fibroblasts induces expression of functional thyrotropin receptor. J Clin Endocrinol Metab. 1999;84(7):2557-62.
23. Aniszewski JP, Valyasevi RW, Bahn RS. Relationship between disease duration and predominant orbital T cell subset in Graves' ophthalmopathy. J Clin Endocrinol Metab. 2000;85(2):776-80.
24. Kumar S, Bahn RS. Relative overexpression of macrophage-derived cytokines in orbital adipose tissue from patients with graves' ophthalmopathy. J Clin Endocrinol Metab. 2003;88(9):4246-50.
25. Rundle FF. Management of exophthalmos and related ocular changes in Graves' disease. Metabolism. 1957;6(1):36-48.
26. Bartalena L, Marcocci C, Tanda ML, Manetti L, Dell'Unto E, Bartolomei MP, et al. Cigarette smoking and treatment outcomes in Graves ophthalmopathy. Ann Intern Med. 1998;129(8):632-5.
27. Pfeilschifter J, Ziegler R. Smoking and endocrine ophthalmopathy: impact of smoking severity and current vs lifetime cigarette consumption. Clin Endocrinol (Oxf). 1996;45(4):477-81.
28. Terwee CB, Prummel MF, Gerding MN, Kahaly GJ, Dekker FW, Wiersinga WM. Measuring disease activity to predict therapeutic outcome in Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2005;62(2):145-55.
29. Bartalena L, Tanda ML, Piantanida E, Lai A, Pinchera A. Relationship between management of hyperthyroidism and course of the ophthalmopathy. J Endocrinol Invest. 2004;27(3):288-94.
30. Bartalena L, Baldeschi L, Dickinson A, Eckstein A, Kendall-Taylor P, Marcocci C, et al. Consensus statement of the European Group on Graves' orbitopathy (EUGOGO) on management of GO. Eur J Endocrinol. 2008;158(3):273-85.
31. Stemberger K, Kahaly GJ, Pitz S. Update on thyroid eye disease. Compr Ophthalmol Update. 2006;7(6):287-98.
32. Bartalena L, Marcocci C, Bogazzi F, Panicucci M, Lepri A, Pinchera A. Use of corticosteroids to prevent progression of Graves' ophthalmopathy after radioiodine therapy for hyperthyroidism. N Engl J Med. 1989;321(20):1349-52.
33. Prummel MF, Mourits MP, Blank L, Berghout A, Koornneef L, Wiersinga WM. Randomized double-blind trial of prednisone versus radiotherapy in Graves' ophthalmopathy. Lancet. 1993;342(8877):949-54.

34. Kahaly GJ, Pitz S, Hommel G, Dittmar M. Randomized, single blind trial of intravenous versus oral steroid monotherapy in Graves' orbitopathy. J Clin Endocrinol Metab. 2005;90(9):5234-40.

35. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis: 2001 update. American College of Rheumatology Ad Hoc Committee on Glucocorticoid-Induced Osteoporosis. Arthritis Rheum. 2001;44(7):1496-503.

36. McKeag D, Lane C, Lazarus JH, Baldeschi L, Boboridis K, Dickinson AJ, et al. Clinical features of dysthyroid optic neuropathy: a European Group on Graves' Orbitopathy (EUGOGO) survey. Br J Ophthalmol. 2007;91(4):455-8.

37. Wakelkamp IM, Baldeschi L, Saeed P, Mourits MP, Prummel MF, Wiersinga WM. Surgical or medical decompression as a first-line treatment of optic neuropathy in Graves' ophthalmopathy? A randomized controlled trial. Clin Endocrinol (Oxf). 2005;63(3):323-8.

38. Prummel MF, Mourits MP, Berghout A, Krenning EP, van der Gaag R, Koornneef L, et al. Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy. N Engl J Med. 1989;321(20):1353-9.

39. Kahaly G, Schrezenmeir J, Krause U, Schweikert B, Meuer S, Muller W, et al. Ciclosporin and prednisone v. prednisone in treatment of Graves' ophthalmopathy: a controlled, randomized and prospective study. Eur J Clin Invest. 1986;16(5):415-22.

40. Kahaly GJ. Effect of intravenous gammaglobulin on organ specific antibodies and lymphocyte subsets. Clin Exp Rheumatol. 1996;14(Suppl 15):S37-S40.

41. Antonelli A, Saracino A, Agostini S, Alberti B, Melosi A, Gambuzza C, et al. [Results of high-dose intravenous immunoglobulin treatment of patients with pretibial myxedema and Basedow's disease. Preliminary findings]. Clin Ter. 1992;141(9 Pt 2):63-8.

42. Antonelli A, Saracino A, Alberti B, Canapicchi R, Cartei F, Lepri A, et al. High-dose intravenous immunoglobulin treatment in Graves' ophthalmopathy. Acta Endocrinol (Copenh). 1992;126(1):13-23.

43. Paridaens D, Lie A, Grootendorst RJ, van den Bosch WA. Efficacy and side effects of 'swinging eyelid' orbital decompression in Graves' orbitopathy: a proposal for standardized evaluation of diplopia. Eye (Lond). 2006;20(2):154-62.

44. Salvi M, Vannucchi G, Campi I, Curro N, Dazzi D, Simonetta S, et al. Treatment of Graves' disease and associated ophthalmopathy with the anti-CD20 monoclonal antibody rituximab: an open study. Eur J Endocrinol. 2007;156(1):33-40.

45. Khanna D, Chong KK, Afifiyan NF, Hwang CJ, Lee DK, Garneau HC, et al. Rituximab treatment of patients with severe, corticosteroid-resistant thyroid-associated ophthalmopathy. Ophthalmology. 2010;117(1):133-9.

46. Kahaly GJ, Roesler HP, Kutzner J, Pitz S, Muller-Forell W, Beyer J, et al. Radiotherapy for thyroid-associated orbitopathy. Exp Clin Endocrinol Diabetes. 1999;107(Suppl 5):S201-7.

47. Mourits MP, van Kempen-Harteveld ML, Garcia MB, Koppeschaar HP, Tick L, Terwee CB. Radiotherapy for Graves' orbitopathy: randomised placebo-controlled study. Lancet. 2000;355(9214):1505-9.

48. Prummel MF, Terwee CB, Gerding MN, Baldeschi L, Mourits MP, Blank L, et al. A randomized controlled trial of orbital radiotherapy versus sham irradiation in patients with mild Graves' ophthalmopathy. J Clin Endocrinol Metab. 2004;89(1):15-20.

49. Kahaly GJ, Rosler HP, Pitz S, Hommel G. Low- versus high-dose radiotherapy for Graves' ophthalmopathy: a randomized, single blind trial. J Clin Endocrinol Metab. 2000;85(1):102-8.

50. Marcocci C, Bartalena L, Rocchi R, Marino M, Menconi F, Morabito E, et al. Long-term safety of orbital radiotherapy for Graves' ophthalmopathy. J Clin Endocrinol Metab. 2003;88(8):3561-6.

51. Jarhult J, Rudberg C, Larsson E, Selvander H, Sjovall K, Winsa B, et al. Graves' disease with moderate-severe endocrine ophthalmopathy-long term results of a prospective, randomized study of total or subtotal thyroid resection. Thyroid. 2005;15(10):1157-64.

52. Karlsson F, Westermark K, Dahlberg PA, Jansson R, Enoksson P. Ophthalmopathy and thyroid stimulation. Lancet. 1989;2(8664):691.

53. Prummel MF, Wiersinga WM, Mourits MP, Koornneef L, Berghout A, van der Gaag R. Effect of abnormal thyroid function on the severity of Graves' ophthalmopathy. Arch Intern Med. 1990;150(5):1098-101.

54. Bartalena L, Marcocci C, Bogazzi F, Manetti L, Tanda ML, Dell'Unto E, et al. Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. N Engl J Med. 1998;338(2):73-8.

55. Tallstedt L, Lundell G, Torring O, Wallin G, Ljunggren JG, Blomgren H, et al. Occurrence of ophthalmopathy after treatment for Graves' hyperthyroidism. The Thyroid Study Group. N Engl J Med. 1992;326(26):1733-8.

56. Bartalena L, Marcocci C, Bogazzi F, Manetti L, Tanda ML, Dell'Unto E, et al. Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. N Engl J Med. 1998;338(2):73-8.

57. Perros P, Baldeschi L, Boboridis K, Dickinson AJ, Hullo A, Kahaly GJ, et al. A questionnaire survey on the management of Graves' orbitopathy in Europe. Eur J Endocrinol. 2006;155(2):207-11.

Netherlands
The Journal of Medicine

REVIEW

The evaluation of spells

I.N. van Loon[1*], J. Lamberts[1], G.D. Valk[2], A.F. Muller[1]

[1]Department of Internal Medicine, Diakonessenhuis, Utrecht, the Netherlands, [2]Department of Internal Medicine, University Medical Centre Utrecht, Utrecht, the Netherlands, *corresponding author: tel.: +31 (0)88- 25 05 901, e-mail: i.n.vanloon@gmail.com

ABSTRACT

The differential diagnosis of spells is broad and includes both innocent and life-threatening conditions with a considerable overlap in clinical presentation. Extensive diagnostic testing is often performed, without reaching a final diagnosis, or resulting in false-positives. A thorough medical history, including family history and medication, and physical examination are required to obtain clues about the cause of a spell. An overview of spells with their stereotypic phenotype in general internal medicine practice is presented in this article. Besides, a diagnostic approach is proposed for the clinical evaluation of spells.

KEYWORDS

Spell, flushing, pheochromocytoma, gastroenteropancreatic neuroendocrine tumours, mastocytosis

Table 1. *Differential diagnosis of episodic symptoms*

Endocrine	**Pharmacological**
Pheochromocytoma	Abrupt withdrawal of adrenergic inhibitor
Thyreotoxicosis	MAO inhibitor in combination with specific food
Hypogonadism (menopause)	Sympathicomimetic
Medullary thyroid carcinoma	Hallucinating drugs (cocaine, LSD)
Pancreatic islet cell tumours (e.g. insulinoma, VIPoma)	Chlorpropamide-alcohol flush
Gastroenteropancreatic neuroendocrine tumours (carcinoid syndrome)	Vancomycin
Hypoglycaemia	Calcium antagonist
Cardiovascular	**Neurological**
Labile hypertension	Autonomic neuropathy
Pulmonary oedema	Migraine
Syncope	Epilepsy
Orthostatic hypotension	
Paroxysmal arrhythmias	**Other**
Angina pectoris	Mastocytosis
Renovascular disease	Recurrent idiopathic anaphylaxis
Psychiatric	
Panic disorder (hyperventilation syndrome)	
Conversion disorder	

INTRODUCTION

A spell is best defined as 'a sudden onset of a symptom or symptoms that are recurrent, self-limited, and stereotypic in nature'.[1] The differential diagnosis of spells is challenging and includes both innocent and life-threatening conditions with a considerable overlap in clinical presentation (*table 1*). This typically results in extensive diagnostic testing without reaching a final diagnosis, or in false-positives. In general, but especially in conditions with a differential diagnosis as extended as spells, testing for a particular disease should only be performed if the pre-test probability is high.

In this article we aim to provide a rational approach to the analysis of spells and spell-like symptoms for the general internist. First, we carried out a systematic literature search on the *systematic* evaluation of spells in general, thereby excluding manuscripts focusing on a specific disorder. Then, we described key components of history and physical examination that are necessary to make a differential diagnosis short list. A description of frequently encountered and commonly sought causes of spells is given. Finally, a proposed work-up for different types of spells is presented.

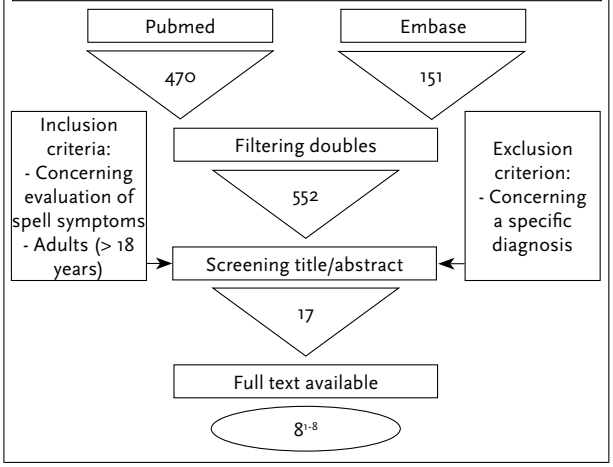
SEARCH STRATEGY

We searched PubMed and Embase databases for publications on the *systematic* evaluation of spells in general (i.e. manuscripts focusing on specific diseases were excluded in this stage) using synonyms for "spell" as shown in the syntaxes (*figure 1*). Since the terms "attack" and "attacks" resulted in many articles concerning neurological or cardiovascular disorders, but not in publications concerning spells in general, we decided to exclude these.

Figure 1. *Search strategy*

Pubmed: (Spell[title/abstract] OR spells[title/abstract] OR flush[title/abstract] OR flushing[title/abstract] OR flushing[MeSH] OR flushes[title/abstract] OR paroxysm[title/abstract] OR paroxysms[title/abstract])AND differential diagnosis[MeSH]

Embase: (Spell:ab,ti OR spells:ab,ti OR flush:ab,ti OR flushing:ab,ti OR flushes:ab,ti OR paroxysm:ab,ti OR paroxysms:ab,ti)AND 'differential diagnosis':ab,ti



When searching PubMed, we used Medical Subject Headings (MeSH terms) for differential diagnosis and flushing to broaden our search. Articles were considered relevant if they described a clinical syndrome fulfilling the criteria of a spell and the study subjects were at least 19 years of age. The search resulted in 17 apparently relevant articles, of which eight were available in full text.[1-8] Two of the articles were obtained by contacting the authors.[2,4] Three publications extensively describe the clinical evaluation of flushing.[5,7,8] Four articles describe a clinical case of a patient presenting with spells and work out the differential diagnosis.[3,6-8] Young *et al.* in particular describe pheochromocytoma, carcinoid syndrome and mastocytosis.[1] None of the publications found focus on the approach to the wide spectrum of spells in general internal medicine, including both rare and frequently observed conditions.

MAKING A DIAGNOSIS

History

The value of a detailed description of the spell cannot be overemphasised. Frequently the patient is able to provide sufficient information but, if this is not the case, it is imperative to obtain a detailed account from those who witnessed the event.

Establish what the patient was doing prior to the event, e.g. activities and position (upright, lying or changing position). Physical or psychological stressors immediately prior to the spell suggest a vasovagal event. Unusual smells, visual disturbances or uncontrolled movements may represent seizure activity, often referred to as an aura. If an attack starts with palpitations or tachycardia this suggests a cardiac cause. Loss or transient alteration of consciousness points to insufficient blood flow to the brain (syncope) or altered brain activity (generalised seizures).

Key components of the spell itself are a description of the specific symptoms, sequence of occurrence, timing, frequency and duration. Besides all of the above, it is important to determine factors which provoke, exacerbate or relieve the attack, such as heat, medications or alcohol. If the attack is accompanied by flushing, one should determine whether it is a 'wet' or a 'dry' flush. A 'wet' flush – e.g. the postmenopausal hot flash – is accompanied by sweating and is of a neurogenic nature (mediated by sympathetic cholinergic neurons that also stimulate sweat glands) whereas a 'dry' flush is the result of direct vasodilatation due to either endogenous (e.g. histamine, prostaglandin and polypeptides) or exogenous (e.g. nicotinic acid an amyl nitrite) substances and is not associated with perspiration.[1] Patients presenting with evident neurological spell-like symptoms are generally referred to the neurologist. It is important to realise that a number of internal diseases, such as porphyria and carcinoid syndrome, can also be accompanied by neurological or psychiatric symptoms. Hypoglycaemia may present with neurological symptoms due to neuroglycopenia. Loss of, or decreased consciousness, may be triggered by cerebrovascular diseases, hypoglycaemia or cardiac arrhythmias. A period of lethargy after the spell can indicate mastocytosis or epilepsy. Listing current medication including recent changes can give important clues pointing to a correct diagnosis, especially in the geriatric population with polypharmacy. Medication associated with flushing is shown in *table 2*.

Regarding family history special attention should be paid to sudden death, hormonal, neurological, psychiatric and sleep disorders.

Physical examination

A key element of the physical examination of a patient presenting with episodic symptoms is the measurement of blood pressure and pulse rate, preferably during the attack. Attention should be paid to the presence of orthostasis, tachycardia and/or an irregular heartbeat. The classical presentation of a pheochromocytoma is intermittent hypertension, but orthostatic hypotension may also occur.[9,10] Orthostatic hypotension may cause episodic dizziness. The thyroid is examined for goitre or palpable nodules. Cardiac murmurs and pulmonary wheezing may be an indication of carcinoid syndrome, which is associated with pulmonary valve stenosis and tricuspid regurgitation,

Table 2. *Medical history and findings*

Spell history
Description of specific symptoms
Sequence of occurrence, timing, frequency, duration of symptoms
Wet flush, dry flush or episodic pallor
Hypertension over time
Lethargy after the spell
Provoking, exacerbating or relieving factors
Cardiac complaints: chest pain, fatigue, dyspnoea, dizziness
Neurological complaints: weakness, sensory loss, altered states of consciousness
Medication
Family history and social history
Physical examination
Blood pressure
Pulse rate and rhythm
Determine orthostatic hypotension
Thyroid goitre or nodules
Cardiac examination: murmurs, deviated ictus cordis
Abdominal examination: Hepatosplenomegaly, abdominal murmur
Skin: urticaria pigmentosa, body hair distribution
Genitalia: testis atrophy

caused by endocardial fibrosis.[11] A deviated ictus cordis, sign of ventricular hypertrophy, can be found in patients with carcinoid syndrome or pheochromocytoma. Examination of the abdomen may reveal hepatomegaly or splenomegaly. The first can be found in metastatic disease (i.e. neuroendocrine tumour), or cardiac failure, whereas isolated splenomegaly may be caused by mastocytosis.[12] An abdominal murmur can indicate pheochromocytoma. The skin is inspected for urticaria pigmentosa (reddish-brown maculae or papules in mastocytosis), and hair pattern, which may differ in hypogonadism. In males suspected of hypogonadism the testis should be examined for testis atrophy.

Mental status can also give important clues. With syncopal events patients quickly regain consciousness whereas post-spell confusion is more typical of a seizure.

DIFFERENTIAL DIAGNOSIS

After taking the history and performing a physical examination, the key elements of which are listed in *table 3,* a differential diagnosis must be made in order to provide guidance in the ordering and interpretation of diagnostic tests. However, given the diversity of diseases and the overlap in clinical presentation we – and others[1] – recommend the following initial laboratory investigations: complete blood count with differential, serum electrolytes, glucose, creatinine, calcium, phosphorus, liver function tests, thyroid-stimulating hormone, luteinising hormone, follicle-stimulating hormone, testosterone or oestradiol, and urine analysis. Further testing should be directed by the differential diagnosis.

Table 3. *Medication associated with flushing (Izikson et al.)*[2]

All vasodilators: nitroglycerin and nitric oxide releasers: e.g. sildenafil	Cholinergic drugs	Vancomycin
All calcium channel blockers	Bromocriptine	Rifampicin
Calcitonin	Chemotherapeutics: cyclosporine, doxorubicin, cisplatin, interferon alfa-2	Contrast media
Beta-blockers	Anti-androgens: flutamide, cyproterone	Combination anaesthesia of isoflurane and fentanyl
Angiotensin-converting enzyme inhibitors	Anti-oestrogens: tamoxifen	Morphine and other opiates
Catecholamines	Prostaglandins	Antiemetics: e.g. metoclopramide
NSAIDs	Caffeine withdrawal	In combination with alcohol: disulfiram, metronidazole, ketoconazole, cephalosporins, anti-malarials
Triamcinolone	Alcohol withdrawal	
Methylprednisolone	Nicotine	

Episodic flushing and palpitations often characterise or accompany an attack. We have therefore designed two decision trees, which can guide in the diagnosis of these symptoms (*figures 2* and *3*). Carcinoid syndrome flushing can last from seconds to hours, usually located in the

Figure 2. *Decision tree for palpitations*

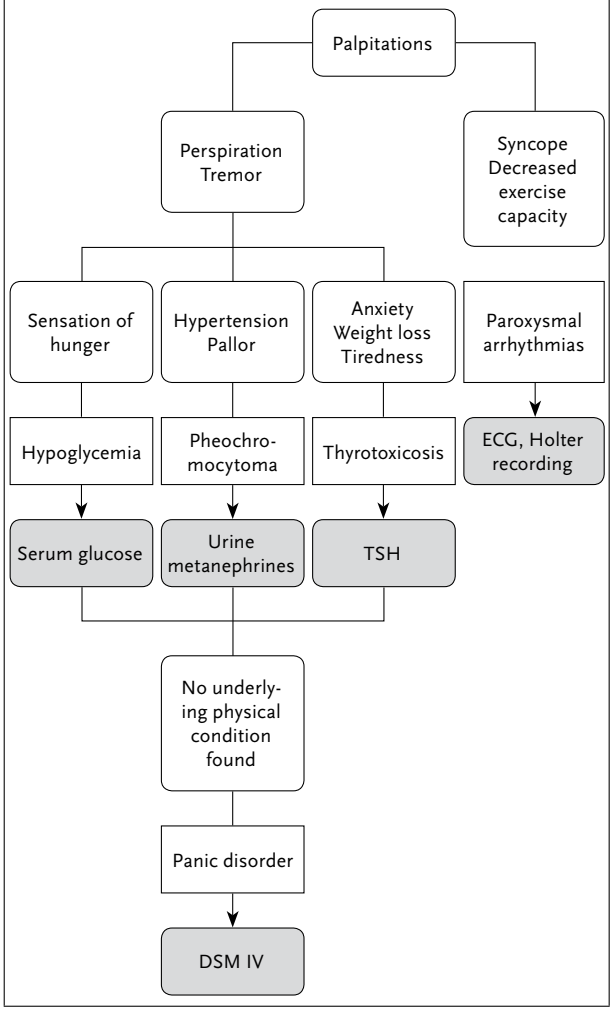
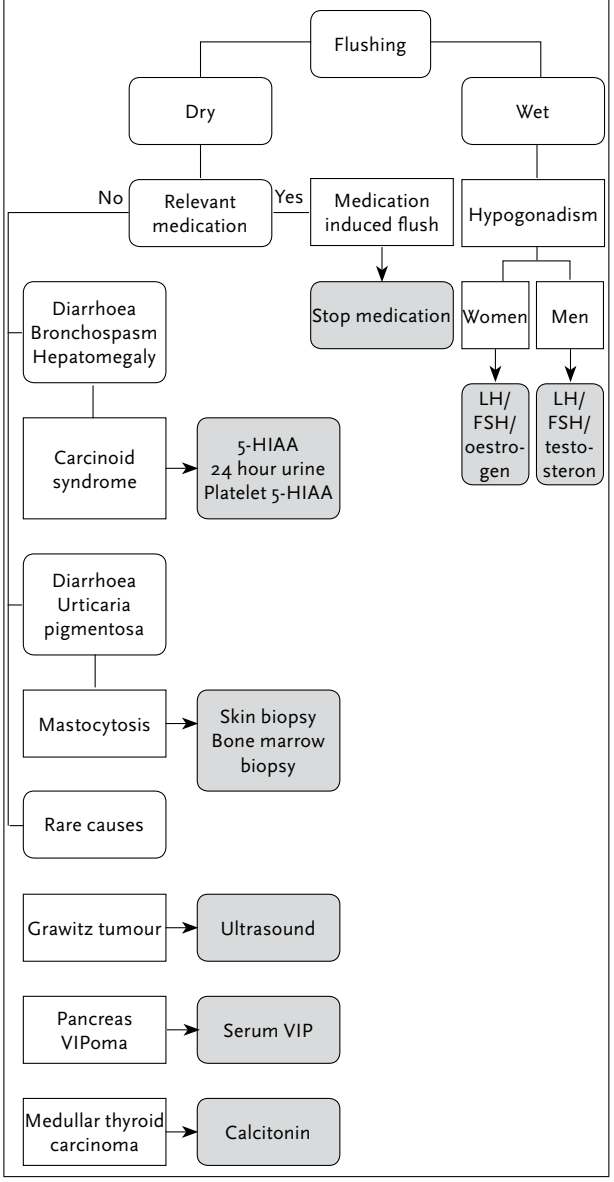


Figure 3. *Decision tree for flushing*



upper half of the body, pink-red to vermilion or dusky blue and may be spontaneous, induced by eating, alcohol, movement or necrosis of tumour deposits.[13] In case of mastocytosis, attacks usually last 15 to 30 minutes with flushing and facial warmth and associated with palpitation, light-headedness and even syncope.

SPECIFIC DISORDERS

In this section we provide some basic information regarding some of the frequently described causes of spells. *Table 4* summarises classical spell phenotypes of these causes. The spell-like symptoms they comprise are listed in *table 5*. Additional diagnostic testing is shown in *table 6*.

Table 4. *Classical spell phenotypes*

Disease	Classic features
Pheochromocytoma	Triad: headaches, palpitations, sweating. Hypertension, pallor
Thyrotoxicosis	Fatigue, anxiety, sweating, palpitations
Mastocytosis	Urticaria pigmentosa, dry flushing, diarrhoea, abdominal pain
Carcinoid syndrome/ GEP-NET	Dry flushing, diarrhoea, bronchospasm, enlarged liver
Hypogonadism	Hot/wet flushes, sweating
Hypoglycaemia	Hunger, tremor, weight gain, symptoms disappear after a meal
Porphyria	Colicky abdominal pain, neurological symptoms
Panic attack	Psychological component, palpitations, chest pain, shortness of breath
Paroxysmal arrhythmias	Palpitations

Hypogonadism – 'Hot flashes'

Hot flashes are a sensation of heat associated with vasodilation and a drop in body temperature. Frequently associated symptoms are: sweating, flushing, palpitations, anxiety, irritability and panic.[14] It is the most common symptom of premenopausal and postmenopausal women.[14] Men on androgen deprivation therapy also have this symptom.[14] In the differential diagnosis of hypogonadism, a distinction is made between primary (ovarian/testicular) and secondary (pituitary/hypothalamic) lack of sex hormones. The pathophysiology of hot flashes is largely unknown.[14] The heat sensation lasts for four minutes on average with a wide variation from seconds to as long as ten minutes.[14] The frequency of such hot flash varies, from several times a day to several times a week.[14] It is important to note that a postmenopausal woman with this typical pattern of complaints is virtually always suffering from climacteric hypogonadism and the diagnosis is a clinical one based on menstrual history and age.[15]

Panic disorder

Panic disorder is a treatable psychiatric disorder which is quite common, with a prevalence of 4 to 7%.[16] Panic disorder is characterised by recurring severe panic attacks and may include ongoing worry about the implications or concern about having other attacks.[16] There are various symptoms: palpitations, chest pain, sweating, shortness of breath, feelings of suffocation and dizziness may occur. In addition, patients experience a fear of dying or doing something uncontrollable during an attack. The criteria of panic disorder according to the Diagnostic and statistical manual of mental disorders (DSM-IV-R) are listed in *table 7*.[17]

After appropriate testing to rule out medical conditions psychiatric referral is in order, especially since 90% of patients with panic disorder will have at least one other psychiatric diagnosis during their lifetime.[16]

Table 5. Occurrence of episodic symptoms

	Pheochromocytoma	Thyrotoxicosis	Mastocytosis	Carcinoid syndrome	Hypogonadism	Hypoglycaemia	Porphyria	Paroxysmal arrhythmias	Panic attack
Weight loss	+/-	+	-	-	-	-	-	-	-
Tachycardia	+/-	+	-	-	-	+	+/-	+	+
Palpitations	++	++	-	-	+	++	+/-	++	++
Tremor	+	+	-	-	-	++	+/-	-	+
Perspiration	++	+	-	-	++	+	+/-	-	+
Flushing	-	-	++	++	++	-	-	-	-
Hypertension	++	+/-	-	-	-	-	+/-	+	-
Abdominal pain	-	+/-	++	++	-	-	++	-	+
Diarrhoea	-	+/-	++	++	--	--	-	--	+/-

-- = never; - = seldom; +/- = sometimes; + = often; ++ = classical symptom.

Table 6. *Additional testing*

Disease	Additional diagnostics
Pheochromocytoma	Plasma free metanephrines or urine fractionated metanephrines
Thyrotoxicosis	TSH, free T4
Mastocytosis	Main criterion: mast cell aggregates of > 15 in bone marrow. Additional criteria: - atypical mast cell morphology - abnormal immunophenotype (CD2,CD25,CD35) - serum tryptase - codon 816 mutation of c-kit cells in affected tissue. For diagnosis: main criterion with an additional criterion, or three additional criteria. Alternative: - urine histamine - skin biopsy: urticaria pigmentosa
GEP-NET/ Carcinoid syndrome	- HIAA in 24-hour urine Platelet 5-HIAA
Hypogonadism	LH, FSH Male: testosterone Women: oestrogen
Hypoglycaemia	Fasting glucose Glucose, insulin, C-peptide, proinsulin, beta-hydroxybutyrate and circulating oral hypoglycaemic agents
Porphyria	Porphobilinogen in 24-hour urine
Panic attack	None
Paroxysmal arrhythmias	ECG, Holter recording

Table 7. *Criteria for diagnosis of panic disorder*[17]

Recurrent unexpected panic attack, defined as a discrete period of intense fear or discomfort in which four (or more) of the following symptoms develop abruptly and reach a peak within 10 minutes:
Palpitations, pounding heart
Sweating
Trembling or shaking
Shortness of breath or choking
Feeling of choking
Chest pain or discomfort
Nausea or abdominal distress
Feeling dizzy, light-headed, or faint
Derealisation or depersonalisation
Fear of losing control or going crazy
Fear of dying
Chills or hot flushes
Paresthesias

At least one of the attacks followed by one month (or more) of one (or more) of the following:
Persistent concern about having additional attacks
Worry about the implications of the attack or its consequences
A clinically significant change in behaviour related to the attacks

Panic attacks not due to the direct physiological effects of an illicit substance (or a prescribed medication) or a general medical condition (e.g., hyperthyroidism)

Panic attacks not better accounted for by another mental disorder, such as social phobia (on exposure to a feared social situation), a specific phobia (during exposure to a specific situation that prompts a phobic response), post-traumatic stress disorder (in response to stimuli associated with a severe stressor), or separation anxiety disorder (in response to being away from home or from a close relative)

Diagnostic and Statistical Manual of Mental Disorders, 4th edition

Systemic mastocytosis

Mastocytosis is characterised by an abnormal increase in the number of tissue mast cells.[12] Mast-cell disease is most commonly seen in the skin, but the skeleton, bone marrow, gastrointestinal tract, and central nervous system may also be involved.[12] In up to 20% of cases syncope and flushing, caused by degranulation of mast cells, is a prominent symptom.[18] A typical attack usually lasts 15 to 30 minutes, and can be accompanied by flushing, dyspepsia, diarrhoea, abdominal pain, musculoskeletal pain, hypotension and syncope.[1] Precipitating factors are: heat, exertion, emotion, sexual arousal and medications such as aspirin and

opioids. An attack can be followed by a period of lethargy. In 90% of patients the skin is involved, manifested by pruritus, urticaria pigmentosa (a red brown macular rash with a wheal and flare reaction) and dermatographism.[18] Therefore, the finding of urticaria pigmentosa in a patient evaluated for unexplained flushing points to a diagnosis of mastocytosis.[4]

Diagnosis is established by demonstration of mast cell proliferation in involved tissues. Biopsy of an urticaria pigmentosa lesion is the least invasive way to obtain histology.[19] Otherwise a bone marrow biopsy with immunohistochemical staining against mast cell markers (CD117 and tryptase) is indicated as this test is positive in about 90% of cases. An alternative approach is to search for elevated levels of biochemical markers of mast cell activation such as serum tryptase or urine histamine and its metabolites.[20,21]

Pheochromocytoma

Pheochromocytoma is a catecholamine-producing tumour arising from the chromaffin cells of the adrenal medulla, or sympathetic ganglia (paraganglioma).[9] Hypertension is not always present but can be paroxysmal and some patients present with hypotension and orthostatic hypotension.[9,22] Symptoms include a traditional triad of headaches, palpitations and sweating with a specificity of 90% when present together.[9,22] A typical pheochromocytoma spell usually lasts 10 to 60 minutes with a wide frequency of occurrence from daily to several times per year.[1] Pallor is more common than flushing with a reported frequency of 40 to 45%; flushing occurs in 10 to 20% of cases only and is due to primary epinephrine and dopamine secretion or a cosecreted peptide such as vasoactive intestinal polypeptide.[1,9] Other signs and symptoms may include nausea, weight loss, tiredness, anxiety or panic, papilla oedema with loss of vision, weight loss, hyperglycaemia, polyuria, polydipsia, increased haematocrit, and cardiomyopathy.[1,9]

Hereditary pheochromocytomas occur in paraganglioma syndromes, multiple endocrine neoplasia type 2, Von Hippel-Lindau syndrome, neurofibromatosis type 1 and paraganglioma syndromes. Frequently, however, pheochromocytoma is not the first manifestation and often other typical symptoms are present.[9]

Diagnosis depends on biochemical evidence of catecholamine overproduction of the tumour and is best based on plasma-free metanephrines or metanephrines and normetanephrines in 24-hour urine specimens.[23,24] The sensitivity of plasma-free metanephrines for the detection of a pheochromocytoma is 99% with a specificity of 89%.[9,23] If determination of plasma metanephrines is unavailable, then the second best diagnostic test is urinary fractionated metanephrines (normetanephrine and metanephrine separately) with a sensitivity and specificity of 97% and 69% respectively.[12,16] However, mildly elevated catecholamines and/or metanephrines may be aspecific and this further aggravates the diagnostic challenge.[25]

Gastroenteropancreatic neuroendocrine tumours

Gastroenteropancreatic neuroendocrine tumours (GEP-NET) are solid tumours arising from the diffuse endocrine system. They originate from enterochromaffin or the closely related enterochromaffin-like cells, present in most organs but primarily in the submucosa of the gut (67%, where they are most common in small intestine (25%), appendix (12%), and rectum (14%)[26]) and the respiratory system.[27] The incidence is estimated at 1 to 2 per 100,000, but is probably higher, since many GEP-NET are indolent, and may secrete hormonal peptides, without the characteristics of a clinical syndrome (nonfunctioning neuroendocrine tumours). This was confirmed by a recent study which showed a substantial discrepancy in numbers of diagnosed GEP-NET between a clinical and a pathological setting.[28]

Neuroendocrine tumours can produce various signalling proteins, of which serotonin (i.e. 5-hydroxytryptamine) is the most prominent. Other substances that may be released include histamine, dopamine, substance P, neurotensin, prostaglandins and kallikrein. Carcinoid syndrome occurs when liver or lung metastases have formed and metabolism of these neurosecretory substances is impaired, thereby causing significant release into the systemic circulation. This includes both episodic symptoms and those signs and symptoms due to structural changes induced by these substances.[13] The medullary thyroid carcinoma and pancreatic VIPoma may present with flushing due to secretion of calcitonin and vasoactive intestinal peptide respectively. Both diseases are also associated with chronic diarrhoea, although VIPoma in particular causes massive diarrhoea. Flushing mainly occurs in medullary thyroid carcinoma when metastases have developed.

Presentation varies with the embryological origin of the tumour, but carcinoid syndrome is mainly associated with neuroendocrine tumours of the stomach and ileum. Typical symptoms are attacks of flushing, diarrhoea and wheezing.[13] A 'carcinoid spell' can be provoked by exercise, or ingestion of blue cheese, chocolate (both containing tyramine) and alcohol. The flush may last from seconds up to hours and is associated with a mild burning sensation, predominantly affecting the upper body half with colour changes from blush pink to blue.[13] As the disease progresses, the flushing periods may become longer and more diffuse and cyanotic. In severe cases tachycardia, hypotension and angio-oedema may be present.[13] Carcinoid heart disease is characterised by pathognomonic plaque-like deposits of fibrous tissue. These deposits occur most commonly on the endocardium

of valvular cusps and leaflets, the cardiac chambers, and occasionally on the intima of the pulmonary arteries or aorta.[11] The right side of the heart is most often affected.[29,30]

The 5-hydroxyindoleacetic acid (5-HIAA) urinary level in a 24-hour sample is most commonly used in the endocrine workup of neuroendocrine tumours.[31,32] However, inference with drugs and food products is common.[31,32] Platelet serotonin is a more discriminating marker for the diagnosis of neuroendocrine tumours, especially in foregut tumours, where urine serotonin may have a poor sensitivity.[33,34]

Thyrotoxicosis

Thyrotoxicosis is a condition showing an excess of circulating thyroid hormone, which activates the adrenergic system.[35] The incidence is 0.5%, with Graves' disease and toxic nodular goitre comprising the majority of new cases.[35-37] Patients may suffer from palpitations, tremor, heat sensitivity, irregular menses in women, anxiety or nervousness.[35,36] In addition, more non-specific symptoms such as fatigue and weight loss may be seen.[35,36] Episodic symptoms can be caused by cardiac arrhythmias which, when present, require thyroid function testing especially in the elderly.[38] Due to the high prevalence of thyrotoxicosis, it may be difficult to establish if symptoms are fully explained by the thyrotoxicosis. In this respect subclinical thyrotoxicosis is only very rarely the cause of significant symptoms.

Hypoglycaemia

Hypoglycaemia raises adrenergic activity, resulting in classical symptoms such as sweating, anxiety, tremor, palpitations, tachycardia, hunger and nausea. If left untreated, neuroglycopenia occurs, resulting in dizziness, headache, visual disturbances, confusion, and ultimately coma and even death.[39,40] Blood glucose lowering drugs and alcohol are the most common causes of hypoglycaemia.[39,41] In critically ill patients, sepsis, renal, hepatic and adrenal failure should be investigated as causes of hypoglycaemia.[39] In patients with a history of gastric bypass surgery, hypoglycaemia resulting from nesidioblastosis is recognised.[42,43] Thus, unexplained spells post-bariatric surgery should raise suspicion of hypoglycaemia. Another less frequent cause of hypoglycaemia is insulinoma. This rare condition should be suspected in patients in which the Whipple's triad is positive (i.e. symptoms of hypoglycaemia, severe hypoglycaemia documented during the attack, relief from the attack by administering glucose.[39,42] Finally, one should always bear in mind that hypoglycaemia can be factitious or even felonious.[39,41]

With regards to diagnosis, it is essential to document hypoglycaemia before pursuing a differential diagnosis of hypoglycaemia. In a patient suspected of insulinoma, a supervised fast should be carried out, with measurement of glucose, insulin, C-peptide, proinsulin, beta-hydroxybutyrate and circulating oral hypoglycaemic agents. When symptoms of hypoglycaemia occur, this should then be corrected with 1.0 mg of glucagon iv with subsequent measurement of glucose. The response will most likely establish diagnosis.[39,40] In an asymptomatic patient in whom fasting hypoglycaemia is suspected a prolonged (72-hour) supervised fast is indicated. When the history is suggestive of postprandial hypoglycaemia an appropriate meal should be served to the patient;[39] for an interpretation of the test result, see *table 3* from Cryer et al.[39]

Porphyria

Porphyria includes several hereditary disorders with a defect in the haeme biosynthetic pathway, with accumulation of porphyrin precursors as a result.[44] One of these conditions is acute intermittent porphyria, classically presenting with colicky abdominal pain and a range of neurological symptoms. Fever, tachycardia, sweating, tremor and hypertension may also be present.[44] In addition, psychiatric symptoms may occur.[45] The urine turns red due to excretion of the precursors of haeme synthesis.[44] A spell can be triggered by various stressors, including alcohol, infections, menstruation, and various medications.[44] Diagnosis is established by demonstrating a markedly increased urinary porphobilinogen in a urine specimen.[44]

Paroxysmal arrhythmias

An underlying cardiac arrhythmia is the most common cause of palpitations. The severity of the clinical symptoms depends on the remaining cardiac output. Decrease in cardiac function can lead to clinical symptoms such as breathlessness, chest pain and syncope. Paroxysmal arrhythmias may also occur in other conditions such as thyrotoxicosis, pheochromocytoma, hypoglycaemia and panic attacks. The mutual underlying pathophysiological mechanism is increased activity of the adrenergic system. Syncope is usually benign but can be the only warning symptom before an episode causing sudden death.[46] If a syncopal event is suspected, an electrocardiogram is indicated[47] and will lead to a diagnosis in 2 to 11% of cases.[47,48] Other causes of transient loss of consciousness are epilepsy, psychogenic disorders and several rare miscellaneous disorders.[49]

DISCUSSION

Some common conditions presenting with episodic symptoms will rarely cause diagnostic problems. Hot flushes in combination with age and menstrual pattern in

women, or in combination with prescribed anti-androgen therapy in men, will usually be readily explained by hypogonadism. In daily practice, most episodes of hypoglycaemia occur in patients known to have diabetes mellitus. If the patient is not known to have diabetes a full work-up is clearly indicated. Tachycardia and palpitations are most common in paroxysmal cardiac arrhythmias or panic disorders. However, in any patient presenting with these complaints, the exclusion of hyperthyroidism as an underlying cause with a single determination of the TSH level is appropriate.

In the category of patients with a more complex spell phenotype resembling pheochromocytoma, carcinoid syndrome or mastocytosis, diagnosis is far more difficult. Hopefully, the tables and figures we present in this overview will provide some guidance.

CONCLUSION

Patients who present with a spell represent a diagnostic challenge. History and physical examination are essential in obtaining clues about the cause of the spell. Hereafter the use of other diagnostic tests should be guided by the differential diagnosis.

REFERENCES

1. Young WF Jr, Maddox DE. Spells: in search of a cause. Mayo Clin Proc. 1995;70:757-65.
2. Izikson L, English JC, Zirwas MJ. The flushing patient: differential diagnosis, workup, and treatment. J Am Acad Dermatol. 2006;55:193-208.
3. Khoo TK, Service FJ. 47-Year-old woman with spells of slurred speech, blurred vision, and loss of consciousness. Mayo Clin Proc. 2006; 81:1495-8.
4. Metcalfe DD. Differential diagnosis of the patient with unexplained flushing/anaphylaxis. Allergy Asthma Proc. 2000;21:21-4.
5. Mohyi D, Tabassi K, Simon J. Differential diagnosis of hot flashes. Maturitas. 1997;27:203-14.
6. Scully RE. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 7-1992. A 57-year-old man with a 20-year history of episodic headache, Flushing, hypotension, and occasional syncope. N Engl J Med. 1992;326:472-81.
7. Stehouwer CD, Gans RO. [Clinical thinking and decision-making in practice. A patient with episodes of flushing]. Ned Tijdschr Geneeskd. 1999;143:792-6.
8. Suchard JR. Recurrent near-syncope with flushing. Acad Emerg Med. 1997;4:718-24.
9. Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Phaeochromocytoma. Lancet. 2005;366:665-75.
10. Samuels MA, Pomerantz BJ, Sadow PM. Case records of the Massachusetts General Hospital. Case 14-2010. A 54-year-old woman with dizziness and falls. N Engl J Med. 362:1815-23.
11. Pandya UH, Pellikka PA, Enriquez-Sarano M, Edwards WD, Schaff HV, Connolly HM. Metastatic carcinoid tumor to the heart: echocardiographic-pathologic study of 11 patients. J Am Coll Cardiol. 2002;40:1328-32.
12. Golkar L, Bernhard JD. Mastocytosis. Lancet. 1997; 349:1379-85.
13. Hodgson HJF. Carcinoid tumours and the carcinoid syndrome. In: Wass JAH, Shapiro MD, editors. Oxford textbook of endocrinology and diabetes. Oxford: Oxford University Press, 2002.
14. Stearns V, Ullmer L, Lopez JF, Smith Y, Isaacs C, Hayes D. Hot flushes. Lancet. 2002; 360:1851-61.
15. Bastian LA, Smith CM, Nanda K. Is this woman perimenopausal? JAMA 2003;289:895-902.
16. Katon WJ. Clinical practice. Panic disorder. N Engl J Med. 2006;354:2360-7.
17. Diagnostic and statistical manual of mental disorders. 4th ed rev.: DSM-IV-R ed. Washington, DC: American Psychiatric Association, 2000.
18. Shome GP, Nangia R, Baldwin JL. Flushing and syncopal episode in a 47-year-old female. Ann Allergy Asthma Immunol. 2001;86:161-5.
19. Topar G, Staudacher C, Geisen F, et al. Urticaria pigmentosa: a clinical, hematopathologic, and serologic study of 30 adults. Am J Clin Pathol. 1998;109:279-85.
20. Granerus G, Olafsson JH, Roupe G. Studies on histamine metabolism in mastocytosis. J Invest Dermatol. 1983;80:410-6.
21. Schwartz LB, Metcalfe DD, Miller JS, Earl H, Sullivan T. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. N Engl J Med. 1987;316:1622-6.
22. Plouin PF, Degoulet P, Tugaye A, Ducrocq MB, Menard J. [Screening for phaeochromocytoma : in which hypertensive patients? A semiological study of 2585 patients, including 11 with phaeochromocytoma (author's transl)]. Nouv Presse Med. 1981;10:869-72.
23. Lenders JW, Pacak K, Walther MM, et al. Biochemical diagnosis of pheochromocytoma: which test is best? JAMA. 2002; 287:1427-34.
24. Pacak K, Linehan WM, Eisenhofer G, Walther MM, Goldstein DS. Recent advances in genetics, diagnosis, localization, and treatment of pheochromocytoma. Ann Intern Med. 2001;134:315-29.
25. Bisschop PH, Corssmit EP, Baas SJ, et al. Evaluation of Endocrine Tests. C: glucagon and clonidine test in phaeochromocytoma. Neth J Med. 2009;67:91-5.
26. Modlin IM, Kidd M, Latich I, Zikusoka MN, Shapiro MD. Current status of gastrointestinal carcinoids. Gastroenterology. 2005;128:1717-51.
27. Lechago J. Gastrointestinal neuroendocrine cell proliferations. Hum Pathol. 1994;25:1114-22.
28. Kuiper P, Verspaget HW, Overbeek LI, Biemond I, Lamers CB. An overview of the current diagnosis and recent developments in neuroendocrine tumours of the gastroenteropancreatic tract: the diagnostic approach. Neth J Med. 2011; 69:14-20.
29. Lundin L, Norheim I, Landelius J, Oberg K, Theodorsson-Norheim E. Carcinoid heart disease: relationship of circulating vasoactive substances to ultrasound-detectable cardiac abnormalities. Circulation. 1988; 77:264-9.
30. Pellikka PA, Tajik AJ, Khandheria BK, et al. Carcinoid heart disease. Clinical and echocardiographic spectrum in 74 patients. Circulation. 1993; 87:1188-96.
31. Feldman JM. Urinary serotonin in the diagnosis of carcinoid tumors. Clin Chem. 1986; 32:840-4.
32. Sjoblom SM. Clinical presentation and prognosis of gastrointestinal carcinoid tumours. Scand J Gastroenterol 1988; 23:779-87.
33. Onaitis MW, Kirshbom PM, Hayward TZ, et al. Gastrointestinal carcinoids: characterization by site of origin and hormone production. Ann Surg. 2000;232:549-56.
34. Meijer WG, Kema IP, Volmer M, Willemse PH, de Vries EG. Discriminating Capacity of Indole Markers in the Diagnosis of Carcinoid Tumors. Clin Chem. 2000; 46:1588-96.
35. Cooper DS. Hyperthyroidism. Lancet. 2003;362:459-68.
36. Brent GA. Clinical practice. Graves' disease. N Engl J Med. 2008;358:2594-605.

37. Muller AF, Berghout A, Wiersinga WM, Kooy A, Smits JW, Hermus AR. Thyroid function disorders--Guidelines of the Netherlands Association of Internal Medicine. Neth J Med. 2008;66:134-42.

38. Boelaert K, Torlinska B, Holder RL, Franklyn JA. Older subjects with hyperthyroidism present with a paucity of symptoms and signs: a large cross-sectional study. J Clin Endocrinol Metab. 95:2715-26.

39. Cryer PE, Axelrod L, Grossman AB, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2009; 94:709-28.

40. Service FJ. Hypoglycemic disorders. N Engl J Med. 1995; 332:1144-52.

41. Marks V, Teale JD. Hypoglycemia: factitious and felonious. Endocrinol Metab Clin North Am. 1999;28:579-601.

42. Service GJ, Thompson GB, Service FJ, Andrews JC, Collazo-Clavell ML, Lloyd RV. Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. N Engl J Med. 2005;353:249-54.

43. Bernard B, Kline GA, Service FJ. Hypoglycaemia following upper gastrointestinal surgery: case report and review of the literature. BMC Gastroenterol. 2010; 10:77.

44. Anderson KE, Bloomer JR, Bonkovsky HL, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med. 2005;142:439-50.

45. Auchincloss S, Pridmore S. Vomiting, burns, and irrational behaviour. Lancet. 2001;358:1870.

46. Grubb BP. Clinical practice. Neurocardiogenic syncope. N Engl J Med. 2005;352:1004-10.

47. Preblick-Salib C, Jagoda A. Spells. Differential diagnosis and management strategies. Emerg Med Clin North Am. 1997;15:637-48.

48. Day SC, Cook EF, Funkenstein H, Goldman L. Evaluation and outcome of emergency room patients with transient loss of consciousness. Am J Med. 1982;73:15-23.

49. Moya A, Sutton R, Ammirati F, et al. Guidelines for the diagnosis and management of syncope (version 2009): the Task Force for the Diagnosis and Management of Syncope of the European Society of Cardiology (ESC). Eur Heart J. 2009; 30:2631-71.

Netherlands
The Journal of Medicine

REVIEW

Alcohol-induced Cushing syndrome

Hypercortisolism caused by alcohol abuse

F. Besemer*, A.M. Pereira, J.W.A. Smit

Department of Internal Medicine and Endocrinology, C4-R, Leiden University Medical Center, Leiden, the Netherlands, *corresponding author: tel.: +31 (0)71-52 63 082

ABSTRACT

Background: Cushing's syndrome (CS), a rare syndrome caused by overexposure to glucocorticoids, is difficult to diagnose. The underlying causes of CS include pituitary and ectopic adrenocorticotropic hormone (ACTH) producing tumours and adrenal adenomas or hyperplasia. Alcoholism, however, can cause similar symptoms, giving rise to a so-called pseudo-Cushing state, which aggravates the differential diagnostic dilemmas of CS.

Aim: To document any specific clinical or biochemical features of alcohol-induced CS.

Methods: A Medline computer-aided search was performed to identify studies that have attempted to differentiate between alcohol-induced pseudo-Cushing and CS. Only original articles, not reviews, written in English were included. A total of 62 articles were included.

Results: Clinical and biochemical abnormalities mimicking increased hypothalamus-pituitary-adrenal (HPA) axis activity were found in the majority of the patients, although the severity of the changes varied widely. The most frequently occurring abnormalities were: insufficient suppression after low-dose dexamethasone or increased 24-hour urinary free cortisol (UFC). After alcohol withdrawal, cortisol decreased and dexamethasone-induced suppression of cortisol increased. No differences were noted between alcoholic and control subjects after an ACTH stimulation test, insulin tolerance test or metyrapone test. Differences were found after a naloxone test and hexarelin test. Studies using corticotropin-releasing hormone stimulation and tests after ethanol ingestion revealed inconclusive results.

Conclusion: There is no clear definition for the alcohol-induced pseudo-Cushing state, and hitherto studies fail to provide clues to differentiate between pseudo-Cushing and Cushing's syndrome. Only cessation of alcohol can normalise biochemical abnormalities and regress hypercortisolic symptoms.

KEYWORDS

Alcohol, cortisol, Cushing's syndrome

INTRODUCTION

Cushing's syndrome (CS) is an extremely rare clinical syndrome characterised by overexposure to glucocorticoids with an annual incidence of two to three per million.[1,2] CS is caused by uncontrolled overexposure of the body, including the central nervous system, to corticosteroids. This results in a phenotype characterised by psychopathology (mainly depression), features of the metabolic syndrome (such as insulin resistance, hypertension, and abdominal fat accumulation), but also proximal muscle wasting, and easy bruisability.[3,4] This uncontrolled exposure to endogenous cortisol is caused by tumours, which overproduce corticotropin-releasing hormone (CRH, in very rare cases), adrenocorticotropic hormone (ACTH, by pituitary adenomas or ectopic tumours), or cortisol (by adrenal tumours). Cushing's syndrome is a fatal condition in the absence of adequate treatment. Although the severe classical phenotype is not difficult to diagnose, some frequently occurring illnesses such as depression, obesity, physical stress, and chronic alcoholism can induce a phenotype that largely overlaps with CS and is also accompanied with increased cortisol exposure, and as a consequence, is called a pseudo-Cushing state. The discrimination between CS and these frequently occurring pseudo-Cushing states is difficult because many symptoms of CS, such as overweight, depressed mood, hypertension and irregular menses, are also prevalent in pseudo-CS, and therefore, biochemical tests often provide equivocal results resulting in a low specificity.[5] This review aims to focus on the diagnosis and therapy for alcohol-induced pseudo-Cushing's syndrome.

METHODS

A computer-aided search was performed in Medline using the search terms "alcohol AND hypercortisolism", "alcohol AND cushing", "alcoholic AND cortisol" and "alcoholic AND cushing". Only articles written in English with an abstract were included. The search was performed on 9 September 2010 and resulted in a total of 423 articles. Based on title and abstracts, 352 were excluded; the remaining 71 articles were retrieved for further evaluation. One article was not available[6] and another eight were excluded after complete evaluation, because they did not present original data[7] or were not relevant for the aim of the present study.[8-14] Finally, a total of 62 articles were included for detailed analysis.

RESULTS

Clinical presentation

Case reports were identified on patients who had symptoms compatible with CS, which eventually proved to be the result of alcoholic addiction, which illustrates that symptoms of the diseases resemble each other.[15-21]

Details on physical examination were reported in all 16 cases; 87.5% had a moon face, 69% hypertension, 81% muscle weakness or tiredness, 12.5% striae and 75% truncal obesity. In Cushing's syndrome, moon face is reported in 82 to 90%, hypertension in 68 to 75%, weakness in 60 to 64%, and obesity in 95% of cases, respectively.[3,4]

Clinical symptoms were reported in 10 out of 16 cases: 50% had weight gain, 30% easy bruisability, 30% sleepiness, 20% headache and only one person suffered from depression. In CS easy bruisability is reported in 65%, whereas headache and sleepiness are not reported.[3,4]

BIOCHEMICAL PRESENTATION

Circulating cortisol concentrations

In the case reports cortisol was elevated in 12 out of 13 patients, which normalised after alcohol abstinence in all patients.[15,16,19-21] Twelve studies investigated plasma cortisol concentrations in alcoholic patients. Six studies found no differences with control subjects, while six other studies found elevated cortisol levels in alcoholics.[28-33] A decrease in mean cortisol after abstinence (7-30 days) was seen in three other studies.[31,34,35] Three studies measured cortisol during 24 hours and observed no differences in circadian rhythms in alcoholics compared with controls,[36-38] although mean cortisol decreased in one study after approximately 30 days of abstinence.[37] Coiro, *et al.* measured cortisol in ten women with alcohol-induced pseudo-Cushing and found a higher fasting cortisol at 8.30 am compared with controls.[39] Frias *et al.* evaluated teenagers and adolescents during acute alcohol intoxication and found elevated cortisol levels, which was more pronounced in females.[40,41] In the study by Bannan and colleagues, 20% of alcoholics were found to have hypercortisolism that appeared to be positively correlated to withdrawal symptoms.[42]

Stalder *et al.* detected higher cortisol in the hair of subjects who had recently stopped drinking alcohol compared with both controls and subjects who had a longer abstinence period.[43]

Salivary cortisol

Salivary cortisol was measured in only two studies: The first (Adinoff *et al.*) reported higher salivary cortisol concentrations at daytime in intoxicated alcoholics and non-intoxicated withdrawal subjects than controls,[44] whereas Beresford noted a higher salivary cortisol in heavy drinking than light drinking alcohol-dependent subjects at awaking and 30 minutes thereafter.[45]

Urinary free cortisol (UFC) excretion

Five studies measured 24-hour UFC.[33,39,46-48] Coiro *et al.* reported higher fasting mean cortisol than control subjects but no difference between pseudo-Cushing and Cushing patients.[39] Stewart *et al.* and Wand *et al.* noted a higher mean 24-hour UFC in 28 alcoholics *vs* 32 controls.[33,47] In contrast Rosman *et al.* reported a lower cortisol in alcoholic subjects, which was to be expected since all his subjects had proven cirrhosis or compelling clinical evidence for it.[46] Willenbring observed normal urinary cortisol that decreased even further after three weeks of alcohol abstinence.[48] Urinary cortisol or corticoids were elevated in three subjects presented in case reports,[16,21] slightly elevated in two[17,18] but not different compared with controls in seven subjects.[15,20]

Dynamic tests to evaluate the HPA axis

Low-dose (1 mg) oral dexamethasone suppression: The dexamethasone suppression test (DST) can be used to demonstrate hypercortisolism. The Endocrine Society advises giving 1 mg dexamethasone orally at 11 pm and taking blood samples the next day at 8 am for determination of plasma cortisol, which should be suppressed to below 50 nmol/l to obtain a specificity greater than 95%.[49] Nine studies took blood samples in alcoholics as recommended, and their results are presented in *table 1*.[27,39,46,50-55] Insufficient suppression was reported in 0 to 75%. Three studies performed the test at admission for alcohol detoxification and found inadequate suppression in 50% (35/70) of patients. After an average of four weeks of alcohol withdrawal, cortisol suppression was still inadequate in seven out of 59 patients.[27,50,53]. In another study,[56] patients also underwent a DST at different periods

Table 1. *Studies reporting the results of a 1 mg dexamethasone suppression test*

Study	N	Age (yrs)	Duration alcoholism (yrs)	Amount alcohol	Time since last drink	Cortisol > 5 μg/dl after DST	Cortisol > 5 μg/dl after abstinence
Bailly	10	27-47	15.8	258	?	60%	10%
Coiro 2000	8	35.7	≥ 5	189.1 g/day	?	75%	
Coiro 2004	10	31.7	≥ 5	240 g/wk	?	60%	
Emsley	36	41-60 30-58	22/22	?	40/6 months	0%	
Oszoy	30	43.03	21.83	330.4 g/day	≥ 4 weeks	22.22%	13.6%
Ravi	30	40	9.6	≥ 15 whiskey on ≥ 3 days	0	63.3%	11.1%
Rosman	17	31-53	10-30	?	?	12.5%	
Swartz 1982	43	44.4/40.8	12.6/9.5	391/326 ml/day	?	4.65%	
Szucs	22	53	?	?	≥ 2weeks	?	

N = number of subjects, DST = dexamethasone suppression test.
Emsley: 19 korsakoff and 17 non-amnesic alcoholists. Swartz: means were reported for non-suppressors and suppressors respectively.

after withdrawal with cortisol measurements at 3 pm. The longer the period of abstinence, the more patients had normalised suppression. In three case reports, abnormal dexamethasone suppression test in alcoholic patients normalised after abstinence.[17,19,21]

Other cortisol suppression tests

Many studies used Carroll's concept for psychiatric research to measure cortisol at 4 and 11 pm after a dexamethasone test.[57] All 11 studies that measured cortisol at 4 pm noticed no suppression in some of the alcoholics, ranging from 6 to 63.3%.[27,48,50,53,54,58-62] Most patients were tested during alcohol withdrawal. Five studies measured cortisol at 11 pm; one of them found normal suppression,[52] the others did not.[27,50,53,61]

Majumdar (1988) observed a non-suppressed cortisol at 4 pm in two out of 20 detoxicated alcoholics after a 2 mg DST.[63] In six of 30 alcoholics who had just stopped drinking non-suppressed cortisol levels were found whereas after abstinence this number decreased to two. Rees also measured a high cortisol after a 2 mg DST in one out of two persons, which normalised after 16 days alcohol abstinence.[21] Proto performed a DST with 2 mg dexamethasone a day for three days and noted normal suppression of cortisol in all six subjects.[20]

Hundt did a DST with 1.5 mg dexamethasone with measurements at 8 am and detected abnormal suppression in four out of 19 alcoholic patients (21%) during alcohol withdrawal.[64]

Fink recruited ten alcoholic patients who were subjected to an intravenous DST with 1 mg/hour of dexamethasone for two hours with subsequent cortisol measurements at 1/2, 1,1 1/2 and 2 hours.[65] Four patients showed abnormal cortisol values.

Combined suppression and stimulation tests

Three studies found no differences in cortisol between alcoholics and controls using the 250 μg ACTH stimulation test.[29,47,55] One of these studies noticed that normal subjects had a cortisol response after a 0.25 μg ACTH stimulation test, which was absent in alcoholics.[47] Another study observed that two out of 13 tested alcoholics did not meet criteria for normal responsiveness; however, four alcoholics had higher levels than normal subjects.[46]

A CRH test (100 μg or 1 μg/kg bolus) was performed in seven studies and three observed no differences in cortisol response in alcoholics and controls,[25,30,50] although two of them found a blunted ACTH response.[25,30] Two others observed a blunted cortisol response[22,47] and two noticed a higher cortisol.[64,66] Three studies retested the subjects after withdrawal, one reported a lower cortisol response,[50] while the others detected no differences between controls and alcoholics anymore.[22,64] Two studies reported no differences in cortisol after an insulin tolerance test (ITT) between non-intoxicated alcoholics and controls,[24,67] yet one noted a blunted ACTH response in alcoholics.[67] ACTH was too high in the subject that underwent an ITT presented by Lamberts.[19] Coiro 2000 performed a hexarelin test in 25 subjects and registered an increase in ACTH and cortisol in controls and subjects with Cushing, but not in alcoholics.[51] Wand performed a metyrapone test in 14 alcoholics and 13 volunteers, and found similar decrements in cortisol in alcoholics and controls.[47] Although the ACTH response and 11 deoxycortisol levels were much lower than in controls and thus 50% of alcoholics met criteria for adrenal insufficiency. Lamberts described a woman with a metyrapone test compatible with Cushing's syndrome.[19] Inder infused 20 mg naloxone in nine alcoholics and nine controls and showed a blunted rise followed by a slower fall to normal values in alcoholics.[25] The controls demonstrated positive correlations between basal cortisol and cortisol increment and ACTH increment, which were not present in the alcoholic subjects.

Nine studies measured cortisol response after alcohol intake. The studies all used different designs. Three of

them found no or a slight change in HPA hormones in alcoholics compared with controls,[29,68,69] three described a higher response[65-67,70-72] and two others observed a lower response.[73,74] One study noticed only changes in cortisol and ACTH in subjects who had gastrointestinal symptoms after drinking alcohol.[75]

The last two studies pointed towards an altered cortisol feedback mechanism in alcoholics. Lovallo measured cortisol after public speaking and detected no cortisol response in alcohol-dependent subjects, but did find this in controls.[76]

Coiro 2007 measured cortisol after an exercise test in alcoholics after four, six and eight weeks of abstinence.[77] After four weeks abstinence, exercise induced no change in cortisol and ACTH in contrast to in controls; after eight weeks the difference between controls and alcoholic had disappeared.

BEST TREATMENT OF ALCOHOL-INDUCED CUSHING

The search did not find any articles about treatment of alcohol-induced Cushing's syndrome. Nevertheless, case reports invariably demonstrated that resolution of symptoms and biochemical disturbances occurs after alcohol withdrawal.[15-21]

DISCUSSION

This review demonstrates that the clinical and biochemical features of the alcohol-induced pseudo-Cushing syndrome vary widely, and that no criteria are available to distinguish between CS and alcohol-induced CS. No stringent criteria were found for the definition of pseudo-Cushing syndrome. Different biochemical tests that evaluate different properties of the HPA axis yielded different results. In addition, standardisation of tests and test criteria varied widely precluding simple generalisations of test results. No laboratory tests yielded sufficient discriminatory power to detect alcohol-induced pseudo-Cushing syndrome. Signs and symptoms tend to normalise after cessation of alcoholism for at least a month. At end of the 1970s the first case reports appeared on patients with symptoms mimicking Cushing's syndrome, caused by alcoholism.[15-21] Tests that were characteristic for hypercortisolism normalised after cessation of alcohol abuse.

Plasma cortisol levels were higher in alcoholics in nine studies[28-33,40-42] and normal in six other studies.[22-27] This discrepancy can be explained by the different study designs: four of six studies that observed no differences in cortisol[22,24,25,27] were done in subjects who had stopped drinking alcohol for at least ten days. In contrast, seven of nine studies that reported a higher cortisol used subjects who stopped drinking for less than one day.[28,30,31,33,40-42] Consequently, the observed higher cortisol could either be provoked by alcohol or by the stress during the first days of abstinence.

Furthermore, blood samples of five out of six studies that observed no difference were taken between 8 and 9 am,[22,24-27] while this was the case in only two out of nine studies that observed differences.(32,33) Other studies did not reveal time or took samples late in the morning, afternoon or night. Since cortisol secretion has a circadian rhythm, time of sampling has a great influence on cortisol level.

Inadequate suppression was measured in 0 to 75% of alcoholics[27,39,46,50-55] during a 1 mg dexamethasone suppression test. However, the studies all used different designs. In the study that observed an appropriate suppression, tests were performed in subjects that were abstinent for at least 12 weeks.[52] One study only stated that there was no difference in plasma cortisol between controls and alcoholics.[55]

After one month of abstinence more suppression was observed using a 1 mg dexamethasone suppression test.[27,50,53] However, these three studies measured cortisol at three different moments and classified suppression as abnormal if at any of these moments suppression was abnormal. Also, they did not mention at which time the suppression was abnormal.

In conclusion, the literature does not provide any evidence for clinical symptoms or laboratory investigations suitable for diagnosing hypercortisolism caused by alcoholism. However, the number of patients studied was limited, different study designs, diverse definitions for alcoholism and pseudo-Cushing were used and tests were performed in various populations. Additionally, most studies were performed during alcoholic withdrawal, which is likely to induce stress and thus hypercortisolism.

Therefore, alcoholic patients diagnosed with CS should have repeat clinical and biochemical work-up after cessation of alcohol before subsequent (radiological) investigations can be performed.

REFERENCES

1. Etxabe J, Vazquez JA. Morbidity and mortality in Cushing's disease: an epidemiological approach. Clin Endocrinol (Oxf). 1994;40(4):479-84.
2. Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, et al. Incidence and late prognosis of cushing's syndrome: a population-based study. J Clin Endocrinol Metab. 2001;86(1):117-23.
3. Boscaro M, Barzon L, Fallo F, Sonino N. Cushing's syndrome. Lancet. 2001;357(9258):783-91.
4. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet. 2006;367(9522):1605-17.

5. Ross EJ, Linch DC. Cushing's syndrome--killing disease: discriminatory value of signs and symptoms aiding early diagnosis. Lancet. 1982;2(8299):646-9.

6. Loosen PT, Chambliss B, Ekhator N, Burns D, Geracioti TD, Orth DN. Thyroid and adrenal dysfunction in abstinent alcoholic men: locus of disturbance. Neuropsychopharmacology. 1993 Dec;9(4):255-66.

7. Jenkins RM. Alcohol-induced Cushingoid syndrome. Biomedicine. 1981;34(4):169-70.

8. Ahmed A, Saksena S, Sherlock M, Olliff SP, Elias E, Stewart PM. Induction of hepatic 11beta-hydroxysteroid dehydrogenase type 1 in patients with alcoholic liver disease. Clin Endocrinol (Oxf). 2008;68(6):898-903.

9. Cobb CF, Van Thiel DH, Gavaler JS, Lester R. Effects of ethanol and acetaldehyde on the rat adrenal. Metabolism. 1981;30(6):537-43.

10. Jeffcoate WJ, Silverstone JT, Edwards CR, Besser GM. Psychiatric manifestations of Cushing's syndrome: response to lowering of plasma cortisol. Q J Med. 1979;48(191):465-72.

11. Nava F, Premi S, Manzato E, Campagnola W, Lucchini A, Gessa GL. Gamma-hydroxybutyrate reduces both withdrawal syndrome and hypercortisolism in severe abstinent alcoholics: an open study vs. diazepam. Am J Drug Alcohol Abuse. 2007;33(3):379-92.

12. Rouach H, Ribiere C, Nordmann J, Nordmann R. Acute ethanol effects on rat liver tryptophan oxygenase and tyrosine aminotransferase. Pharmacol Biochem Behav. 1980;13(Suppl 1):139-43.

13. Zimmermann US, Buchmann A, Steffin B, Dieterle C, Uhr M. Alcohol administration acutely inhibits ghrelin secretion in an experiment involving psychosocial stress. Addict Biol. 2007;12(1):17-21.

14. Hu J, Xia Y, Wu Z, Liu L, Tang C. Fluoxetine might alleviate brain damage and hypercortisolemia related to chronic alcohol in rats. J Stud Alcohol Drugs. 2010;71(2):290-4.

15. Aron DC, Tyrrell JB, Fitzgerald PA, Findling JW, Forsham PH. Cushing's syndrome: problems in diagnosis. Medicine (Baltimore). 1981;60(1):25-35.

16. Endo Y, Chuhbachi A. Hyperlipemia in alcohol-induced pseudo-Cushing's syndrome. Jpn J Med. 1986;25(2):184-5.

17. Jordan RM, Jacobson JM, Young RL. Alcohol-induced Cushingoid syndrome. South Med J. 1979;72(10):1347-8.

18. Kapcala LP. Alcohol-induced pseudo-Cushing's syndrome mimicking Cushing's disease in a patient with an adrenal mass. Am J Med. 1987;82(4):849-56.

19. Lamberts SW, Klijn JG, de Jong FH, Birkenhager JC. Hormone secretion in alcohol-induced pseudo-Cushing's syndrome. Differential diagnosis with Cushing disease. JAMA. 1979;242(15):1640-3.

20. Proto G, Barberi M, Bertolissi F. Pseudo-Cushing's syndrome: an example of alcohol-induced central disorder in corticotropin-releasing factor-ACTH release? Drug Alcohol Depend. 1985;16(2):111-5.

21. Rees LH, Besser GM, Jeffcoate WJ, Goldie DJ, Marks V. Alcohol-induced pseudo-Cushing's syndrome. Lancet. 1977;1(8014):726-8.

22. Adinoff B, Martin PR, Bone GH, Eckardt MJ, Roehrich L, George DT, et al. Hypothalamic-pituitary-adrenal axis functioning and cerebrospinal fluid corticotropin releasing hormone and corticotropin levels in alcoholics after recent and long-term abstinence. Arch Gen Psychiatry. 1990;47(4):325-30.

23. del Arbol JL, Aguirre JC, Raya J, Rico J, Ruiz-Requena ME, Miranda MT. Plasma concentrations of beta-endorphin, adrenocorticotropic hormone, and cortisol in drinking and abstinent chronic alcoholics. Alcohol. 1995;12(6):525-9.

24. Eisenhofer G, Johnson RH, Lambie DG. Growth hormone, vasopressin, cortisol, and catecholamine responses to insulin hypoglycemia in alcoholics. Alcohol Clin Exp Res. 1984;8(1):33-6.

25. Inder WJ, Joyce PR, Ellis MJ, Evans MJ, Livesey JH, Donald RA. The effects of alcoholism on the hypothalamic-pituitary-adrenal axis: interaction with endogenous opioid peptides. Clin Endocrinol (Oxf). 1995;43(3):283-90.

26. Leggio L, Malandrino N, Ferrulli A, Cardone S, Miceli A, Gasbarrini G, et al. Is cortisol involved in the alcohol-related fat mass impairment? A longitudinal clinical study. Alcohol Alcohol. 2009;44(2):211-5.

27. Ozsoy S, Esel E. Hypothalamic-pituitary-adrenal axis activity, dehydroepiandrosterone sulphate and their relationships with aggression in early and late alcohol withdrawal. Prog Neuropsychopharmacol Biol Psychiatry. 2008;32(2):340-7.

28. Burra P, Franklyn JA, Ramsden DB, Elias E, Sheppard MC. Severity of alcoholic liver disease and markers of thyroid and steroid status. Postgrad Med J. 1992;68(804):804-10.

29. Elias AN, Meshkinpour H, Valenta LJ, Grossman MK. Pseudo-Cushing's syndrome: the role of alcohol. J Clin Gastroenterol. 1982 Apr;4(2):137-9.

30. Heuser I, von Bardeleben U, Boll E, Holsboer F. Response of ACTH and cortisol to human corticotropin-releasing hormone after short-term abstention from alcohol abuse. Biol Psychiatry 1988;24(3):316-21.

31. Kutscher S, Heise DJ, Banger M, Saller B, Michel MC, Gastpar M, et al. Concomitant endocrine and immune alterations during alcohol intoxication and acute withdrawal in alcohol-dependent subjects. Neuropsychobiology. 2002;45(3):144-9.

32. Stewart A, Johnston DG, Alberti KG, Nattrass M, Wright R. Hormone and metabolite profiles in alcoholic liver disease. Eur J Clin Invest. 1983;13(5):397-403.

33. Stewart PM, Burra P, Shackleton CH, Sheppard MC, Elias E. 11 beta-Hydroxysteroid dehydrogenase deficiency and glucocorticoid status in patients with alcoholic and non-alcoholic chronic liver disease. J Clin Endocrinol Metab. 1993;76(3):748-51.

34. Majumdar SK, Shaw GK, Bridges PK. Relationship between plasma adrenocorticotrophic hormone and cortisol concentrations in chronic alcoholic patients with depression. Drug Alcohol Depend. 1989;23(2):111-6.

35. Soardo G, Donnini D, Varutti R, Milocco C, Basan L, Esposito W, et al. Effects of alcohol withdrawal on blood pressure in hypertensive heavy drinkers. J Hypertens. 2006;24(8):1493-8.

36. Bertello P, Agrimonti F, Gurioli L, Frairia R, Fornaro D, Angeli A. Circadian patterns of plasma cortisol and testosterone in chronic male alcoholics. Alcohol Clin Exp Res. 1982;6(4):475-81.

37. Iranmanesh A, Veldhuis JD, Johnson ML, Lizarralde G. 24-hour pulsatile and circadian patterns of cortisol secretion in alcoholic men. J Androl. 1989;10(1):54-63.

38. Mukai M, Uchimura N, Hirano T, Ohshima H, Ohshima M, Nakamura J. Circadian rhythms of hormone concentrations in alcohol withdrawal. Psychiatry Clin Neurosci. 1998;52(2):238-40.

39. Coiro V, Volpi R, Galli P, Manfredi G, Magotti MG, Saccani-Jotti G, et al. Serum total prostate-specific antigen assay in women with Cushing's disease or alcohol-dependent pseudo-Cushing's State. Horm Res. 2004;61(3):148-52.

40. Frias J, Rodriguez R, Torres JM, Ruiz E, Ortega E. Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adolescents of both sexes. Life Sci. 2000;67(9):1081-6.

41. Frias J, Torres JM, Miranda MT, Ruiz E, Ortega E. Effects of acute alcohol intoxication on pituitary-gonadal axis hormones, pituitary-adrenal axis hormones, beta-endorphin and prolactin in human adults of both sexes. Alcohol Alcohol. 2002 Mar;37(2):169-73.

42. Bannan LT, Potter JF, Beevers DG, Saunders JB, Walters JR, Ingram MC. Effect of alcohol withdrawal on blood pressure, plasma renin activity, aldosterone, cortisol and dopamine beta-hydroxylase. Clin Sci (Lond). 1984;66(6):659-63.

43. Stalder T, Kirschbaum C, Heinze K, Steudte S, Foley P, Tietze A, et al. Use of hair cortisol analysis to detect hypercortisolism during active drinking phases in alcohol-dependent individuals. Biol Psychol. 2010;85:357-60.

44. Adinoff B, Ruether K, Krebaum S, Iranmanesh A, Williams MJ. Increased salivary cortisol concentrations during chronic alcohol intoxication in a naturalistic clinical sample of men. Alcohol Clin Exp Res. 2003;27(9):1420-7.

45. Beresford TP, Arciniegas DB, Alfers J, Clapp L, Martin B, Beresford HF, et al. Hypercortisolism in alcohol dependence and its relation to hippocampal volume loss. J Stud Alcohol. 2006;67(6):861-7.

46. Rosman PM, Farag A, Benn R, Tito J, Mishik A, Wallace EZ. Modulation of pituitary-adrenocortical function: decreased secretory episodes and blunted circadian rhythmicity in patients with alcoholic liver disease. J Clin Endocrinol Metab. 1982;55(4):709-17.

47. Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. J Clin Endocrinol Metab. 1991;72(6):1290-5.

48. Willenbring ML, Morley JE, Niewoehner CB, Heilman RO, Carlson CH, Shafer RB. Adrenocortical hyperactivity in newly admitted alcoholics: prevalence, course and associated variables. Psychoneuroendocrinology. 1984;9(4):415-22.

49. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2008;93(5):1526-40.

50. Bailly D, Dewailly D, Beuscart R, Couplet G, Dumont P, Racadot A, et al. Adrenocorticotropin and cortisol responses to ovine corticotropin-releasing factor in alcohol dependence disorder. Preliminary report. Horm Res. 1989;31(1-2):72-5.

51. Coiro V, Volpi R, Capretti L, Caffarri G, Chiodera P. Desmopressin and hexarelin tests in alcohol-induced pseudo-Cushing's syndrome. J Intern Med. 2000;247(6):667-73.

52. Emsley RA, Roberts MC, Aalbers C, Taljaard FJ, Kotze TJ. Endocrine function in alcoholic Korsakoff's syndrome. Alcohol Alcohol. 1994;29(2):187-91.

53. Ravi SD, Dorus W, Park YN, Collins MC, Reid RW, Borge GF. The dexamethasone suppression test and depressive symptoms in early and late withdrawal from alcohol. Am J Psychiatry. 1984;141(11):1445-8.

54. Swartz CM, Dunner FJ. Dexamethasone suppression testing of alcoholics. Arch Gen Psychiatry. 1982;39(11):1309-12.

55. Szucs N, Varga I, Patocs A, Toth M, Jakab C, Glaz E, et al. Plasma 6beta-hydroxycortisol measurements for assessing altered hepatic drug metabolizing enzyme activity. Acta Physiol Hung. 2003;90(3):217-23.

56. Balldin J, Berggren U, Bokstrom K, Lindstedt G, Sjoberg J, Wendestam C. Dexamethasone suppression test in alcohol withdrawal: relationship to depression and liver function. Drug Alcohol Depend. 1992;30(2):175-9.

57. Carroll BJ. Dexamethasone suppression test for depression. Adv Biochem Psychopharmacol. 1984;39:179-88.

58. Abou-Saleh MT, Merry J, Coppen A. Dexamethasone suppression test in alcoholism. Acta Psychiatr Scand. 1984;69(2):112-6.

59. Carson SW, Halbreich U, Yeh CM, Galanter M. Liver function, plasma dexamethasone, and DST results in detoxified alcoholics. Psychiatry Res. 1989;30(2):217-21.

60. Del Porto JA, Monteiro MG, Laranjeira RR, Jorge MR, Masur J. Reversal of abnormal dexamethasone suppression test in alcoholics abstinent for four weeks. Biol Psychiatry. 1985;20(11):1156-60.

61. Khan A, Ciraulo DA, Nelson WH, Becker JT, Nies A, Jaffe JH. Dexamethasone suppression test in recently detoxified alcoholics: clinical implications. J Clin Psychopharmacol. 1984;4(2):94-7.

62. Muller N, Hoehe M, Klein HE, Nieberle G, Kapfhammer HP, May F, et al. Endocrinological studies in alcoholics during withdrawal and after abstinence. Psychoneuroendocrinology. 1989;14(1-2):113-23.

63. Majumdar SK, Shaw GK, Bridges PK. The dexamethasone suppression test in chronic alcoholics with and without depression and its relationship to their hepatic status. Drug Alcohol Depend. 1988;21(3):231-5.

64. Hundt W, Zimmermann U, Pottig M, Spring K, Holsboer F. The combined dexamethasone-suppression/CRH-stimulation test in alcoholics during and after acute withdrawal. Alcohol Clin Exp Res. 2001;25(5):687-91.

65. Fink RS, Short F, Marjot DH, James VH. Abnormal suppression of plasma cortisol during the intravenous infusion of dexamethasone to alcoholic patients. Clin Endocrinol (Oxf). 1981;15(1):97-102.

66. Fonzi S, Solinas GP, Costelli P, Parodi C, Murialdo G, Bo P, et al. Melatonin and cortisol circadian secretion during ethanol withdrawal in chronic alcoholics. Chronobiologia. 1994;21(1-2):109-12.

67. Berman JD, Cook DM, Buchman M, Keith LD. Diminished adrenocorticotropin response to insulin-induced hypoglycemia in nondepressed, actively drinking male alcoholics. J Clin Endocrinol Metab. 1990;71(3):712-7.

68. Danel T, Vantyghem MC, Touitou Y. Responses of the steroid circadian system to alcohol in humans: Importance of the time and duration of intake. Chronobiol Int. 2006;23(5):1025-34.

69. Ida Y, Tsujimaru S, Nakamaura K, Shirao I, Mukasa H, Egami H, et al. Effects of acute and repeated alcohol ingestion on hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal functioning in normal males. Drug Alcohol Depend. 1992;31(1):57-64.

70. Dai X, Thavundayil J, Santella S, Gianoulakis C. Response of the HPA-axis to alcohol and stress as a function of alcohol dependence and family history of alcoholism. Psychoneuroendocrinology. 2007;32(3):293-305.

71. Linkola J, Fyhrquist F, Ylikahri R. Renin, aldosterone and cortisol during ethanol intoxication and hangover. Acta Physiol Scand. 1979;106(1):75-82.

72. Valimaki MJ, Harkonen M, Eriksson CJ, Ylikahri RH. Sex hormones and adrenocortical steroids in men acutely intoxicated with ethanol. Alcohol. 1984;1(1):89-93.

73. Ireland MA, Vandongen R, Davidson L, Beilin LJ, Rouse IL. Acute effects of moderate alcohol consumption on blood pressure and plasma catecholamines. Clin Sci (Lond). 1984;66(6):643-8.

74. Waltman C, Blevins LS, Jr., Boyd G, Wand GS. The effects of mild ethanol intoxication on the hypothalamic-pituitary-adrenal axis in nonalcoholic men. J Clin Endocrinol Metab. 1993;77(2):518-22.

75. Inder WJ, Joyce PR, Wells JE, Evans MJ, Ellis MJ, Mattioli L, et al. The acute effects of oral ethanol on the hypothalamic-pituitary-adrenal axis in normal human subjects. Clin Endocrinol (Oxf). 1995;42(1):65-71.

76. Lovallo WR, Dickensheets SL, Myers DA, Thomas TL, Nixon SJ. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. Alcohol Clin Exp Res. 2000;24(5):651-8.

77. Coiro V, Casti A, Jotti GS, Rubino P, Manfredi G, Maffei ML, et al. Adrenocorticotropic hormone/cortisol response to physical exercise in abstinent alcoholic patients. Alcohol Clin Exp Res. 2007;31(5):901-6.

Netherlands
The Journal of Medicine

REVIEW

False-negative tests in breast cancer management

A. Coolen[1], K. Leunen[1], J. Menten[2], W. van Steenbergen[3], P. Neven[1*]

Departments of [1]Gynaecology and Obstetrics, [2]Radiotherapy/Oncology, [3]Internal Medicine/Hepatology, Multidisciplinary Breast Centre (MBC), University Hospital Gasthuisberg, Leuven, Belgium, *corresponding author: tel.: +32 16 34 46 34, fax: +32 16 34 42 05, e-mail: patrick.neven@uz.kuleuven.ac.be

ABSTRACT

We review the sensitivity of different diagnostic tests for breast cancer management based on recent experience in a 34-year-old patient. False-negative tests at diagnosis of early disease and of relapse resulted in diagnostic and therapeutic delays.

Initial mammography and breast ultrasonography were falsely negative despite a palpable breast lump. Clinical examination and axillary ultrasound missed macroscopically involved lymph nodes. At relapse, metastatic lesions were missed despite symptoms, three years after primary treatment. CA 15-3 was normal; bone and liver metastases were missed by standard and more advanced imaging techniques including liver ultrasonography, nuclear bone scan and PET-CT scan. Worsening of clinical symptoms, lab results and abnormal tissue biopsies finally led to the diagnosis of extensive metastatic disease. Genetic screening showed an abnormality within the BRCA-1 region of unknown clinical importance.

This review highlights 1) that diagnostic tests managing symptomatic breast cancer patients may have a low sensitivity, 2) the importance of clinical findings and other markers for disease, such as lactate dehydrogenase and 3) the need for diagnostic biopsies for clinically suspect symptoms despite normal imaging and biochemistry.

KEYWORDS

Breast cancer, investigations, metastases, sensitivity

INTRODUCTION

In women, breast cancer is common and leads to significant morbidity and mortality. Early diagnosis of the disease and accurate recognition of life-threatening relapses affect disease outcome. Under the age of 40, breast cancer is mostly symptomatic with a palpable lump as the most frequent presenting symptom. The diagnosis is then confirmed by a diagnostic mammography, breast ultrasound and image-guided core biopsy. If staging excludes metastases, local and systemic therapies follow. Follow-up is advised with clinical examination at regular intervals and a yearly mammogram. Appropriate technical and biochemical tests are done when suspect symptoms arise.[1] Routine diagnostic investigations in general are sensitive enough to confirm/reject the initial diagnosis or relapse. We critically review this sensitivity following the case of a recent patient who died of metastatic breast cancer with false-negative tests both at primary diagnosis and relapse.

PATIENT

A 34-year-old healthy parous woman presented with a palpable lump in the left breast. In the family history, her mother was premenopausal when diagnosed with ovarian cancer and she died at the age of 50 years. The sister of her maternal grandmother had postmenopausal breast cancer and her father was treated for prostate cancer.

Four months before referral to the hospital, the same breast lump was evaluated by her general practitioner as non-suspect because the mammogram and breast ultrasound were normal; the lump was attributed to residual breast congestion due to recently stopped breastfeeding. Since the lump became firmer, she was referred to our breast unit. Clinical examination revealed a suspicious left breast lump of 35 x 30 mm in the upper inner quadrant without palpable lymph nodes (cT2N0). The repeated mammography remained normal; there was no architectural distortion or asymmetrical density. Breast ultrasound, however, showed a hyperechoic solid mass and

magnetic resonance imaging (MRI) was suspicious for a unifocal cancer. Lymph nodes were considered normal at imaging. An ultrasound-guided core biopsy revealed a grade 3 invasive and *in situ* ductal adenocarcinoma. There were no clinical signs of metastatic disease and this was confirmed by all preoperative biochemical (liver tests, serum calcium and CA 15.3) and imaging tests (chest X-ray, ultrasound of the liver and nuclear bone scan). A modified radical mastectomy with axillary clearance was performed. Pathology confirmed the malignancy, section margins were clear, the lympho-vascular space was involved and five of 14 axillary lymph nodes contained macrometastatic deposits (pT2N2). Oestrogen and progesterone receptors were moderately positive, HER-2-Neu was completely absent. Six courses of adjuvant chemotherapy (3x FEC q3w and 3x Taxotere 100 q3w) were given, followed by loco-regional radiotherapy with a total dose of 50 Gy in 25 daily fractions to the chest wall and median subclavian and parasternal lymph node area. Hormonal treatment was started during radiotherapy with monthly injections of the luteinising hormone-releasing hormone (LHRH)-agonist gosereline (Zoladex®) and a daily tablet of 20 mg of tamoxifen (Nolvadex D®). BRCA 1/2 mutation testing showed a mutation in exon 18 of the BRCA1 gene, a rare variant of unknown significance.

She was well for almost three years, but then started complaining of bone pain located in the pelvis and neck, temporarily improving with physiotherapy; biochemical markers were normal. Two months later, she was referred because of more symptoms (anorexia, bone pain, uncontrollable weight loss) and a doubled but almost normal tumour marker level: CA 15.3: 31 kU/l (normal: <30 kU/l). A bone scintigraphy and CT scan of chest and abdomen were normal. Six weeks later, CA 15.3 had risen to 81 kU/l. Lactate dehydrogenase (LDH), which had not been measured for six months, mounted to 1122 U/l (normal: 240-480 U/l), alanine aminotranferease and aspartate aminotransferase were 59 U/l and 77 U/l respectively (normal <31 U/l), whereas alkaline phosphatase was normal. A FDG-PET-CT scan at this stage was completely negative. Based on the clinical complaints and the increased CA 15.3 and LDH levels, tamoxifen was switched to the oral aromatase inhibitor anastrozole (Arimidex®) while the goserelin implants were continued. Given the discordance between the negative imaging and the patient's symptoms and biochemistry suggesting disease progression, an at random liver biopsy was performed. Frozen section of the biopsies was normal. The biopsy was complicated by severe intra-abdominal bleeding enhanced by an isolated thrombocytopenia ($32*10^9$/l; normal range: 150 to $450*10^9$/l). A platelet count had been done before the biopsy but unfortunately the result had been unnoticed. Blood platelets further dropped and levels of liver function tests were mounting. A hypovolaemic shock had to be stabilised with colloids, packed cells, platelet transfusion and intensive care management. The final pathology report of the liver biopsy stated necrotic hepatitis, most likely 'drug-related'.

All current medication (anastrazole, mirtazapin, antioxidants, zolpidem) and mistletoe, which she informed us she was using as complementary therapy to affect disease progression, were immediately stopped. The platelet count did not improve. A bone marrow aspiration and bone biopsy were performed, both confirming metastatic disease. Additional immuno-histochemical stainings on the earlier liver biopsy revealed tumour cells. Chemotherapy with paclitaxel (80 mg/m^2) was given every two out of three weeks because of haematological intolerance. The patient improved temporarily with normalising biochemistry. A CT scan after six courses showed diffuse blastic bone metastases that remained stable on CT following the 18th course. Bone scintigraphy and liver ultrasound remained negative for metastases. Shortly after interruption of the chemotherapy, again, an important elevation of liver tests and LDH occurred (*figure 1*). Her condition deteriorated and comfort therapy was started with the help of the palliative support team. She died 10 months after the diagnosis of metastatic breast cancer.

REVIEW OF THE LITERATURE

Standard diagnostic tests in managing breast cancer may sometimes fail in detecting loco-regional or metastatic disease, as was seen in our patient. Therefore we have reviewed the frequency of false-negative tests in breast cancer management and their impact on treatment and outcome. *Table 1* reviews the frequency each test is falsely negative.

Tests at diagnosis

1. Breast lump: An early compared with late diagnosis of breast cancer ensures a more favourable outcome.[1] Delay in diagnosis can occur at different phases during the diagnostic process.[2] Any breast lump requires a clinical examination, imaging of the breast and breast biopsy, also known as 'triple diagnosis'.[3-7] Normal imaging and a hypothesis of 'residual milk retention' were the reason why our patient did not undergo a breast biopsy, which undoubtedly delayed diagnosis. Delay in diagnosis is the commonest basis for litigation in breast cancer management, but the effect of delay in diagnosis on survival remains controversial once a breast lump is palpable.[8] The adverse impact of delay in presentation on survival of breast cancer is mainly seen if delay leads to a more advanced stage of disease.[9-11] Furthermore, the impact of a false-negative mammogram on breast cancer

Figure 1. *Change of lactate dehydrogenase (LDH), platelets and CA 15.3 from diagnosis of metastatic disease until death*

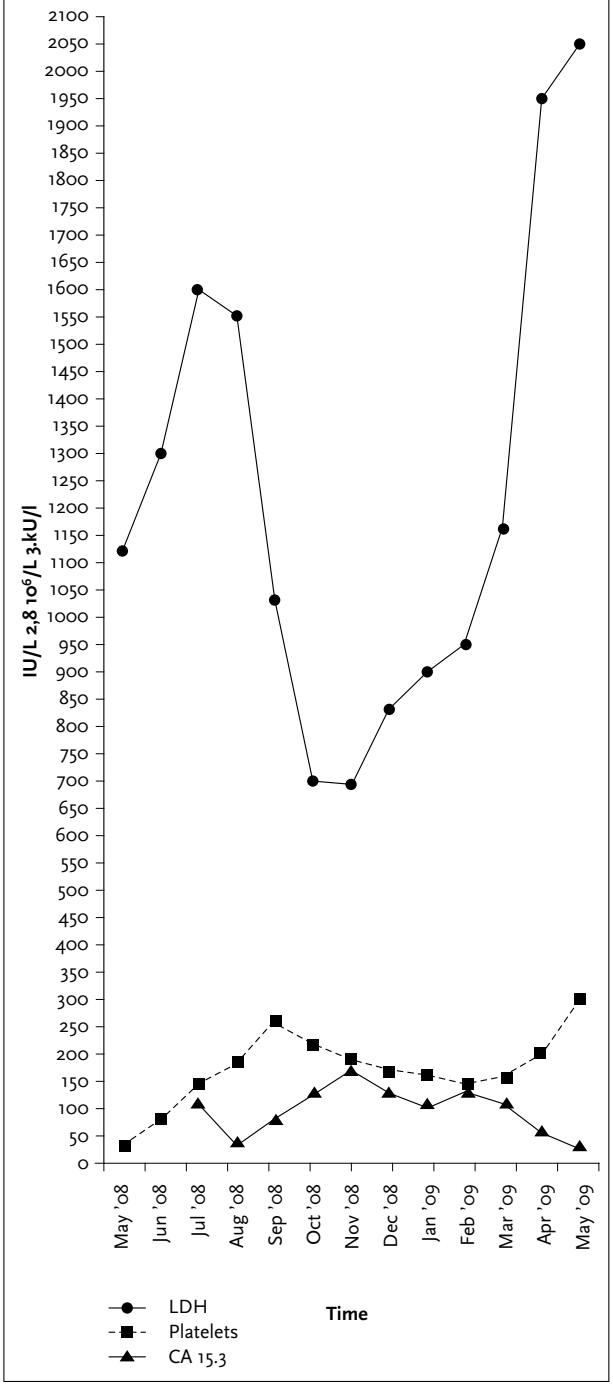


Table 1. *Overview of false-negative tests*

Problem	False-negative test	Discussion
Primary diagnosis		
Palpable malignant breast lump	Mammography/ ultrasound	Sensitivity variable (30-90%), improving with addition of ultrasound (94-97%)[3-7] More difficult in premenopausal women and in case of dense breasts
	MRI	Indicated if other tests are inconclusive, search for occult tumours, lobular cancers, monitoring response to neoadjuvant therapy, women with implants, suspected lesions in a scar, screening high risk patients. No place as routine preoperative imaging[14,15]
Positive lymph node involvement	Clinical investigation	Involvement can be estimated by several risk factors. False-negative in 30-60%, false-positive in 30-40%[17-19]
	Ultrasound	In expert hands moderately sensitive 26-87%, fairly specific 56-98%[20]
Progressive disease		General complaints Rising CA 15.3, LDH, deteriorating liver function and thrombocytopenia *(see table 2)*
Liver metastases	Ultrasound of liver	Considered appropriate, limitations due to operator variability, body habitus, patient compliance, evaluation of subcostal area. Small lesions within fatty/necrotic liver difficult to depict[1]
	CT scan	Identified in porto-venous phase. Hypervascular lesions can be missed. Sensitivity 92%[1]
	PET/CT scan	FDG uptake in many lesions as high as in healthy liver tissue. Small lesions can be missed. Sensitivity 76%. Superior to conventional imaging, performance comparable with CT scan[1,25]
	Liver biopsy	Necrotic hepatitis, most likely toxic in origin. Tamoxifen, anastrazole and mistletoe are described to cause hepatotoxicity and were stopped immediately.[36-41] Extra assessment with cytokeratin 7 immunostain was necessary to detect metastatic cells
Bone metastases	Bone scintigraphy	Sensitivity 67-92%, specificity 80-99%, especially for osteoblastic or mixed metastases[1,24]
	CT scan	Sensitivity only 67%[1]
	PET/CT-scan	Relatively low detection rate, especially those of osteoblastic type. Sensitivity 87-92%, specificity 92%.[1,24,26] ! Bone core biopsy was necessary for obtaining diagnosis of osteoblastic metastases

survival, in symptomatic breast cancer, may therefore be of less importance than when this happens in asymptomatic disease.[12]

Mammograms have an overall sensitivity of 30 to 90%.[3-7,13] The largest study of false-negative mammograms in women with a malignant symptomatic breast lump has shown that mammograms in this population are negative in 10%. Of these false-negative results, 42% are considered to be potentially avoidable oversights.[13] Patients with false-negative mammograms are likely to be younger, usually with denser breast tissue, smaller tumours and more tumours located in the upper outer quadrant. Although some studies recognise a high rate (24%) of a simultaneous false-negative ultrasound with a false-negative mammogram, ultrasound has been reported to significantly raise the sensitivity of breast cancer

imaging to 94 to 97% and may become a very valuable adjunctive diagnostic tool.[13] MRI has sensitivity rates of approximately 90%, but low specificity rates and high cost, and is of most use in select cases (*table 1*).[14,15]

Overall, young age as a poor prognostic factor might have been more important in our case than delay in diagnosis. In a multivariate model with stage and adjuvant therapy amongst different prognostic variants, young age remained a bad prognostic factor for breast cancer specific survival, especially in case of triple negative and HER-2 positive breast cancers.[16] Although the overall sensitivity of breast cancer imaging is high (up to 94%), it should be emphasised that a negative mammogram and ultrasound should not influence the management of a suspect clinical lesion.

Lymph nodes: The preoperative detection of nodal and systemic metastases in those most likely to have disseminated disease affects patient management and prognosis. In our case, the preoperative evaluation of the axilla was falsely negative. A normal clinical assessment of the axilla is of little value.[17-19] Axillary ultrasound is more sensitive and specific to select patients eligible for the sentinel lymph node procedure.[20] Since the sentinel lymph node procedure was not considered in our case, this false-negative finding did not affect management of our patient.

There have been many published reports and models on the correlation between patient and tumour characteristics and lymph node status. Tumour size seems to be the most powerful predictor of axillary node involvement.[19,21-23] Voogd *et al.* showed an odds ratio for node positivity of 3.53 with tumours >3 cm.[19] On the other hand it has been found that 1 to 15% of patients with a negative sentinel node biopsy still have other affected lymph nodes in the same node region.[20]

Tests at relapse

In our patient, when progressive disease was suspected based on clinical symptoms and haematological changes, several imaging tests were again falsely negative (see also *table 1*). CA 15.3 was increasing over time, although remaining within reference values until two months before the start of palliative chemotherapy. An increase in LDH, a deteriorating liver function and thrombocytopenia all pointed towards systemic disease whilst bone scintigraphy and both ultrasound and CT scan of the liver remained normal. Although there is no consensus on the most sensitive imaging method, conventional imaging procedures to screen for metastatic disease are chest X-ray, abdominal ultrasound and bone scintigraphy. The last-mentioned is widely accepted for detection of osteoblastic or mixed osteolytic-osteoblastic bone metastases (sensitivity 67 to 92%; specificity 80 to 99%), knowing that additional imaging procedures are not uncommonly needed to determine the nature of such lesions.[1,24] Contrast-enhanced CT scan has a high sensitivity in the detection of visceral metastases, but small lesions may be missed. Generally, liver metastases from breast cancer are readily identified in the porto-venous phase on CT, although hypervascular lesions can potentially be missed. Sensitivity of CT for the detection of liver metastases is 92%; for bone metastases it is only 67%.[1] While ultrasound of the liver is considered appropriate, its limitations are mainly due to operator expertise, body habitus, patient compliance, and the evaluation of the subcostal area. Small lesions within a fatty or necrotic liver are difficult to depict.[1] Other investigations that are used in oncology are PET and PET/CT scan. Characterisation of malignant liver lesions is hampered by the fact that 18F-fluorodeoxyglucose (FDG) uptake in most lesions is as high as in healthy liver tissue. Therefore, small metastases, as well as lesions with low metabolic activity, can potentially be missed.[25] Sensitivity for detecting liver metastases is about 76%.[1] PET scan also has a relative low detection rate for bone metastases, especially those of the osteoblastic type.[26] Reported sensitivity to detect bone metastases is 87 to 92%, with a specificity of 92%.[1,24] Overall PET scan is superior to conventional imaging for detection of distant breast cancer metastases and its diagnostic performance is comparable with that of contrast-enhanced CT scan.[1] PET scan lacks precise anatomical localisation and morphological characterisation of metastases. This problem can be overcome by using a contrast-enhanced PET/CT scan. Another advantage of this combined investigation is that the entire patient is analysed during a single non-invasive total body investigation.[1]

In breast cancer, tumour-associated markers can reflect the total tumour cell load. They may help in determining prognosis and in monitoring response or resistance to specific therapies.[27] See *table 2* for more detailed discussion on the markers that were important in our patient.[28-36]

Oestrogen receptor expression is important for the behaviour of breast cancer cells and is reflected in gene expression patterns of breast tumours.[37]

The current, first-generation genomic prognostic markers, which were developed from combined analysis of all breast cancer subtypes, appear to classify almost all oestrogen receptor negative or grade 3 patients as high risk and therefore have limited value to risk stratify this clinical group. However, these molecular markers can subdivide oestrogen receptor positive breast cancers (with or without endocrine therapy) into lower- and higher-risk groups, and therefore if clinical variables are equivocal, they may provide some clinical value. Some recent data suggest that multivariate prognostic models including oestrogen receptor, HER2, and Ki-67, with or without tumour size and nodal status, determined in a central pathology

Table 2. *Haematological changes*

Marker	Discussion
LDH	Potential as marker of breast cancer activity. Involved in anaerobic glycolysis; tumour hypoxic environment with anaerobic metabolism. Serum levels 2-3 fold higher with localised disease, >5 fold with distant metastases. Level correlates well with tumour load. Increase is linked to poorer prognosis and survival rates.[28-30]
CA 15.3	Detect expression of MUC-1 antigen. Serial measurement can result in early detection of recurrent disease and indicate efficacy of therapy. Limited sensitivity and specificity Concentrations are increased in about 10% of patients with stage I disease, 20% with stage II, 40% with stage III, and 75% with stage IV disease, but can also be increased in benign conditions; limiting its use for early stage breast cancer.[27,31,32] In 25-30% of metastatic disease no increase. Levels can increase before radiological or clinical evidence of disease relapse, but the contrary can also be true, especially in case of bone metastases Unclear if introduction of early treatment improves overall survival or quality of life in case of metastatic disease.[33-35] Routine surveillance after primary surgery not recommend by most guidelines.[27,32]
Thrombocytopenia	Metastatic breast cancer with bone marrow involvement and pronounced thrombocytopenia as sole haematological abnormality is not often seen, although platelet depression can be the only finding of bone marrow metastases. Often accompanied by signs of suppressed erythropoiesis and leucopoiesis, elevated alkaline phosphatise and/or hypercalcaemia, but can be absent as in our case.[36]

laboratory could yield prognostic information very similar to the 21-Gene Recurrence Score assay.[38,39]

Another investigation that was false-negative at first was the pathology of the liver biopsy. The histopathology of biopsy tissue can provide otherwise unobtainable qualitative information regarding the structural integrity of the liver and the type and degree of injury and/or fibrosis.[40] There are several methods for procuring liver tissue: percutaneous, transjugular, laparoscopic, or ultrasound or CT-guided fine needle aspiration (FNA). It is common practice to obtain a prothrombin time, platelet count and a complete blood count prior to biopsy. In our patient, the thrombocytopenia was noticed too late. Significant intra-peritoneal haemorrhage is a serious complication of liver biopsy. Normally the risk is less than 0.1%. The biopsy specimen showed a necrotic hepatitis, most likely toxic in origin. It was only after an extra assessment with a cytokeratin 7 immunostain, a basic cytokeratin found on glandular and transitional epithelia and usually present in adenocarcinomas such as that of the breast and useful in discriminating primary from metastatic adenocarcinoma, that metastatic cells in the liver were also diagnosed. Hepatotoxicity can occur with a variety of drugs and other products,[41-44] such as those used in alternative medicine or in soft herbal remedies. Most adverse reactions take place following a short exposure period, although they sometimes only become manifest after a longer period.[41-]

Finally, a last item that has to be emphasised in this patient is that she had a mutation in exon 18 of the BRCA 1 gene. Several mutations are reported in the highly penetrating BRCA 1 and 2 genes (normally being involved in DNA repair). Although a positive family history is reported by 15 to 20% of women with breast cancer, only 5 to 6% of all breast cancers are associated with an inherited gene mutation.[45] Mutations are rare, occurring in approximately 0.3% of the general population, resulting in a tenfold increased risk of developing breast cancer. Somatic mutations in these genes or their expression products can also be involved in sporadic breast cancers. The clinical implication of the mutation retained in this patient is unknown for the moment, although the family history (ovarian cancer, breast and pancreatic cancer in first and second degree relatives) is very suggestive. As BRCA-related breast cancers are often early-onset breast cancers, detection rate in dense and dysplastic young breast tissue is challenging. Moreover, BRCA-associated breast cancers tend to exhibit histological and histochemical evidence of aggressive biological behaviour and to be highly proliferative leading to more interval cancers in comparison with the sporadic breast cancers. An expansive growth pattern with pushing borders seems a feature characterising the BRCA phenotype, which can sometimes make these tumours indistinguishable from fibroadenomas, appearing as well-defined, roundish, hypoechoic masses without acoustic shadowing on ultrasound, without associated microcalcifications on mammography and with strong wash-out phenomenon on breast MRI.[46] It is not known if there is an association between BRCA mutations and false-negative tests. In contrast to many BRCA 1 related breast cancers, our patient's breast cancer was not triple negative. In the future, further investigations of these 'unclassified variants are warranted.

CONCLUSION

Our literature review highlights the relative sensitivity of the diagnostic tests we encounter in our daily practice managing breast cancer patients.

An explanation or reason for a specific diagnostic test being false-negative is not always clear, often multi-factorial, test-related or specific and clearly inherent to each particular test.

We believe that, if a patient is clinically suspect for a primary breast cancer or progressive disease, but imaging and biochemistry are not (yet) confirming this suspicion, the clinical status of the patient has to guide the physician for further diagnostic work-up. A suspect clinical sign is far more important than a negative technical result.

REFERENCES

1. Mahner S, Schirrmacher S, Brenner W, et al. Comparison between position emission tomography using 2-[fluorine-18]fluoro-2-deoxy-D-glucose, conventional imaging and computed tomography for staging of breast cancer. Ann Oncol. 2008;19:1249-54.
2. Nichols S, Waters WE, Fraser JD, Wheeller MJ, Ingham SK. Delay in the presentation of breast symptoms for consultant investigation. Commun Med. 1981;3:217-5.
3. Berg WA, Blume JD, Cormack JB, et al. Combined Screening with Ultrasound and Mammography vs Mammography alone in Women at Elevated Risk of Breast Cancer. JAMA. 2008;299:2151-63.
4. Kolb Tm, Lichy J, Newhouse JH. Comparison of the performance of screening mammography, physical examination, and breast US and evaluation of factors that influence them: an analysis of 27,825 patient evaluations. Radiology. 2002;225:165-75.
5. Wang JM, Chang KJ, Kuo WH, Lee HT, Ting-Fang Shih T. Efficacy of Mammographic Evaluation of Breast Cancer in Women less than 40 years of Age: Experience from a Single Medical Center in Taiwan. J Formos Med Assoc. 2007;106:736-47.
6. Houssami N, Irwig L, Simpson JM, et al. Sydney Breast Imaging Accuracy Study- comparative sensititvity and specificity of mammography and sonography in young women with symptoms. Am J Roentgenol. 2003;180:935-40.
7. Flobbe K, Bosch AM, Kessels AG, et al. The additional diagnostic value of ultrasonography in the diagnosis of breast cancer. Arch Intern Med. 2003;163:1194.
8. Macleod U, Mitchell ED, Burgess C, Macdonald S, Ramirez AJ. Risk factors for delayed presentation and referral of symptomatic cancer: evidence for common cancers. Br J Cancer. 2009;101(Suppl 2):S92-S101.
9. Richards MA, Smith P, Ramirez AJ, Fentiman IS, Rubens RD. The influence on survival of delay in the presentation and treatment of symptomatic breast cancer. Br J Cancer. 1999;79:858-64.
10. Richards MA, Westcombe AM, Love SB, Littlejohns P, Ramirez AJ. Influence of delay on survival in patients with breast cancer: a systematic review. Lancet. 1999;353:1119-26.
11. Joensuu H, Asola R, Holli K, Kumpulainen E, Nikkanen V, Parvinen LM. Delayed diagnosis and large size of breast cancer after a false negative mammogram. Eur J Cancer. 1994;30A(9):1299-302.
12. Kavanagh AM, Giles GG, Mitchell H, Cawson JN. The sensitivity, specificity, and positive predictive value of screening mammography and symptomatic status. J Med Screen. 2000;7(2):105-10.
13. Murphy [fill in reference]
14. Bluemke DA, Gatsonis CA, Chen MH, et al. Magnetic resonance imaging of the breast prior to biopsy. JAMA. 2004;292:2735-42.
15. Peters NH, Borel Rinkes IH, Zuithoff NP, et al. Meta-analysis of MR imaging in the diagnosis of breast lesions. Radiology. 2008;246:116-24.
16. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. PLoS One. 2009;4(11):e7695.
17. Lanng C, Hoffmann J, Galatius H, Engel U. Assessment of clinical palpation of the axilla as a criterion for performing the sentinel node procedure in breast cancer. ESJO. 2007;33:281-4.
18. Specht MC, Fey JV, Borgen PI, Cody III HS. Is the clinically positive axial in breast cancer really a contraindication to sentinel lymph node biopsy? J Am Coll Surg. 2005;200:10-4.
19. Voogd AC, Coebergh J-WW, Repelaer van Driel OJ, et al. The risk of nodal metastases in breast cancer patients with clinically negative lymph nodes: a population-based analysis. Breast Cancer Res Treat. 2000;62:63-9.
20. Alvarez S, Anorbe E, Alcorta P, Lopez F, Alonso I, Cortés J. Role of sonography in the diagnosis of axillary lymph node metastases in breast cancer: a systematic review. Am J Radiol. 2006;186:1342-8.
21. Barranger E, Coutant C, Flahault A, Delpech Y, Darai E, Uzan S. An axilla scoring system to predict non-sentinel lymph node status in breast cancer patients with sentinel lymph node involvement. Breast Cancer Res Treat. 2005;91(2):113-9.
22. Fein DA, Fowble BL, Hanlon AL, et al. Identification of women with T1-T2 breast cancer at low risk of positive axillary nodes. Surg Oncol. 1997;65(1):34-9.
23. Ravdin PM, De Laurentis M, Vendely T, Clark GM. Prediction of axillary lymph node status in breast cancer patients by use of prognostic indicators. J Nat Cancer Inst. 1994;86:1771-5.
24. Gallowitsch HJ, Kresnik E, Gasser J, et al. F-18 fluorodeoxyglucose positron-emission tomography in the diagnosis of tumor recurrence and metastases in the follow-up of patients with breast carcinoma: a comparison to conventional imaging. Invest Radiol. 2003;38:250-6.
25. Brix G, Ziegler SI, Bellemann ME, et al. Quantification of [18F]FDG uptake in the normal liver using dynamic PET: impact and modelling of the dual hepatic blood supply. J Nucl Med. 2001;42:1265-73.
26. Cook GJ, Houston S, Rubens R, et al. Detection of bone metastases in breast cancer by 18FDG PET: differing metabolic activity in osteoblastic and osteolytic lesions. J Clin Oncol. 1998;16:3375-9.
27. Duffy MJ. Serum tumor markers in breast cancer: Are they of clinical value? Clin Chem. 2006;52:345-51.
28. Harris AL. Hypoxia: a key regulatory factor in tumour growth. Nat Rev Cancer. 2002;2:38-47.
29. Seth RK, Kharb S, Kharb DP. Serum biochemical markers in carcinoma breast. Ind J Med Sol. 2003;57:350-4.
30. Lee YT. Biochemical and haematological tests in patients with breast carcinoma: correlations with extent of disease, site of relapse, and prognosis. J Surg Oncol. 1985;29:242-8.
31. Sturgeon CM, Lai LC, Duffy MJ. Serum tumour markers: how to order and interpret them. BMJ. 2009;339:b3527.
32. Harris L, Fritsche H, Mennel R, et al. American Society of Clinical Oncoly 2007. Update of Recommendations for the Use of Tumor Markers in Breast Cancer. J Clin Oncol. 2007;25:5287-312.
33. Nicolini A, Carpi A, Michelassi C, et al. Tumor marker guided salvage treatment prolongs survival of breast cancer patients: final report of a 7-year study. Biomed Pharmacother. 2003;57:452-9.
34. Kovner F, Merimsky O, Hareuveni M, et al. Treatment of disease negative but mucin like carcinoma associated antigen positive breast cancer patients with tamoxifen: preliminary results of a prospective controlled randomized trial. Cancer Chemother Pharmacol. 1994;35:80-3.
35. Chourin S, Veyret C, Chevier A, et al. Routine Use of serial plasmatic CA 15-3 determinations during the follow-up of patients treated for breast cancer. Evaluation as factor of early diagnosis of recurrence. Ann Biol Clin (Paris). 2008;66:385-92.
36. Sar R, Aydogdu I, Ozen S, et al. Metastatic bone marrow tumours: a report of six cases and review of the literature. Hematologia (Budap). 2001;31:215-23.
37. Kok M, Linn SC. Gene expression profiles of the oestrogen receptor in breast cancer. Neth Med J. 2010;68:291-302.
38. Bueno-de-Mesquita JM, van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). Lancet Oncol. 2007; 8:1079-87.
39. Goldstein LJ, Gray R, Badve S, et al. Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features. J Clin Oncol 2008;26:4063-71.
40. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med. 2001;344:495-500.
41. Zapata E, Zubiaurre L, Bujanda L, Pierola A. Anastrozole-induced hepatotoxicity. Eur J Gastroenterol Hepat. 2006;18:1233-4.
42. Harvey J, Colin-Jones DG. Mistletoe hepatitis. Br Med J. (Clin Res Ed). 1981;282:186-7.
43. Lasso De La Vega MC, Zapater P, Such J, et al. Toxic hepatits associated with tamoxifen use. A case report and literature review. Gastroenterol Hepatol. 2002;25:247-50.
44. Murata Y, Ogawa Y, Saibara T, et al. Tamoxifen induced non-alcoholic steatohepatitis in patients with breast cancer: determination of a suitable biopsy for diagnosis. Oncol Rep. 2003;10:97-100.
45. Malone KE, Daling JR, Thompson JD, et al. BRCA1 mutations and breast cancer in the general population: Analyses in women before age 35 years and in women before age 45 years with first-degree family history. JAMA. 1998;279:922-9.
46. Kuhl CK. Familial breast cancer: what the radiologist needs to know. Breast. 2005;14:480-6.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Catechol-*O*-methyltransferase (*COMT*) gene variants and pain in chronic pancreatitis

A.A.J. van Esch*, E. de Vries, R.H.M. te Morsche, M.G.H. van Oijen, J.B.M.J. Jansen, J.P.H. Drenth

[1]Department of Gastroenterology and Hepatology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24-36 14 760, fax: +31 (0)24-35 40 103, e-mail: A.vanEsch@MDL.umcn.nl

ABSTRACT

Background: Pain is the major symptom of chronic pancreatitis. The role of genetics in pancreatic pain is unclear. Catechol-*O*-methyltransferase (COMT) regulates enkephalin levels and influences pain perception. The *COMT* gene contains functional polymorphisms that have been found to influence human pain perception. The aim of our study was to investigate *COMT* single-nucleotide polymorphisms (SNPs) and diplotypes in chronic pancreatitis patients and healthy controls.

Methods: We genotyped four *COMT* gene SNPs: *c.1-98A>G* (rs6269), *c.186C>T (p.=)* (rs4633), *c.408C>G (p.=)* (rs4818) and *c.472G>A (p.Val158Met)* (rs4680) using a dual-colour discrimination assay in 240 chronic pancreatitis patients and 445 controls. We generated five diplotypes with a frequency >0.5% and compared prevalence between patients and controls.

Results: There was no significant association between the SNPs in the *COMT* gene and chronic pancreatitis. The diplotype ATCA/ACCG was more prevalent in controls compared with patients (OR 0.48, 95% CI 0.24 to 0.93, p=0.03) where the most common diplotype GCGG/ATCA served as reference. However, after correction for multiple testing, this is not a significant difference. The distribution of other diplotypes was not significantly different between patients and controls.

Conclusion: *COMT* SNPs and diplotypes are not associated with chronic pancreatitis. As a consequence, our results do not support a significant role for the *COMT* gene in chronic pancreatitis.

KEYWORDS

Chronic pancreatitis, polymorphism, COMT, pain

INTRODUCTION

Chronic abdominal pain is the major presenting symptom of chronic pancreatitis and the majority of patients will have pain at a given time during the course of their disease. A large majority of patients with chronic pancreatitis presented with pain in a survey of the Asia-Pacific region, varying from 60% in Japan, to 90% in Australia, South Korea and South India and 100% in Singapore.[1] The inter- and intra-individual variation of pain in chronic pancreatitis is high, with pain duration varying from intermittent to persistent and pain intensity ranging from mild to disabling.[2] The inter-individual differences in the response to pain suggest that genetic factors can be involved in its modulation.[3,4]

Recently, several studies have investigated the association between the catechol-*O*-methyltransferase (*COMT*) gene and pain sensitivity.[5-13] In some studies, there was a positive association between *COMT* gene SNPs and pain.[5,10-13] This was not confirmed by other studies.[6-8] Other studies have focused on the association between COMT and the efficacy of pain therapy, such as morphine.[14,15] The COMT enzyme metabolises catecholamines, thereby acting as a key modulator of dopaminergic and adrenergic/noradrenergic neurotransmission.[16,17] Low activity of COMT is associated with activation of dopaminergic neurons, a reduction in the neuronal content of enkephalin and an increase in the regional concentration of μ-opioid system receptors. The μ-opioid system is activated in response to stressors, pain and other salient environmental stimuli, typically reducing pain and stress responses.[9,18] COMT inhibition results in increased pain sensitivity via a $\beta_{2/3}$-adrenergic mechanism.[19]

The *COMT* gene is located on the long arm of chromosome 22, at the gene map locus of 22q11.2. The human *COMT* gene encodes two distinctive proteins: soluble COMT

(S-COMT) and membrane-bound (MB-COMT) through the use of alternative translation initiations sites and promoters.[20] There are different single-nucleotide polymorphisms (SNPs) in the *COMT* gene, which induce important functional alterations of the enzyme. The *COMT* gene contains a common functional polymorphism: *c.472G>A (p.Val158Met)* (rs4680). This substitution is associated with a reduction in thermostability and activity of the enzyme.[21] Individuals with the Val^{158}/Val^{158} genotype have the highest activity of COMT and have been found to be less susceptible to pain compared with other genotypes. Individuals with the Met^{158}/Met^{158} genotype showed diminished regional μ-opioid system responses to pain compared with heterozygotes.[9] The exact mechanism by which diminished COMT activity influences pain perception is not known. However, associations between the low-activity Met^{158} allele are often inconsistent.[22] This suggests that additional SNPs in the *COMT* gene modulate COMT activity. There are three other SNPs in the *COMT* gene that exhibit a strong linkage disequilibrium with the $Val^{158}Met$ variation. One is located in the *S-COMT* promoter region: *c.1-98A>G* (rs6269). The two other SNPs are located in the *MB-COMT* coding region: *c.186C>T (p.=)* (rs4633) and *c.408C>G (p.=)* (rs4818).[23] Furthermore, haplotypes of the *COMT* gene that have functional consequences with respect to COMT enzyme activity have been revealed. Diatchenko identified three different haplotypes formed by the four different SNPs.[23] The use of haplotype reconstruction is preferred because combinations of SNPs might have a synergistic effect on COMT protein function. Since each person has two haplotypes for each gene, one can determine the variation on both haplotypes simultaneously: the diplotype.

The aim of this study was 1) to compare four *COMT* SNPs and the diplotypes between patients with CP and controls and 2) examine the effect of *COMT* gene variants on presence and severity of pain in CP.

MATERIALS AND METHODS

Subjects

We included patients diagnosed with chronic pancreatitis who visited the outpatient clinic at the Department of Gastroenterology and Hepatology of the Radboud University Nijmegen Medical Centre between 1980 and 2009. We sampled patients and performed a cross-sectional study. Therefore, we collected venous blood samples for DNA analysis in these patients at our outpatient clinic. The clinical diagnosis of chronic pancreatitis was based on one or more of the following criteria: presence of typical complaints (recurrent upper abdominal pain, radiating to the back, relieved by leaning forward or sitting upright and increased after eating), suggestive radiological findings, such as pancreatic calcifications or pseudo cysts, and pathological findings (pancreatic ductal irregularities and dilatations) revealed by endoscopic retrograde pancreaticography or magnetic resonance imaging of the pancreas before and after stimulation with secretin. We collected data regarding the cause of pancreatitis. Patients who had an estimated intake of alcohol of more than 60 g (females) or 80 g (males) daily for more than two years were classified as chronic pancreatitis of alcoholic origin. The diagnosis hereditary pancreatitis was established by fulfilling the international diagnostic criteria for hereditary pancreatitis: two first-degree relatives or three or more second-degree relatives, in two or more generations with recurrent acute pancreatitis and/or chronic pancreatitis for which there were no known precipitating factors.[24] Idiopathic pancreatitis was diagnosed if precipitating factors such as alcohol abuse, bile stones, trauma, medication, infection, metabolic disorders, and a positive family history were absent. Patients with other causes of pancreatitis, such as anatomic or tropical, were classified as miscellaneous causes. The controls were unrelated, healthy individuals from the Netherlands who were not suffering from pancreatic disease. We matched cases and controls on gender, while gender is a significant covariate in genetic studies of human pain.

A positive family history for pancreatic diseases was absent in all controls. In addition there was no chronic alcohol abuse (<60 g for females and <80 g for males) in our population. These data were collected through interviews.

Ethics

The study was conducted in accordance with the principles of the Helsinki Declaration. The study protocol was approved by the local medical ethics review committee, the Institutional Review Board from the Radboud University Nijmegen Medical Centre (CWOM no. 0011-0242). All subjects gave their informed consent. The informed consent was obtained verbally in the presence of a witness and documented in the patient's medical file.

Genotyping

All patients donated a venous blood sample. Genomic DNA was extracted from 300 μl whole blood using the Puregene® genomic DNA isolation kit (Gentra Systems, Minneapolis, USA). The 4 *COMT* SNPs (*c.1-98A>G*, *c.186C>T (p.=)*, *c.408C>G (p.=)* and *c.472G>A*) were analysed by a dual-colour discrimination assay, using the iCycler iQ Multicolour Real-Time Detection System (Bio-Rad Laboratories; Hercules, USA). The PCR amplifications were carried out in a final volume of 25 μl, which contained 200 ng of genomic DNA, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 3 mM $MgCl_2$ 0.25 mM dNTP's, 200 nM of forward and reverse primer, 200 nM of both probes complementary to the two alleles of each SNP labelled at the 5' end with the fluorophore Fam or Hex and at the 3' end with BHQ1 as quencher (primer

sequences available on request) and 3.0 units of Taq-DNA polymerase. Genomic DNA was denatured at 95 °C for five minutes. Forty cycles were carried out, each composing denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 63 °C, and extension for 30 seconds at 72 °C. Genotype assignment was conducted using the iCycler iQ Optical System Software version 3.1 (Bio-Rad Laboratories; Hercules, USA) using the final fluorescent signals.

Statistical methods

After testing for Hardy-Weinberg equilibrium (HWE) among controls, frequency tables were provided for the distribution of the four studied SNPs.[25] Differences between continuous variables were tested using Student's t-test and categorical variables by the χ^2 test. Combination of haplotypes, diplotypes, were generated based on the four studied SNPs; missing SNPs were imputed.

The relative risk associated with minor alleles was estimated as an odds ratio (OR) with a 95% confidence interval (CI) with the most common diplotype as a reference. Statistical significance was defined as $p<0.05$. For diplotypes that were only present in either the patient population or healthy controls, no odds ratios could be calculated. Statistical analysis was carried out with SPSS 16.0 for Windows. Pair-wise linkage disequilibrium estimations between polymorphisms and haplotype reconstruction were performed with Haploview version 4.0.[26]

RESULTS

Characteristics of patients and controls

Samples of 685 subjects were included in our study cohort. The characteristics of the patients and controls are shown in *table 1*. The cohort consisted of 240 chronic pancreatitis patients (157 males, 83 females), with a mean age of 48 years (range 17 to 78 years). We included 445 controls (294 male, 150 female) with a mean age of 53 years (range 19 to 90 years). The patients and controls were Caucasians. Of the patients, 44% had alcohol-related chronic pancreatitis. Healthy controls were significantly older (3 years).

The genotyping completion rate was 100%. The observed and expected frequencies of the different SNPs in controls were in Hardy-Weinberg equilibrium. The allele frequencies of the four SNPs in chronic pancreatitis patients and healthy controls are shown in *table 2*. There was no significant association between the SNPs and chronic pancreatitis.

Table 1. *Demographic and clinical characteristics of chronic pancreatitis patients and healthy controls*

	Patients	Controls	*P* value
N	240	445	
Age (mean, range, in years)	48 (17-78)	53 (19-90)	0.001*
Sex (male:female)	157;83	294;150; 1 N/A	0.83
Tobacco use			
• Smoking	158		
• Non-smoking	63		
• Unknown	19	445	
Cause of chronic pancreatitis			
• Alcoholic	106		
• Hereditary	14		
• Idiopathic	103		
• Miscellaneous	17		

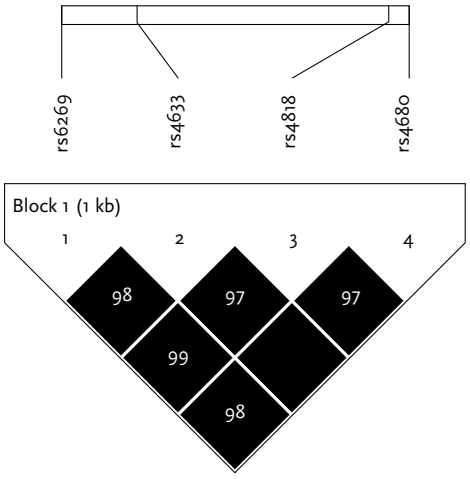
Table 2. *Allele frequencies of the four SNPs in chronic pancreatitis patients and healthy controls*

	Alleles	Patients (n=240)	Controls (n=445)	*P* value
rs6269				0.25
	A/A	84 (35%)	164 (37%)	
	A/G	123 (51%)	202 (45%)	
	G/G	33 (14%)	79 (18%)	
rs4633				0.14
	T/T	70 (29%)	122 (27%)	
	T/C	127 (53%)	214 (48%)	
	C/C	43 (18%)	109 (25%)	
rs4818				0.26
	C/C	82 (34%)	165 (37%)	
	C/G	126 (53%)	206 (46%)	
	G/G	32 (13%)	74 (17%)	
rs4680				0.18
	A/A	70 (29%)	120 (27%)	
	A/G	127 (53%)	218 (49%)	
	G/G	43 (18%)	107 (24%)	

Diplotype analysis

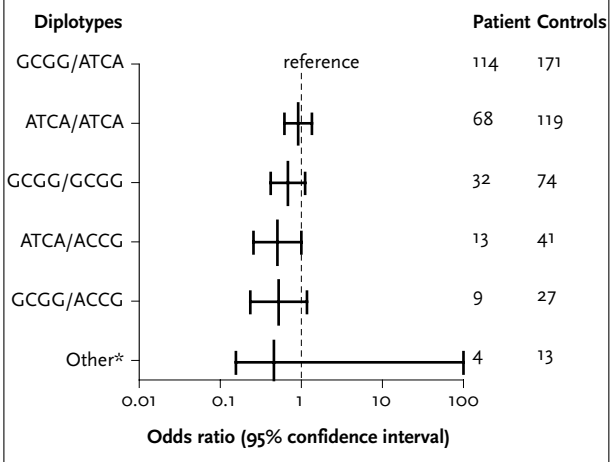
Linkage analysis between the four SNPs showed that they were closely linked (*figure 1*). We then determined haplotypes and combinations of haplotypes (diplotypes). Based on the SNP distribution, five diplotypes with a frequency >0.5% were generated, three of them representing 84% of all diplotypes observed in this study. Diplotype GCGG/ATCA is most prevalent in both groups, but more frequent in patients compared with controls (47.5% *vs* 38.4%). This haplotype served as reference in calculating the odds ratios for the remaining diplotypes (*figure 2*). ATCA/ACCG was more prevalent in controls compared with patients (9.2 *vs* 5.4%, OR 0.48, 95% CI 0.24 to 0.93, p=0.03). After correction for multiple testing, this was no longer a significant difference. The distribution of other diplotypes was not significantly different between patients and controls.

Figure 1. *Linkage disequilibrium plot*



Linkage disequilibrium (LD) plot across the COMT. The box at the top indicates the *COMT* gene with the four investigated SNPs. The LD plot is based on the measure of D'. Each diamond indicates the pair-wise magnitude of LD, with dark grey diamonds indicating strong LD (D' > 0.8).
LD = linkage disequilibrium is the non-random association of alleles at two or more loci, not necessarily on the same chromosome. Linkage disequilibrium describes a situation in which some combinations of alleles or genetic markers occur more or less frequently in a population than would be expected from a random formation of haplotypes from alleles based on their frequencies.

Figure 2. *Diplotype distribution of chronic pancreatitis patients and healthy controls*



Distribution of diplotypes in patients with chronic pancreatitis and healthy controls. The diplotypes are compared with the most prevalent diplotype GCGG/ATCA.

DISCUSSION

This study investigated the association between four SNPs in the *COMT* gene in a large cohort of patients with chronic pancreatitis and healthy controls. We considered the *COMT* gene a candidate in chronic pancreatitis for several reasons. First, COMT has been associated with several chronic pain conditions, such as fibromyalgia syndrome, neuropathic pain and temporomandibular disorder. Second, pain is a major symptom in chronic pancreatitis, which ultimately will be present in nearly all patients and it causes substantial impairments in health-related quality of life in these patients.

Gene association studies in chronic pancreatitis have so far focussed on the presence *vs* absence of the disease.[27] For example, mutations in pancreatic serine protease inhibitor Kazal type 1 (*SPINK 1*) are enriched in patients with idiopathic chronic pancreatitis as well in alcoholic pancreatitis in comparison with background population.[28] Likewise, the G191R variant of anionic trypsinogen gene (*PRSS2*) affords protection against various forms of chronic pancreatitis when compared with healthy controls.[29] We tried to take this further and search for genetic variants that determine an important symptom in chronic pancreatitis: pain.

In our study, we investigated if COMT polymorphisms are associated with chronic pancreatitis, but we were actually interested in the question whether COMT polymorphisms are associated with pain in patients with chronic pancreatitis. COMT itself has no role in the aetiology of CP per se, but its genetic variants have a role in altered pain perception. Our chronic pancreatitis group consisted of patients experiencing pain varying from intermittent to persistent and pain intensity ranging from disabling to no pain or mild pain. We did not directly quantify pain, which makes it difficult to study the exact correlation between COMT and pain due to chronic pancreatitis in this population. It is very complex to investigate pain, due to different levels of pain that patients' experience, the use of analgesic drugs and different pain scales. Furthermore, the difficulty in measuring pain is that there is no validated objective measurement of pain associated with chronic pancreatitis. This is partially due to the unpredictable course of chronic pancreatitis with relapses and remission. Pain in chronic pancreatitis is highly variable and it varies greatly during the lifetime of the disease. But ultimately, the majority of the patients with chronic pancreatitis will experience pain.

Moreover, there are several confounding variables, such as dependence of analgesic drugs and the use of alcohol or other narcotic agents. However, since almost every patient with chronic pancreatitis will experience pain during the course of their disease, we lumped the patients together and investigated COMT in chronic pancreatitis patients from our cohort.

We did not limit ourselves to a single *COMT* SNP, but rather elected to perform haplotype (and diplotype) association studies. Haplotype and diplotype reconstruction, rather than individual SNPs, better predicts variability in pain sensitivity. Diplotype GCGG/ATCA is most prevalent in both groups and more frequent in patients than in controls. ATCA/ACCG was more prevalent in controls compared with patients (OR 0.48,

95% CI 0.24 to 0.93). However, after correction for multiple testing this is not a significant difference.

Furthermore, we demonstrated no association between the SNPs *c.1-98A>G* (rs6269), *c.186C>T (p.=)* (rs4633), *c.408C>G (p.=)* (rs4818) and *c.472G>A (p.Val158Met)* (rs4680) and chronic pancreatitis. As a consequence, our results do not support a significant role for the *COMT* gene in the chronic pancreatitis.

A possible limitation of our study is that we do not have detailed insights into nicotine and alcohol use in our healthy controls. Numerous studies have explored the association of COMT with alcohol dependence. The Met^{158} allele has been associated with late-onset alcoholism in men, but not in the development of early-onset alcoholism with severe antisocial behaviour.[30,31] Second, the Met^{158} allele has also been associated with elevated weekly alcohol consumption in male social drinkers.[32] However, these findings are not consistent, because others failed to find evidence to support an association between alcohol dependence and variation in COMT.[33] In addition, we do not know if the pain pattern is different between patients with idiopathic and alcoholic chronic pancreatitis.

In conclusion, our study shows that the SNPs of the *COMT* gene are not associated with chronic pancreatitis. Because our results do not answer the complete complex of pain, future studies are needed to characterise the joint effect of multiple genes affecting pain.

REFERENCES

1. Garg PK, Tandon RK. Survey on chronic pancreatitis in the Asia-Pacific region. J Gastroenterol Hepatol. 2004;19(9):998-1004.
2. Van Esch AA, Wilder-Smith OH, Jansen JB, et al. Pharmacological management of pain in chronic pancreatitis. Dig Liver Dis. 2006;38(7):518-26.
3. Mogil JS. The genetic mediation of individual differences in sensitivity to pain and its inhibition. Proc Natl Acad Sci U S A. 1999;96(14):7744-51.
4. Reimann F, Cox JJ, Belfer I, et al. Pain perception is altered by a nucleotide polymorphism in SCN9A. Proc Natl Acad Sci USA. 2010;107(11):5148-53.
5. Diatchenko L, Nackley AG, Slade GD, et al. Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. Pain. 2006;125(3):216-24.
6. Hagen K, Pettersen E, Stovner LJ, et al. The association between headache and Val158Met polymorphism in the catechol-O-methyltransferase gene. J Headache Pain. 2006;7(2):70-4.
7. Hagen K, Pettersen E, Stovner LJ, Skorpen F, Zwart JA. No association between chronic musculoskeletal complaints and Val158Met polymorphism in the Catechol-O-methyltransferase gene. The HUNT study. BMC Musculoskelet Disord. 2006;7:40.
8. Armero P, Muriel C, Santos J, et al. COMT (Val158Met) polymorphism is not associated to neuropathic pain in a Spanish population. Eur J Pain. 2005;9(3):229-32.
9. Zubieta JK, Heitzeg MM, Smith YR, et al. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 2003;299(5610):1240-3.
10. Vargas-Alarcon G, Fragoso JM, Cruz-Robles D, et al. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis Res Ther. 2007;9(5):R110.
11. Jensen KB, Lonsdorf TB, Schalling M, et al. Increased sensitivity to thermal pain following a single opiate dose is influenced by the COMT val(158)met polymorphism. PLoS One. 2009;4(6):e6016.
12. Lee PJ, Delaney P, Keogh J, et al. Catecholamine-o-methyltransferase polymorphisms are associated with postoperative pain intensity. Clin J Pain. 2011;27(2):93-101.
13. McLean SA, Diatchenko L, Lee YM, et al. Catechol O-methyltransferase haplotype predicts immediate musculoskeletal neck pain and psychological symptoms after motor vehicle collision. J Pain. 2011;12(1):101-7.
14. Reyes-Gibby CC, Shete S, Rakvag T, et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain. Pain. 2006;17:1-7.
15. Rakvag TT, Klepstad P, Baar C, et al. The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients. Pain. 2005;116(1-2):73-8.
16. Cumming P, Brown E, Damsma G, et al. Formation and clearance of interstitial metabolites of dopamine and serotonin in the rat striatum. J Neurochem. 1992;59(5):1905-14.
17. Li PP, Warsh JJ, Godse DD. Formation and clearance of norepinephrine glycol metabolites in mouse brain. J Neurochem. 1984;43(5):1425-33.
18. Zubieta JK, Smith YR, Bueller JA, et al. Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science. 2001;293(5528):311-5.
19. Nackley AG, Tan KS, Fecho K, et al. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both beta2- and beta3-adrenergic receptors. Pain. 2007;128(3):199-208.
20. Mannisto PT, Kaakkola S. COMT: biochemistry, molecular biology, pharmacology, and clinical efficacy of the new selective COMT inhibitors. Pharmacol Rev. 1999;51(4):593-628.
21. Lotta T, Vidgren J, Tilgmann C, et al. Kinetics of human soluble and membrane-bound catechol O-methyltransferase. Biochemistry. 1995;34(13):4202-10.
22. Nackley AG, Shabalina SA, Lambert JE, et al. Low enzymatic activity haplotypes of the human catechol-O-methyltransferase gene: enrichment for marker SNPs. PLoS One. 2009;4(4):e5237.
23. Diatchenko L, Slade GD, Nackley AG, et al. Genetic basis for individual variations in pain perception and the development of a chronic pain condition. Hum Mol Genet. 2005;14(1):135-43.
24. Howes N, Lerch MM, Greenhalf W, et al. Clinical and genetic characteristics of hereditary pancreatitis in Europe. Clin Gastroenterol Hepatol. 2004;2(3):252-61.
25. www.genetik.charite.de. Tests for Hardy-Weinberg equilibrium.
26. www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview.
27. Derikx MH, Drenth JP. Genetic factors in chronic pancreatitis; implications for diagnosis, management and prognosis. Best Pract Res Clin Gastroenterol. 2010;24(3):251-70.
28. Drenth JP, te Morsche R, Jansen JB. Mutations in serine protease inhibitor Kazal type 1 are strongly associated with chronic pancreatitis. Gut. 2002;50(5):687-92.
29. Witt H, Sahin-Toth M, Landt O, et al. A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. Nat Genet. 2006;38(6):668-73.
30. Hallikainen T, Lachman H, Saito T, et al. Lack of association between the functional variant of the COMT gene and early-onset alcoholism associated with severe antisocial behavior. Am J Med Genet. 2000;96(3):348-52.
31. Tiihonen J, Hallikainen T, Lachman H, et al. Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism. Mol Psychiatry. 1999;4(3):286-9.
32. Kauhanen J, Hallikainen T, Tuomainen TP, et al. Association between the functional polymorphism of catechol-O-methyltransferase gene and alcohol consumption among social drinkers. Alcohol Clin Exp Res. 2000;24(2):135-9.
33. Foroud T, Wetherill LF, Dick DM, et al. Lack of association of alcohol dependence and habitual smoking with catechol-O-methyltransferase. Alcohol Clin Exp Res. 2007;31(11):1773-9.

Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Endocarditis: effects of routine echocardiography during Gram-positive bacteraemia

F.J. Vos[1,2,6*], C.P. Bleeker-Rovers[1,6], P.D. Sturm[3,6], P.F.M. Krabbe[4], A.P.J. van Dijk[5], W.J.G. Oyen[2,6], B.J. Kullberg[1,6]

Departments of [1]Internal Medicine, [2]Nuclear Medicine, [3]Medical Microbiology, [4]Epidemiology Biostatics & Health Technology Assessment, [5]Heart Lung Centre, Department of Cardiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, [6]Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)24-361 88 19, fax: +31(0)24-354 17 34, e-mail: f.vos@aig.umcn.nl

ABSTRACT

Background: Despite firm recommendations to perform echocardiography in high-risk patients with Gram-positive bacteraemia, routine echocardiography is not embedded in daily practice in many settings. The aim of this study was to evaluate whether a regime including routine echocardiography results in better outcome.

Methods: A total of 115 patients with Gram-positive bacteraemia and at least one risk factor for developing metastatic infection were prospectively included. Routine echocardiography was advocated and facilitated in these patients. Results were compared with a matched historical control group of 230 patients in whom echocardiography was performed at the discretion of the attending physician. Endocarditis was diagnosed according to the Duke criteria.

Results: Echocardiography was performed more often in the study group (82 *vs* 27%, p<0.001). Endocarditis was diagnosed more often among study patients, 22/115 (19%) *vs* 17/230 (7%) in the control group (p=0.002). In the study group echocardiography revealed vegetations in 22 of 94 (23%) patients, compared with 17 of 64 (27%) control patients (p=0.7). In the absence of heart murmurs, 70% of patients underwent echocardiography in the study group against 21% in the control group (p<0.001). No differences in adherence to American Heart Association guidelines concerning treatment of endocarditis were noticed. In patients with endocarditis, overall mortality was 23% in study patients and 59% in controls (p=0.04).

Conclusion: Routinely performed echocardiography in patients with Gram-positive bacteraemia resulted in diagnosing endocarditis in a larger proportion of patients, which was associated with a significant decrease in mortality rates. In the past, endocarditis was probably detected in a more advanced stage.

KEYWORDS

Bacteraemia, echocardiography, infective endocarditis, *Staphylococcus aureus*, *Streptococcus* spp

INTRODUCTION

Timely recognition of infective endocarditis is the first step in successful management and improved outcome of this potentially devastating disease. However, classical signs of endocarditis, such as new-onset heart murmurs and skin lesions, are absent in up to 57% of patients.[1,2] The importance of echocardiography to reveal cardiac sequelae has been emphasised in several studies.[3] In the American Heart Association (AHA) and European Society of Cardiology (ESC) guidelines, echocardiography is recommended for all patients with high-risk *Staphylococcus aureus* bacteraemia to reveal disseminated disease.[4,5] Although the recommendations from the literature are clear, routine echocardiography is not performed in a substantial number of patients with *S. aureus* bacteraemia in many clinical settings: in several studies, patients with *S. aureus* bacteraemia underwent echocardiography in only 27 to 59% of cases.[2,6-9]

We performed a unique prospective case-control study in patients with Gram-positive bacteraemia and at least one risk factor for the presence of metastatic infection comparing two echocardiographic regimes.[10-12] We added patients with Gram-positive bacteraemia because risk factors predicting the presence of metastatic infection were suggested to be identical to those in *S. aureus* bacteraemia.[13] In this prospective study, routine echocardiography was facilitated in all patients within two weeks after admission in the study group and was compared with a matched historical control group in the same hospital in whom echocardiography was performed at the discretion of the attending physician. The aim of the study was to evaluate whether routine performance of echocardiography in a high-risk patient group with Gram-positive bacteraemia leads to an increased incidence of the diagnosis of endocarditis and a better outcome when compared with clinically driven requests for echocardiography.

PATIENTS AND METHODS

Study design

This study is part of a prospective case-control study in which patients with a positive blood culture growing *Staphylococcus aureus*, *Streptococcus* species (excluding *S. pneumoniae*), or *Enterococcus* species at high risk for developing secondary metastatic foci between November 2005 and January 2008 were assigned to undergo an FDG-PET scan within 14 days after their first positive blood culture. Echocardiography was advocated and facilitated in all patients. High-risk bacteraemia was defined as the presence of at least one of the known risk factors for metastatic infectious disease, i.e., community acquisition, treatment delay for more than 48 hours after the onset of symptoms, persisting fever for more than 72 hours after initiation of antibiotic treatment, or the presence of persistently positive blood cultures for more than 48 hours after starting appropriate treatment. The original study was registered in the ISRCTN database, number 76425553. The protocol was approved by the institutional review board. Written informed consent was obtained from all patients.

All charts of subsequent adult, non-neutropenic patients with positive blood cultures were revised within the first working day in order to check for eligibility. Patients primarily admitted to the ICU were only included if they were discharged from the ICU within 14 days. Exclusion criteria were polymicrobial infection and pregnancy.

A historical control group was assigned by revising all charts of patients with the same type of bacteraemia during the four years before the study started. For this purpose, the database of the department of medical microbiology was used. Only patients with the same risk profile were eligible, including an ICU stay of less than 14 days. Matching criteria were the microorganism, community acquisition, and the presence or absence of one of the remaining risk factors. For every study patient, two of the best matching controls were included.

Endocarditis and metastatic infection

Endocarditis was defined according to the modified Duke criteria.[14] The AHA guidelines were used for the treatment of infective endocarditis. Choice and timing of starting antibiotics were evaluated in all patients diagnosed with endocarditis. No relevant changes were noticed between subsequent AHA guidelines throughout the study period.[4,15] The portal of entry of bacteraemia was defined as a localised focus of infection preceding bacteraemia. A central venous catheter (CVC) was considered a portal of entry if there was evidence of inflammation at the insertion site or if culture of the catheter tip grew the same microorganism as the blood culture in the absence of evidence for another source of infection. Metastatic infection was discriminated from the portal of entry of infection. Metastatic infection was defined as true haematogenous spreading of infection, e.g. endocarditis or spondylodiscitis. In addition, infectious foci without anatomic relations to the portal of entry or direct spreading outside the anatomic borders of the portal of entry were defined as metastatic infection, e.g., deep tissue abscess complicating a surgical wound infection.

Echocardiography

Echocardiography was strongly recommended and facilitated in all study patients by the principal investigator. Transthoracic echocardiography (TTE) was only performed first in the absence of prosthetic valves, but had to be followed by transoesophageal echocardiography (TEE) when no signs of vegetations were visualised. An echocardiogram was judged to be positive for endocarditis when vegetations, defined as oscillating intracardiac structures, were visualised on valves or their adjacent structures or in the path of a regurgitant jet, or on implanted material in the absence of an alternative anatomic explanation.[4] The outcomes of TTE and TEE were compared.

Patients

The site of acquisition of infection was determined in all patients. Bacteraemia was considered to be nosocomial if only blood cultures taken after >48 hours of hospitalisation were positive and clinical signs of the infection were absent at the time of admission. All other infections were considered community-acquired. Blood cultures were routinely taken during the first three days of admission and every second day as long as blood cultures remained positive. Except for echocardiography and FDG-PET,

which were routinely performed in all study patients, other diagnostic tests were ordered at the discretion of the attending physician. Special attention was paid to the presence of heart murmurs, immunological or vascular skin features, and the presence of guiding symptoms and signs supporting possible metastatic infectious foci. Patient follow-up ended six months after admission. Mortality data, epidemiological data, number, type and results of all diagnostic tests and treatment data were registered in a structured database (Microsoft Access).

Endpoints

The primary endpoint was the incidence of endocarditis as defined by the Duke criteria.[14] Secondary outcome measures were overall mortality, other metastatic foci of infection, and outcome of TTE and TEE.

Statistical evaluation

Differences between groups were tested with Fisher's exact tests for categorical variables. Differences for continuous variables were tested with unpaired Student t-tests. Differences were considered to be statistically significant at a p value less than 0.05 (two-sided). Cox proportional hazard models were performed to analyse outcome after adjustment for potential confounding differences between both groups. SPSS, version 17.0, was used for the analyses.

Table 1. *Baseline characteristics of study and control patients*

	Study patients % (n)	Control patients % (n)
Total number of patients	(115)	(230)
Male	56% (65)	52% (120)
Mean age (years)	59 ± 16	58 ± 16
Blood culture results:		
- *S. aureus**	64% (73)	64% (146)
- *Haemolytic streptococcus*	11% (13)	13% (29)
- *Viridans streptococcus*	15% (17)	13% (31)
- *Enterococcus* spp	10% (12)	10% (24)
Community acquisition	70% (81)	68% (156)
Treatment delay >48 hrs	27% (31)	45%[#] (104)
Persistent fever >72 hrs	46% (53)	37% (86)
Portal of entry unknown	52% (60)	46% (106)
CVC not removed within 48 hrs	5% (6)	8% (18)
Persistently positive blood cultures	16% (18)	6% (14)
Prior ICU admission	11% (13)	10% (24)
Diabetes mellitus	29% (33)	13%[b] (31)
Malignancy	14% (16)	17% (39)
Immune suppression	23% (26)	17% (39)
Prosthetic heart valves	6% (7)	4% (9)

***All *S. aureus* isolates were methicillin-susceptible. [#]p<0.05 (treatment delay was not a significant confounder for survival in a Cox proportional hazards model (not shown). p<0.05 (repetitive blood cultures were not routinely taken in the control group). No intravenous drug use was seen in the two groups.**

Table 2. *Number of patients in whom echocardiography was performed*

	Study patients (n=115)	Control patients (n=230)
TTE only	34% (39)	18% (43)*
TEE only	9% (10)	3% (7)*
TEE following TTE	39% (45)	6% (14)*
Total percentage of echocardiography	82% (94)	27% (64)*

TTE = transthoracic echocardiography; TEE = transoesophageal. p<0.05.

RESULTS

A total of 148 eligible patients with Gram-positive bacteraemia were identified during the study period. Of those, 22 were excluded because of prolonged ICU stay, and 11 refused informed consent. The remaining 115 patients were included in the study. A matched control group of 230 patients was identified out of a pool of 294 eligible patients. Baseline characteristics did not differ significantly between the two groups except for a treatment delay of more than 48 hours, which was found significantly more often in the control group, persistently positive blood cultures and diabetes mellitus, which were found significantly more often in the study group (*table 1*). Treatment delay was not a significant confounder for survival in a Cox proportional-hazards model (not shown, p=0.9). Follow-up blood culture samples had not been routinely drawn in the control group. Echocardiography was performed significantly more often in the study group (82% (94/115) *vs* 27% (64/230), p<0.001, *table 2*). In the study group two patients died before echocardiography took place, six patients refused echocardiographic evaluation and in 13 patients echocardiographic evaluation was not performed for various other reasons. The mean time between the first positive blood culture and echocardiography was 6.4 *vs* 6.8 days in the study and control group respectively, with a median of five days in both groups. Endocarditis was diagnosed significantly more often among study patients, 22/115 (19%) *vs* 17/230 (8%) in the control group (p=0.002). In the control group, endocarditis was first detected by autopsy in another two patients. Echocardiography revealed vegetations in 22 of 94 (23%) study patients in whom echocardiography was performed, compared with 17 of 64 (27%) patients in the control group (p=0.7). A negative TTE was followed by TEE in 50% of study patients (36/72) *vs* 11 of 48 control patients (23%, p=0.26). In six of 22 patients with definite endocarditis (27%), TTE did not show any signs of endocarditis while only TEE did. This was also the case in three of 17 (18%) control patients with endocarditis.

S. aureus was the causative microorganism in 13 patients with endocarditis (59%) in the study group and in eight patients (42%) in the control group (*table 3*). Seven patients in the study group had a prosthetic valve (6.1%) compared with nine patients in the control group (3.9%, p=0.42). In the study group, 4/7 of these patients were diagnosed with prosthetic valve endocarditis *vs* 3/9 in the control group (p=0.6). Only a minority of patients had vascular or immunological skin phenomena supporting a diagnosis of endocarditis: 3.5% (n=4) in the study group *vs* 0.9% (n=2) in the control group (p=0.1). Of the patients diagnosed with endocarditis, 64% had a new or existing heart murmur in the study group *vs* 79% among controls (p=0.32, *table 3*). Of the patients without a heart murmur, 71% (46/65) underwent echocardiography in the study group against 21% (39/190) in the control group (p<0.001). In patients with endocarditis, relevant metastatic foci of infection requiring supplementary therapy were found in 13 (59%) of study patients and in eight patients (42%) in the control group (p=0.54, *table 4*). In some of these patients a truly haematogenous focus of infection was the first presentation on admission, leading to repetitive echocardiography in order to identify the source of persistently positive blood cultures.

AHA guidelines concerning the choice of antibiotic treatment were followed in all study patients compared with 18 of 19 (95%, p=0.46) in the control group. Seven patients in both groups underwent valve replacement (p=0.25). The mean duration of symptoms in patients diagnosed with endocarditis before blood culture results were present was 16 days in the study group *vs* 26 in the control group. The median duration of treatment among study patients diagnosed with endocarditis was 42 (range 15 to 140) days compared with 38 (range 1 to 182) days in the control group. Two patients in the study group and six patients in the control group died within one month after admission while on antibiotic treatment. When these patients are excluded, median duration of treatment was 44 (range 13 to 140) and 42 (range 7 to 182) days, respectively. In patients without definite endocarditis or other complicating infectious foci, median treatment duration was 14 days in both groups.

Mortality rates at six months of follow-up differed significantly between the two groups in favour of the study group. Overall mortality was 19% (22/115) in study patients compared with 32% (74/230) in control patients (p=0.011). This difference was also found regarding infection-related mortality (14/115 *vs* 56/230, p=0.049). In the patients diagnosed with endocarditis during life, mortality was 23% (5/22) in the study group and 59% (10/17) in controls (p=0.04).

Table 3. *Baseline characteristics of patients with endocarditis**

	Study patients (n=22)	Control patients (n=19)
Median age (years)	58	59
Community acquisition	91% (20)	63% (12)
Duration of symptoms prior to blood culture results (mean number of days)	16	26
Unknown portal of entry	82% (18)	63% (12)
Cardiac murmur	64% (14)	79% (15)
Skin phenomena	18% (4)	11% (2)
Prosthetic valve	14% (3)	21% (4)
Microorganism		
- *S. aureus*	59% (13)	42% (8)†
- *Haemolytic Streptococcus*	9% (2)	5% (1)
- *Viridans Streptococcus*	27% (6)	42% (8)
- *Enterococcus* spp	5% (1)	11% (2)
Adherence to AHA guideline	100% (22)	95% (18)

***Endocarditis was found in 22/115 (19%) of study patients and in 19/230 (8%) of matched historical controls, p=0.005. In 2 of 19 control patients endocarditis was only diagnosed post-mortem. No right-sided endocarditis was detected. † p=0.006**

Table 4. *Other metastatic infectious foci in patients with endocarditis*

	Study patients (n=22)	Control patients (n=19)
Patient without metastatic foci	9	9
Patients with metastatic foci	13	10
- Lung	0	1
- CNS	3	2
- Endovascular	4	1
- Soft tissue	2	1
- Spondylodiscitis	3	3
- Arthritis	6	2
- Psoas abscess	1	0
- Spleen	1	0
- Eye	1	0

CNS = central nervous system.

DISCUSSION

This is the first study directly comparing two echocardiographic regimes. Routinely facilitating echocardiography in patients with Gram-positive bacteraemia resulted in the detection of significantly more patients with definite endocarditis compared with a matched historical control group in the same hospital, in whom echocardiography was ordered at the discretion of the treating physician. This was associated with significantly reduced mortality rates at six-month follow-up in the study group.

Although the recommendations to perform echocardiography have been clear for a long time, the percentage of control patients in whom echocardiography

was performed (27%) was low, completely in agreement with other population-based studies (22 to 59%).[3,6,16] The incidence of definite endocarditis in our control group (8%) is comparable with those studies (5 to 13%).[6,8,9,16] Reported incidence rates are higher (25 to 32%) in studies on selected populations in whom echocardiography was routinely performed.[17,18] No previous study, however, has directly compared two echocardiographic regimens in two identical groups, as was done in the current study. In a study on patients with *S. aureus* bacteraemia, the impact of recommendations of an infectious disease consultant were studied.[6] In patients not treated according to these recommendations (n=132), relapse rate and mortality were higher compared with patients in whom the recommendations of an infectious disease consultant were followed. In half (65/132) of patients in that study noncompliance to the recommendations concerned the failure to perform any form of echocardiography.

Although the recommendation to perform echocardiography in all patients with *S. aureus* bacteraemia is supported by both treatment guidelines and previous studies, the decision to perform echocardiography in clinical practice appears to be largely driven by the presence of certain symptoms.[1,3-5,18] This might be the most important caveat explaining the proportion of patients (18%) who did have echocardiographic examination even in our study group. This is, however, favourable compared with other studies evaluating the effects of consultation with an infectious disease specialist recommending echocardiographic evaluation in patients with *S. aureus* bacteraemia. Echocardiographic evaluation was not performed in 27 to 34% of patients in those studies.[6,9,19] In our historical control group, echocardiography was mainly restricted to patients with cardiac murmurs. Cardiac murmurs, however, were only present in 64% of patients with definite endocarditis in our prospective study group. Also, endocarditis-associated vascular and immunological phenomena of the skin and mucosa were only found in a very small minority of cases in our study group. This is in line with several other studies.[2,3,20] Patients with possible endocarditis, therefore, cannot be selected by the presence of clinical findings alone. Our findings support recommendations to perform echocardiography in all patients with *S. aureus* bacteraemia.[4,5] In previous studies, risk factors for metastatic infection in patients with other Gram-positive bacteraemia were identical to those in patients with *S. aureus* bacteraemia.[13] The present study suggests that routine echocardiography should also be performed in patients with bacteraemia caused by other Gram-positive microorganisms and at least one risk factor for metastatic complications. In 27% (6/22) of patients, infective endocarditis was only revealed by TEE following normal TTE. This is comparable with other studies (14 to 19%) comparing both echocardiographic techniques.[17,20,21]

An important difference between the present study and previous studies is that a matched control group was included in order to compare two strategies of a diagnostic approach in high-risk patients with Gram-positive bacteraemia. The use of a historical control group might have introduced potential bias. Patients were, however, accurately matched regarding risk profiles and microorganisms. In addition, the control group was large and within the timeframe of this study, no changes in diagnostic work-up or therapy regarding Gram-positive bacteraemia or endocarditis had occurred in our hospital. It is therefore most likely that endocarditis remained undetected in many control patients, as the incidence of endocarditis was significantly lower in the control group. This was also supported by the fact that endocarditis was first diagnosed by autopsy in two patients in the control group and that mortality rates were significantly higher in the control group. Autopsy had only been performed in a minority of patients in both patient groups. The antibiotic regimens for diagnosed endocarditis were similar in the two groups, regarding both choice and timing of antibiotic treatment. However, more patients in the study group underwent valve replacement. In the absence of definite endocarditis or metastatic infectious foci, no differences in treatment duration were found, suggesting that the percentage of patients with prolonged antibiotic treatment due to suspected but unproven endocarditis or metastatic foci did not differ between patient groups. Most likely early detection and intervention led to the lower mortality in patients with endocarditis in the study group, resulting in favourable mortality rates (23%) in line with previous studies (22 to 46%).[3,22] In contrast, in the past endocarditis was presumably diagnosed in an advanced stage in most patients. This was further supported by the difference in duration of symptoms prior to diagnosis. This may also be an important explanation for higher mortality rates in endocarditis patients in the past in studies on the positive effects of infectious diseases specialist consultation. An increase in the number of patients with *S. aureus* bacteraemia who underwent echocardiographic evaluation as a result of consultation with an infectious disease specialist was described in some of these studies.[6,9,19] Overall mortality rates (32%) in the control group were in line (17 to 43%) compared with studies that introduced consultation of an infectious disease specialist in order to improve diagnostics and treatment.[6,19] No details on mortality in patients with endocarditis are provided in those studies. Mortality (59%) in patients with endocarditis in the control group in the present study is in line with mortality (71 to 74%) in patients with advanced stages of endocarditis, including those with metastatic disease and congestive heart failure and patients with a contraindication for surgery.[3,22] The fact that a retrospective control group was included makes it impossible to evaluate

in detail if the non-significant difference in patients who underwent valve replacement reflects contraindications for surgery due to the advanced stage alone, or a change in acceptance for cardiothoracic surgery in the timeframe of the study. No such changes, however, were noticed in internal guidelines in our hospital.

Relevant additional infectious foci were found in more than half of patients with endocarditis. Several of these foci, such as spondylodiscitis and mycotic aneurisms, required prolonged antibiotic treatment or surgical interventions. This underscores the possibility of metastatic infectious foci being the cause of treatment failure and thus the need for a directed search for these complicating foci in addition to echocardiography. FDG-PET appears to be a valuable diagnostic technique for this purpose.[13,23]

CONCLUSION

Routine performance of echocardiography in all patients with Gram-positive bacteraemia and at least one risk factor for complicated infection is associated with the detection of a significantly higher percentage of endocarditis cases when compared with a strategy in which echocardiography was performed based on signs and clinical suspicion of endocarditis. Patients selected by a clinically driven echocardiographic regime were characterised by higher mortality rates. This probably reflects the fact that only advanced cases of endocarditis are detected relying on clinical signs and symptoms of endocarditis alone. Our results also support the finding by others that TEE is more sensitive than TTE in a substantial proportion of endocarditis patients. Therefore, transthoracic echocardiography should be performed routinely in all patients with Gram-positive bacteraemia and at least one risk factor for complicated infection. If transthoracic echocardiography does not reveal signs of endocarditis, transoesophageal echocardiography should be performed.

REFERENCES

1. Slabbekoorn M, Horlings HM, van der Meer JT, Windhausen A, van der Sloot JA, Lagrand WK. Left-sided native valve *Staphylococcus aureus* endocarditis. Neth J Med. 2010;68:341-7.
2. Espersen F, Frimodt-Møller N. *Staphylococcus aureus* endocarditis. A review of 119 cases. Arch Intern Med. 1986;146:1118-21.
3. Røder BL, Wandall DA, Frimodt-Møller N, Espersen F, Skinhøj P, Rosdahl VT. Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. Arch Intern Med. 1999;159:462-9.
4. ACC/AHA 2006 guidelines for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2006;114:e84-231.
5. Habib G, Hoen B, Tornos P, et al. Guidelines on the prevention, diagnosis, and treatment of infective endocarditis (new version 2009): the Task Force on the Prevention, Diagnosis, and Treatment of Infective Endocarditis of the European Society of Cardiology (ESC). Endorsed by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society of Chemotherapy (ISC) for Infection and Cancer. Eur Heart J. 2009;30:2369-413.
6. Fowler VG Jr, Sanders LL, Sexton DJ, et al. Outcome of *Staphylococcus aureus* bacteremia according to compliance with recommendations of infectious diseases specialists: experience with 244 patients. Clin Infect Dis. 1998;27:478-86.
7. Figueiredo LT, Ruiz-Junior E, Schirmbeck T. Infective endocarditis (IE) first diagnosed at autopsy: analysis of 31 cases in Ribeirao Preto Brazil. Rev Inst Med Trop Sao Paulo. 2001;43: 213-6.
8. Chang FY, MacDonald BB, Peacock JE Jr, et al prospective multicenter study of *Staphylococcus aureus* bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance. Medicine (Baltimore). 2003;82:322-32.
9. Jenkins TC, Price CS, Sabel AL, Mehler PS, Burman WJ. Impact of routine infectious diseases service consultation on the evaluation, management, and outcomes of *Staphylococcus aureus* bacteremia. Clin Infect Dis. 2008;46:1000-8.
10. Fowler VG Jr, Olsen MK, Corey GR, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med. 2003;163:2066-72.
11. Lesens O, Hansmann Y, Storck D, Christmann D. Risk factors for metastatic infection in patients with *Staphylococcus aureus* bacteremia with and without endocarditis. Eur J Intern Med. 2003;14:227-31.
12. Lesens O, Hansmann Y, Brannigan E, et al. Positive surveillance blood culture is a predictive factor for secondary metastatic infection in patients with *Staphylococcus aureus* bacteraemia. J Infect. 2004;48:245-52.
13. Cuijpers ML, Vos FJ, Bleeker-Rovers CP, et al.Complicating infectious foci in patients with *Staphylococcus aureus* or *Streptococcus* species bacteraemia. Eur J Clin Microbiol Infect Dis. 2007;26:105-13.
14. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633-8.
15. Bonow RO, Carabello B, de Leon AC Jr, et al. Guidelines for the management of patients with valvular heart disease: executive summary. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients with Valvular Heart Disease). Circulation. 1998;98:1949-84.
16. Jensen AG, Wachmann CH, Espersen F, Scheibel J, Skinhoj P, Frimodt-Moller N. Treatment and outcome of *Staphylococcus aureus* bacteremia: a prospective study of 278 cases. Arch Intern Med. 2002;162:25-32.
17. Fowler VG Jr, Li J, Corey GR, et al. Role of echocardiography in evaluation of patients with *Staphylococcus aureus* bacteremia: experience in 103 patients. J Am Coll Cardiol. 1997;30:1072-8.
18. Abraham J, Mansour C, Veledar E, Khan B, Lerakis S. *Staphylococcus aureus* bacteremia and endocarditis: the Grady Memorial Hospital experience with methicillin-sensitive *S. aureus* and methicillin-resistant *S. aureus* bacteremia. Am Heart J. 2004;147:536-9.
19. Rieg S, Peyerl-Hoffmann G, de With K, et al. Mortality of *S. aureus* bacteraemia and infectious diseases specialist consultation-a study of 521 patients in Germany. J Infect. 2009;59:232-9.
20. Durante-Mangoni E, Bradley S, Selton-Suty C, et al. Current features of infective endocarditis in elderly patients: results of the International Collaboration on Endocarditis Prospective Cohort Study. International Collaboration on Endocarditis Prospective Cohort Study Group. Arch Intern Med. 2008;168:2095-103.
21. Sullenberger AL, Avedissian LS, Kent SM. Importance of transesophageal echocardiography in the evaluation of *Staphylococcus aureus* bacteremia. J Heart Valve Dis. 2005;14:23-8.
22. Hill EE, Herijgers P, Claus P, Vanderschueren S, Herregods MC, Peetermans WE. Infective endocarditis: changing epidemiology and predictors of 6-month mortality: a prospective cohort study. Eur Heart J. 2007;28:196-203.
23. Vos FJ, Bleeker-Rovers CP, Sturm PD, et al. 18F-FDG PET/CT for detection of metastatic infection in gram-positive bacteremia. J Nucl Med. 2010;51:1234-40.

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CASE REPORT

Spontaneous remission of immunotactoid glomerulopathy

I.M. Rood[1], L.G. Lieverse[3], E.J. Steenbergen[2], J.F. Wetzels[1], J.K. Deegens[1]*

Departments of [1]Nephrology and [2]Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, [3]Department of Internal Medicine, Maxima Medisch Center, Eindhoven, the Netherlands, *corresponding author: tel.: +31 (0)24-361 47 61, fax: +31 (0)24-354 00 22, e-mail: j.deegens@nier.umcn.nl

ABSTRACT

Immunotactoid glomerulopathy (ITG) is a rare cause of nephrotic syndrome, occurring in approximately 0.1% of native kidney biopsies. We describe a 43-year-old woman who presented with a nephrotic syndrome. Renal biopsy revealed a membranous pattern of glomerular injury. In electron microscopy the subepithelial deposits were comprised of 40 nm wide tubular structures, confirming ITG. During follow-up the patient developed a remission of proteinuria with only supportive treatment.

KEYWORDS

Immunotactoid glomerulopathy, membranous nephropathy, nephrotic syndrome, spontaneous remission

What was known on this topic?

Immunotactoid glomerulonephritis (ITG) is a rare cause of nephrotic syndrome. On electron microscopic examination, ITG is characterised by glomerular deposits of microtubules arranged in a parallel fashion. ITG can be idiopathic or secondary to an underlying disease including hepatitis C and lymphoproliferative disorders. The clinical course of patients with idiopathic ITG is difficult to predict because published series have been too small and the follow-up too short. To date, there is no proven effective therapy for idiopathic ITG. The response to immunosuppressive therapy and prognosis may differ according to light microscopic findings.

What does this case add?

There are two principal points that this case report adds. First, electron microscopic examination is important for the correct diagnosis of the underlying cause of nephrotic syndrome. ITG can only be diagnosed by electron microscopy. Moreover, early membranous nephropathy can be difficult to distinguish from minimal change disease without electron microscopic examination. Second, this case report is the first to show that patients with ITG and a membranous pattern of glomerular injury on light microscopy can attain a spontaneous remission of proteinuria, i.e. with only supportive treatment and not induced by immunosuppressive therapy.

INTRODUCTION

Glomerular diseases, characterised by the presence of organised deposits, are a rare cause of nephrotic syndrome. The most common form is amyloidosis. Immunotactoid glomerulonephritis (ITG) is reported in less than 0.1% of renal biopsies. ITG can mimic membranous nephropathy in light microscopy and immunofluorescence examination. We present a patient with ITG with a membranous pattern of glomerular injury. This case illustrates that ITG may be missed in the absence of electron microscopic studies. Furthermore, remissions can occur without immunosuppressive therapy.

CASE REPORT

A 43-year-old woman was referred to the outpatient clinic with progressive tiredness, leg cramps, oedema and proteinuria. She was not using any medication. Her past medical history revealed relapsing-remitting multiple sclerosis (MS) of more than ten years' duration. She

had received several courses of intravenous methylprednisolone. Six years before presentation, treatment with interferon was started, resulting in a complete remission of her MS. Treatment with interferon was discontinued two years before presentation.

Physical examination was unremarkable, except for pitting oedema in the lower extremities. At the time of her initial review, her blood pressure was 130/70 mmHg. Laboratory testing showed proteinuria of 4.9 g/24 hours and hypoalbuminaemia (25 g/l). Renal function was normal (eGFR using the Chronic Kidney Disease Epidemiology Collaboration formula was 110 ml/min/1.73 m^2). Additional work-up showed normal complement levels, no antinuclear or double-stranded-DNA antibodies, no cryoglobulins, and serum and urine protein electrophoresis revealed no signs of monoclonal gammopathy. Serology for hepatitis B and C was also negative. A renal biopsy was performed, showing normal glomeruli with negative immunofluorescence and a diagnosis of minimal change disease was made. Electron microscopic examination was not performed. In the absence of a definite diagnosis and because of the mild symptoms and normal renal function, immunosuppressive therapy was withheld. Supportive therapy was initiated consisting of a loop diuretic, an ACE inhibitor and a statin. Over the next six months her renal function remained stable, but nephrotic range proteinuria and hypoalbuminaemia persisted despite a well-regulated blood pressure <125/75 mmHg. The patient was referred for treatment advice and review of the renal biopsy. Upon light microscopic review of the initial renal biopsy, subtle abnormalities of the basal membrane were seen in some glomeruli raising the suspicion of membranous nephropathy. Unfortunately, no material was available for additional immunofluorescence and electron microscopic examination.

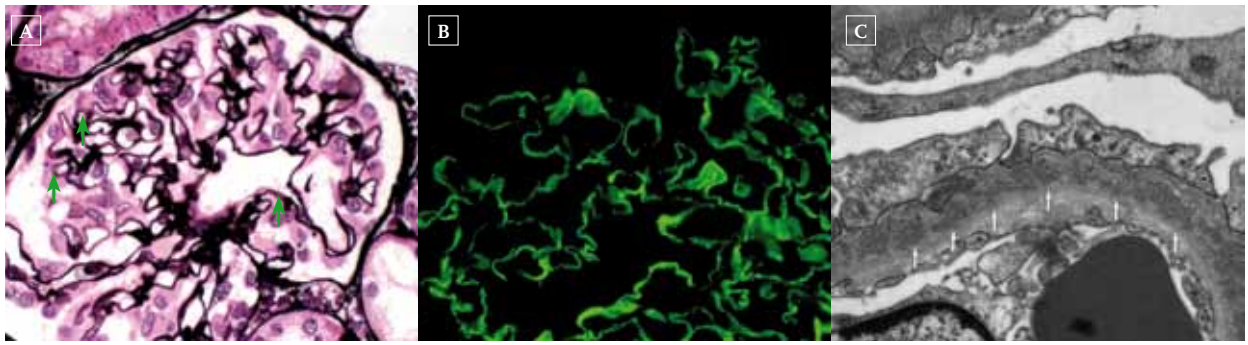
A new renal biopsy was performed (*figure 1*). Light microscopy showed ten glomeruli, two of which were globally sclerosed. Of the remainder, many glomeruli showed subtle abnormalities of the basal membrane consisting of capillary loop thickening and small areas of lucency were seen in the silver methenamine stain. No endocapillary hypercellularity was observed. There were no abnormalities of the endothelium, mesangium, Bowman's capsule or the juxtaglomerular apparatus. Congo-red staining was negative. Immunofluorescence showed fine granular deposits of IgG, C3, kappa and lambda as seen in membranous nephropathy. However, electron microscopy revealed subepithelial immune deposits consisting of microtubules of 40 nm diameter with hollow centres arranged in parallel stacks, compatible with a diagnosis of ITG. Mesangial deposits were not observed.

The patient was diagnosed with idiopathic ITG. Therapy was directed at the findings of membranous nephropathy on light microscopy. Urinary excretion of β2-microglobulin (0.15 µg/min) and IgG (76 mg/24 hours) were both low. Therefore, supportive therapy was continued. During follow-up the serum creatinine has remained stable and proteinuria decreased. Currently, the patient has attained a remission of proteinuria (0.22 g/day) with a stable serum albumin (36 g/l; *figure 2*), which persists after discontinuation of all supportive medication.

DISCUSSION

ITG belongs to the class of glomerular diseases characterised by the deposition of organised often fibrillary structures.[1-4] A schematic overview is given in *figure 3*.[5] Silver methenamine staining can be used to distinguish between disorders replacing the normal

Figure 1. *Renal biopsy findings*



(A) Light microscopy showing small areas of lucency in the glomerular basement membrane (green arrows). (B) Immunofluorescence microscopy showing finely granular (almost linear) deposits of IgG along the glomerular basement membrane.(C) Electron microscopic examination (original magnification 15000x) showing highly organised subepithelial deposits made up of microtubules (hollow centre) approximately 40 nm wide (arrows).

Figure 2. *Clinical course after diagnosis. Proteinuria showed a steady decline to 0.22 g/24 hours at last follow-up. Serum albumin increased to 36 g/l at last follow-up.*

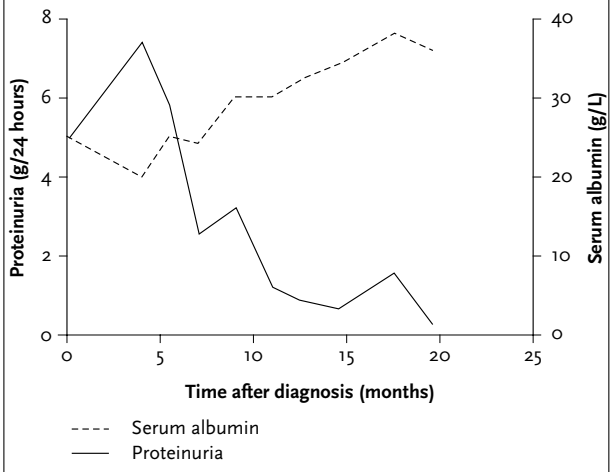
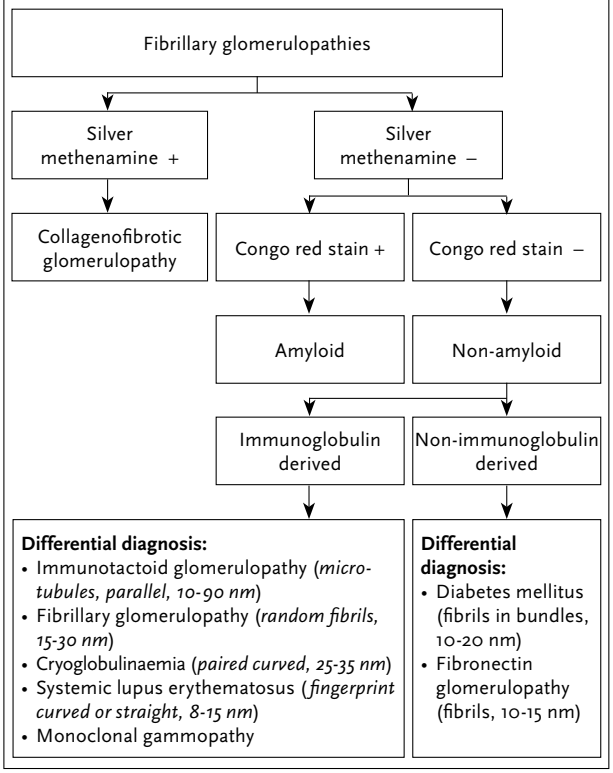


Figure 3. *Algorithmic approach to the interpretation of glomerular diseases with organised deposits (adapted from Herrera[5])*



mesangial matrix (methenamine negative) or disorders resulting in an increase of mesangial extracellular matrix (methenamine positive). Silver methenamine negative disorders with organised deposits are divided into two categories consisting of Congo red positive diseases, encompassing all types of amyloidoses, and Congo red negative disorders.[2] Most Congo red negative organised deposits are immunoglobulin derived. The deposits cannot be recognised by light microscopy, thus a diagnosis can only be made with certainty if renal tissue is appropriately examined by electron microscopy. Moreover, the specific features of organised deposits (size, shape, arrangement; *figure 3*) on electron microscopy can further assist in characterisation of the various disorders. Our case emphasises the importance of electron microscopic examination of renal biopsies from patients with nephrotic proteinuria. Electron microscopy was not only necessary to detect organised deposits but also to distinguish early membranous lesions from minimal change disease. Initially a diagnosis of minimal change disease was made based on light microscopy, and negative immunofluorescence examination. However, as noted by previous studies, early membranous nephropathy does not always show capillary loop thickening by light microscopy and may lack convincing immunofluorescence findings.[6,7] A study by Haas showed that electron microscopy provides useful diagnostic information in nearly half of native renal biopsies.[7]

ITG is a rare disease; in a large series it comprised only 0.1% of native kidney biopsies.[1] In series of adult patients with nephrotic syndrome, ITG constituted less than 4% of the biopsies.[2,8,9] ITG is characterised by the deposition of microtubules with a hollow core, generally measuring more than 30 nm in diameter and arranged in a parallel fashion.[5,10] The deposits are usually confined to the glomerulus, specifically the mesangium and subendothelial space. Some cases also have subepithelial or intramembranous deposits.[1-3] The deposits of ITG are immunoglobulin derived, and can be either polyclonal (30 to 40%) or monoclonal (60 to 70%).[1] Interestingly, in a few patients with ITG and chronic lymphocytic leukaemia or related B-cell lymphoma, monoclonal deposits with a microtubular diameter <30 nm have been observed.[11] Light microscopic findings associated with ITG are nonspecific and include membranoproliferative, diffuse proliferative and atypical membranous patterns.[1,3]

There is some debate as to whether ITG should be distinguished from fibrillary glomerulonephritis (FGN), a glomerulopathy characterised by randomly arranged, nonbranching fibrils in the mesangium and glomerular basement membrane with an average diameter of 20 nm.[1,2,8,12] Some regard ITG and FGN as a single disease with different ultrastructural variants, referring to both as ITG.[12,13] However, several studies have shown important clinical and immunopathological differences between these two entities. Patients with ITG exhibit a higher incidence of monoclonal deposits and lymphoproliferative disorders (33 to 50% *vs* 0 to 7%) compared with patients with FGN.[1,3,8,14]

ITG can occur at any age, with a peak occurrence at 60 years of age.[1-3,8] Patients with ITG present with proteinuria, which is in the nephrotic range in more than half of the patients.[1,3,8] Other findings include haematuria, hypertension and renal insufficiency. Patients with ITG have a predisposition to an underlying lymphoproliferative disease.[1-3,8] A lymphoproliferative disorder should especially be considered in patients with monoclonality of the deposits on renal biopsy.[1,8] ITG has also been reported in association with hepatitis C infection, HIV, leucocytoclastic vasculitis with hypocomplementaemia, lupus nephritis and cryoglobulinaemia.[3] Thus, a diagnosis of ITG should lead to a search for an underlying disorder. In the present case, we were unable to identify an underlying condition. Our patient had previously been diagnosed with MS; however, there is no known association between ITG and with MS.

The clinical course of patients with ITG is difficult to predict because published series have been too small, the follow-up was too short and/or they included patients with ITG secondary to underlying disorders.[1-3] Nevertheless, treatment directed at the underlying disorder can lead to remission of nephrotic syndrome in patients with secondary ITG.[2] In one study 83% of nephrotic patients with lymphoproliferative disease and/or paraproteinaemia attained a complete or partial remission.[3] To date, there is no proven effective therapy for idiopathic ITG and the response to immunosuppressive therapy is generally poor.[12] However, according to a recent study in patients with FGN, prognosis may differ according to histology, with the membranous type having the best prognosis.[1] We therefore decided to treat our patient according to our protocol for idiopathic membranous nephropathy.[15] For low-risk patients (normal renal function and low urinary excretion of β2-microglobulin and IgG), a wait-and-see policy is advised. During supportive therapy our patient attained a remission of proteinuria.

To our knowledge, this is the first case report describing a remission of proteinuria not induced by immunosuppressive therapy in a patient with idiopathic ITG. Admittedly, our case is somewhat different from previous reports.[1,3,16] In contrast to the previously described atypical membranous nephropathy lesions associated with mesangial matrix expansion, immune deposits in our patient were typical of membranous nephropathy and mesangial deposits were completely absent. This may explain the benign course in our patient, which is similar to idiopathic membranous nephropathy.

In conclusion, we demonstrate a female patient with idiopathic ITG and a typical membranous pattern on light microscopy and immunofluorescence, who attained a spontaneous remission of proteinuria. The present case also demonstrates that a diagnosis of membranous nephropathy and/or ITG can be missed by light microscopy and immunofluorescence. Therefore, electron microscope examination is mandatory in patients with a nephrotic syndrome.

REFERENCES

1. Rosenstock JL, Markowitz GS, Valeri AM, et al. Fibrillary and immunotactoid glomerulonephritis: Distinct entities with different clinical and pathologic features. Kidney Int. 2003;63:1450-61.
2. Fogo A, Qureshi N, Horn RG. Morphologic and clinical features of fibrillary glomerulonephritis versus immunotactoid glomerulopathy. Am J Kidney Dis. 1993;22:367-77.
3. Bridoux F, Hugue V, Coldefy O, et al. Fibrillary glomerulonephritis and immunotactoid (microtubular) glomerulopathy are associated with distinct immunologic features. Kidney Int. 2002;62:1764-75.
4. van Ginneken EE, Assmann KJ, Koolen MI, Jansen JL, Wetzels JF. Fibrillary-immunotactoid glomerulopathy with renal deposits of IgAlambda: a rare cause of glomerulonephritis. Clin Nephrol. 1999;52:383-9.
5. Herrera GA. Renal diseases associated with hematopoietic disorders or organized deposits. In: Zhou XJ, Laszik Z, Nadasky T, D'Agati V, Silva F, editors. Silva's diagnostic renal pathology. New York: Cambridge University Press, 2009:345-406.
6. Pearson JM, McWilliam LJ, Coyne JD, Curry A. Value of electron microscopy in diagnosis of renal disease. J Clin Pathol. 1994;47:126-8.
7. Haas M. A reevaluation of routine electron microscopy in the examination of native renal biopsies. J Am Soc Nephrol. 1997;8:70-6.
8. Pronovost PH, Brady HR, Gunning ME, Espinoza O, Rennke HG. Clinical features, predictors of disease progression and results of renal transplantation in fibrillary/immunotactoid glomerulopathy. Nephrol Dial Transplant. 1996;11:837-42.
9. Korbet SM, Schwartz MM, Lewis EJ. Immunotactoid glomerulopathy. Am J Kidney Dis. 1991;17:247-57.
10. Schwartz MM, Lewis EJ. The quarterly case: nephrotic syndrome in a middle-aged man. Ultrastruct Pathol. 1980;1:575-82.
11. Bridoux F, Hugue V, Coldefy O, et al. Fibrillary glomerulonephritis and immunotactoid (microtubular) glomerulopathy are associated with distinct immunologic features. Kidney Int. 2002;62:1764-75.
12. Korbet SM, Schwartz MM, Lewis EJ. Immuotactoid glomerulopathy (fibrillary glomerulonephritis). Clin J Am Soc Nephrol. 2006;1:1351-6.
13. Schwartz MM, Korbet SM, Lewis EJ. Immunotactoid glomerulopathy. J Am Soc Nephrol. 2002;13:1390-7.
14. Iskandar SS, Falk RJ, Jennette JC. Clinical and pathologic features of fibrillary glomerulonephritis. Kidney Int. 1992;42:1401-7.
15. du Buf-Vereijken PW, Branten AJ, Wetzels JF. Idiopathic membranous nephropathy: outline and rationale of a treatment strategy. Am J Kidney Dis. 2005;46:1012-29.
16. Muller-Hocker J, Weiss M, Sitter T, Samtleben W. Fibrillary glomerulonephritis mimicking membranous nephropathy--a diagnostic pitfall. Pathol Res Pract. 2009;205:265-71.

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PHOTO QUIZ

A soft-tissue mass on the forehead

S. Nadery[1*], E.M.S. Leyten[2]

[1]Department of Internal Medicine, Academic Medical Centre, University of Amsterdam, the Netherlands,
[2]Department of Internal Medicine, Medical Center Haaglanden, The Hague, the Netherlands,
[*]corresponding author: tel.: +31 (0) 20-566 22 22, fax: +31 (0)20-566 92 23, e-mail: s.nadery@amc.uva.nl

CASE REPORT

A 25-year-old Indonesian woman was referred to the surgeon for excision of a subcutaneous soft-tissue mass on her forehead. She noticed the swelling three months earlier, which seemed to progress slowly and was only slightly sensitive. She did not mention any other symptoms. On physical examination a 3-4 cm subcutaneous, fixed, soft-tissue swelling on the left side of her forehead was observed (*figure 1*). Magnetic resonance imaging (MRI) showed a small defect in the frontal bone underlying the subcutaneous mass. There was no involvement of the brain (*figure 2a,b*). She was referred to internal medicine for further analysis. A careful medical history revealed a backache, tiredness, 5 kg weight loss and night sweats without fever. Physical examination showed no further abnormalities except for a localised pain over the thoracic spine. Laboratory results were normal besides an erythrocyte sedimentation rate (ESR) of 79 mm/h and a C-reactive protein (CRP) of 14 mg/l. A human immunodeficiency virus (HIV) test was negative.

Figure 1. *Patient with soft swelling on forehead*

Figure 2a. *MR image showing the lesion in the frontal bone with some extension in the subcutis; no brain invasion is seen*

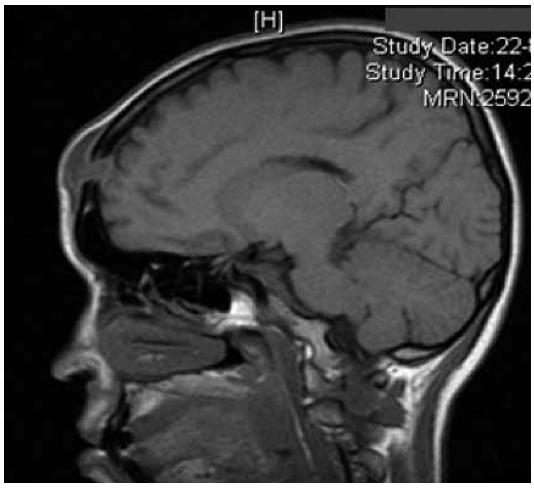
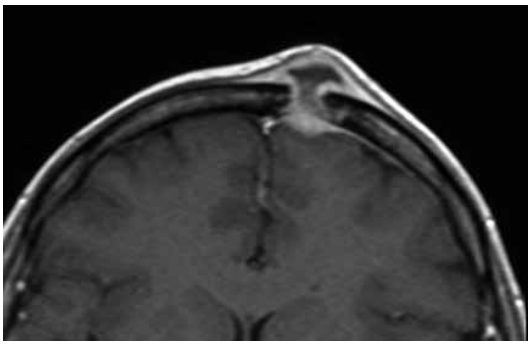


Figure 2b. *Axial image after gadolinium administration; rim enhancement of the lesion and some dural enhancement*



WHAT IS YOUR DIAGNOSIS?

See page 348 for the answer to this photo quiz.

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PHOTO QUIZ

A Sézary cryptogram

I.M.E. Desar[1*], W.B.C. Stevens[1], P.E. Verweij[2], W.J.F.M. van der Velden[1]

Departments of [1]Hematology and Oncology and [2]Medical Microbiology, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands, *corresponding author: tel.: +31 (0)25 351 03 53, e-mail: i.desar@aig.umcn.nl

CASE REPORT

A 77-year-old women was admitted to the haematology department because of fever. Her medical history consisted of a cutaneous T cell non-Hodgkin's lymphoma (Sézary syndrome) diagnosed in 2003, for which she had received several lines of chemotherapy, but was currently only taking prednisone 5 mg twice daily. The day before presentation she had collapsed after which she started to complain of headaches. Physical examination revealed no neurological abnormalities, in particular no signs of meningitis. There were no signs of a urinary, abdominal or pulmonary infection. The skin showed features of the Sézary syndrome, but also a large red swelling (20 cm in length) on the inner left thigh. This lesion was sharply demarcated, and painful on palpation. The laboratory results showed an elevated C-reactive protein of 62 mg/l (normal value <5 mg/l) and a leucocyte count of 9.4×10^9/l (with 72% neutrophils and 21% lymphocytes). A chest X-ray showed no abnormalities, and cerebral scanning CT revealed no intracerebral haemorrhages or lesions. The lesion was surgically drained, as shown in *figure 1*. Purulent material was sent to the microbiology laboratory for microscopic investigation and culture. The microscopy is shown in *figure 2*.

Figure 1. *Skin lesion*

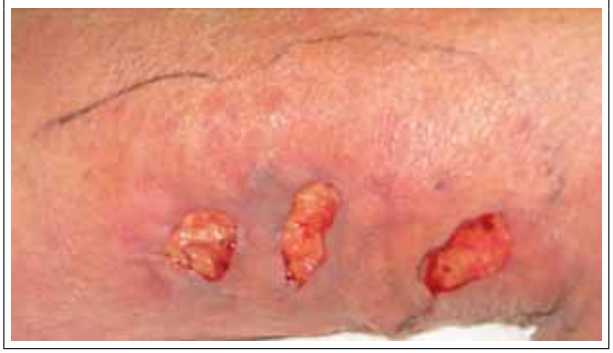


Figure 2a. *Indian ink staining*

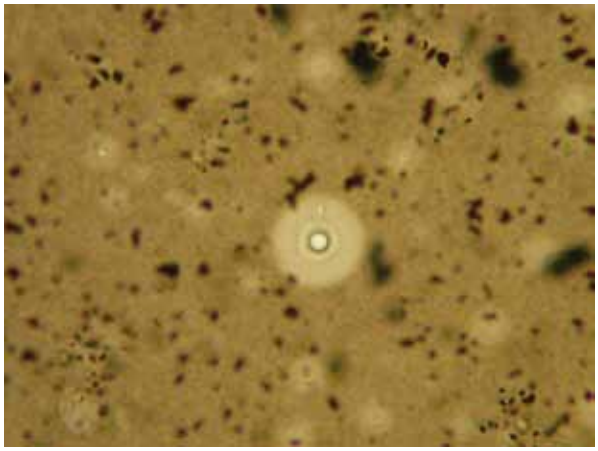
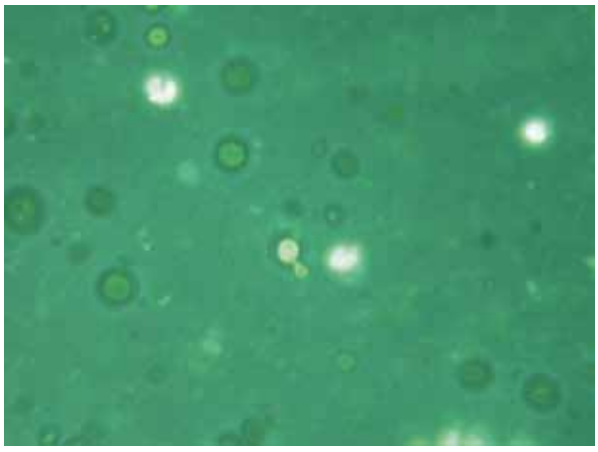


Figure 2b. *Blankophor P staining*



WHAT IS YOUR DIAGNOSIS?

See page 349 for the answer to this photo quiz.

Netherlands
The Journal of Medicine

PHOTO QUIZ

Multiple lesions in upper jaw

K.E. Verweij[1]*, H.J.H. Engelkens[2], C.A. Bertheux[3], A. Dees[1]

Departments of [1]Internal Medicine, [2]Dermatology, [3]Oral and Maxillofacial Surgery, Ikazia Hospital, Rotterdam, the Netherlands, *corresponding author: e-mail: evelyneverweij@gmail.com

CASE REPORT

A 25-year-old woman, without relevant medical history, presented at the outpatient clinic with multiple masses of the maxilla, just above the teeth. She had been aware of the slow but steady enlargement of the masses over the past five years. She did not experience discomfort or pain. There was no family history of similar lesions or intestinal polyps.

Physical examination of the oral cavity revealed large, bilateral overgrowths located on the buccal aspect of the maxilla in the premolar and molar areas *(figure 1)*. The lesions were bony-hard on palpation. The overlying mucosa was normal. Further physical examination was unremarkable. Radiographic examination revealed well-defined ovoid radiopacities superimposed over the roots of the premolars *(figure 2)*.

Figure 1. *Buccal overgrowths of the maxilla*

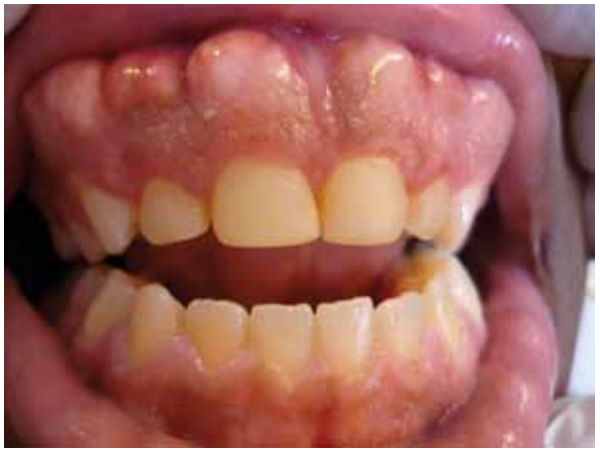
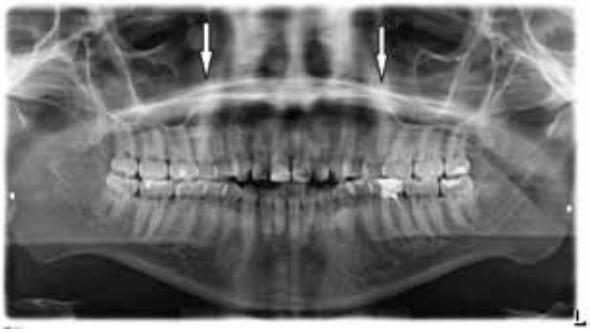


Figure 2. *Radiographic examination*



WHAT IS YOUR DIAGNOSIS?

See page 350 for the answer to this question.

ANSWER TO PHOTO QUIZ (PAGE 345)

A SOFT-TISSUE MASS ON THE FOREHEAD

DIAGNOSIS

This report describes a rare presentation of extrapulmonary tuberculosis (EPTB) localised in the frontal bone. Further examination revealed that she also had tuberculous spondylitis of Th5 and Th10, also known as Pott's disease, which is a more classical presentation of EPTB. Ziehl-Nielsen (ZN) and auramine staining of aspirates of the frontal swelling and of the epidural abscess were negative. Empirical tuberculosis (TB) treatment was initiated because the suspicion of TB remained high. Later, the diagnosis of EPTB was confirmed by PCR and *Mycobacterium tuberculosis* was cultured. She was treated with tuberculostatic drugs for nine months. She completely recovered and the osteolytic defect in the frontal bone restored spontaneously.

EPTB accounts for approximately 45% of TB cases in the Netherlands.[1] Skeletal tuberculosis accounts for 1 to 3% of all TB cases.[2] TB of the flat bone or skull is rare. The most common clinical symptoms of skeletal TB include low-grade fever, night sweats, weight loss and localised pain. Radiological imaging typically shows osteolytic defects in the bone, often in combination with a cold abscess in the adjacent soft tissue. Multifocal skeletal TB can easily be mistaken for a malignancy, due to the osteolytic defects in the bones in combination with only mildly elevated inflammatory markers.

EPTB is a serious clinical condition, which is difficult to diagnose. The tuberculin skin test (TST) is of limited value as it can be both false-positive due to cross reactivity with BCG vaccination and false-negative in case of extensive disease.[3] In our patient the TST was positive; however, this could as also be attributed to her BCG vaccination in childhood or a latent *M. tuberculosis* infection as she originated from a region where TB is endemic.

For diagnosis of EPTB a biopsy or aspirate of a suspected lesion should be taken for histological and microbiological examinations. Culture of *M. tuberculosis*, the gold standard test for diagnosing TB, takes weeks and is frequently negative, as the diagnosis of EPTB could be confirmed by culture in only 40 to 66% of the cases.[1,2]

Direct stains, ZN and auramine, have a low sensitivity,[2] as was illustrated by our case. Novel diagnostic modalities such as PCR are slightly more sensitive. The new *M. tuberculosis*-specific immunodiagnostic assays are highly specific for *M. tuberculosis* as they do not cross-react with BCG.[3] Although promising at first, several studies have now shown that these assays have a limited sensitivity (70 to 90%) for active TB.[4,5] Further, as with the TST, they do not discriminate between latent infection and active disease.[6]

This case illustrates that TB can manifest practically throughout the whole body. We emphasise the importance of considering EPTB in patients who present with osteolytic bone defects, in particular in persons from TB-endemic regions. If clinical suspicion of EPTB is high, empirical treatment should be initiated, even though ZN and PCR are negative. However, prior to initiating TB treatment adequate specimens should be collected for culture, as *M. tuberculosis* culture not only provides a defined diagnosis but also permits resistance testing.

ACKNOWLEDGEMENT

The authors would like to thank Dr. G.J. Lycklama à Nijeholt from the Department of Radiology, Medical Center Haaglanden (MCH) for his contribution to this case report.

REFERENCES

1. KNCV Tuberculosis Foundation – Royal Dutch Association for the Fight Against Tuberculosis. 'Tuberculose in Nederland 2009', Surveillancerapport over de tuberculosesituatie in Nederland; Nov 2010. Available at (accessed February 2011): http://www.kncvtbc.nl/Site/Professional.aspx.
2. Marconda M, Cozzolino A, Attingenti P, Cozzolino F, Milano C. Osteoarticular tuberculosis in a developed country. J Inf. 2007;54:375-80.
3. Arend SM, Andersen P, van Meijgaarden KE, et al. Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. J Infect Dis. 2000;181:1850-4.
4. Song KH, Jeon JH, Park WB, et al. Usefulness of the whole-blood interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis. Diagn Microbiol Infect Dis. 2009;63:182-7.
5. Pai M, Zwerling A, Menzies D. Systematic Review: T-Cell–based Assays for the Diagnosis of Latent Tuberculosis Infection: An Update. Ann Intern Med. 2008;149:177-84.
6. Leyten EM, Arend SM, Prins C, Cobelens FG, Ottenhoff TH, van Dissel JT. Discrepancy between Mycobacterium tuberculosis-specific gamma interferon release assays using short and prolonged in vitro incubation. Clin Vaccine Immunol. 2007;14:880-5.

ANSWER TO PHOTO QUIZ (PAGE 346)

A SÉZARY CRYPTOGRAM

DIAGNOSIS

The Indian ink staining (*figure 2a*) showed round yeast cells surrounded by a halo, and a blankophor P stain showed narrow-based budding (*figure 2b*), both consistent with cryptococcosis. The culture finally revealed *Cryptococcus neoformans*. Blood cultures taken on admission also became positive, and the serum cryptococcal antigen titre was 1:1024. Within two days after admission the patient developed progressive neurological signs, including headache, drowsiness, palsy of the facial nerve, and nausea. A lumbar puncture was performed and the cerebral fluid contained *Cryptococcus*. So we concluded that this patient suffered from a disseminated cryptococcal infection with fungaemia, meningo-encephalitis, and skin abscesses.

Only one previous case of a patient with a Sézary syndrome suffering from a cutaneous cryptococcal infection (*Cryptococcus albidus*) has been described, underlining the rareness of the complication.[1] In addition, also in other haematological malignancies *Cryptococcus neoformans* infections, such as pneumonia and meningitis, have been reported only sporadically.[2] Sézary syndrome is characterised by the classic triad of generalised erythroderma (>80% of the body surface area), generalised lymphadenopathy or other systemic manifestations of the lymphoma, and the presence of more than 5% of large atypical T cells with cerebriform nuclei (called Sézary cells).[3] Nowadays, the diagnosis is largely based on the combination of the typical clinical picture with erythroderma and flow cytometry of the blood showing a clone of T cells with a CD4+ phenotype with aberrant surface marker expression. Patients with Sézary syndrome suffer from compromised specific cellular immune responses. The pathogenesis of this immune deficiency is regarded to be multi-factorial, but the use of prednisone was probably the most important factor causing the immunocompromised status in this patient.[4,5]

Our patient was initially treated with a combination of liposomal amphotericin B and fluconazole, followed by the combination of flucytosine and fluconazole because of unsatisfactory clinical recovery. She recovered with improvement of the neurological signs. Long-term maintenance therapy with fluconazole 400 mg, once daily, was started at discharge, because of the persisting immune deficiency, resulting from the necessity to continue prednisone for the Sezary syndrome.

REFERENCES

1. Narayan S, Batta K, Colloby P, Tan CY. Cutaneous cryptococcus infection due to *C. albidus* associated with Sezary syndrome. Br J Dermatol. 2000;143:632-4.
2. Rhew DC, Gaultier CR, Daar ES, Zakowski PC, Said J. Infections in patients with chronic adult T-cell leukemia/lymphoma: case report and review. Clin Infect Dis. 1995;21:1014-6.
3. Hwang ST, Janik JE, Jaffe ES, Wilson WH. Mycosis fungoides and Sezary syndrome. Lancet. 2008;371:945-57.
4. Kim EJ, Hess S, Richardson SK, Newton S, Showe LC, Benoit BM, et al. Immunopathogenesis and therapy of cutaneous T cell lymphoma. J Clin Invest. 2005;115:798-812.
5. Yawalkar N, Ferenczi K, Jones DA, Yamanaka K, Suh KY, Sadat S, et al. Profound loss of T-cell receptor repertoire complexity in cutaneous T-cell lymphoma. Blood. 2003;102:4059-66.

ANSWER TO PHOTO QUIZ (PAGE 347)

MULTIPLE LESIONS IN UPPER JAW

DIAGNOSIS

The diagnosis is (typical) multiple buccal exostoses.

The multiple masses in the maxilla are consistent with multiple buccal exostoses, which are bony protuberances that arise from the cortical plates in the maxilla and mandible.[1] They usually occur in the late teens and early adult years, and many continue to enlarge slowly over time 2. The aetiology of the multiple exostoses remains unknown, although it has been suggested to be the outcome of a mild, chronic periosteal inflammation.[3]

The diagnosis of a buccal exostoses is based on clinical and radiographic findings.[1] An additional biopsy for diagnostic support is usually not recommended. It remains important to distinguish exostoses from early osteosarcomas and chondrosarcomas. Furthermore, the patient should be evaluated for Gardner's syndrome if he or she presents with multiple bony growths not in the classic buccal exostoses locations. Intestinal polyposis and cutaneous cysts or fibromas are other common features of the autosomal dominant Gardner's syndrome. The importance of this syndrome is the development of multiple intestinal polyps, which have a very high potential for malignant transformation. When Gardner's syndrome is suspected, the patient should be referred to the dermatologist; a colonoscopy should also be performed.[4]

Buccal exostoses are benign lesions that do not possess malignant potential, in contrast to the polyps in Gardner's syndrome. Therefore, they usually do not require treatment.[1] However, surgical resection is sometimes indicated if the bony outgrowths become so large that they interfere with function and denture placement.[1]

In this case, it was assumed that the presence of multiple bony changes of the maxilla warranted further investigation. Investigation of the skin and ileocolonoscopy excluded Gardner's syndrome as a diagnosis in this patient. In retrospect, investigation for Gardner's syndrome might not have been required because of the typical buccal location of the exostoses. Finally, the exostoses were removed by surgical excision because of cosmetic reasons.

ACKNOWLEDGEMENTS

We thank Prof. I. van der Waal, Department of Oral and Maxillofacial Surgery, VU University Medical Centre, Amsterdam, the Netherlands, for his helpful advice.

REFERENCES

1. Neville BW, Damm DD, Allan CM, Bouquot JE. Oral and Maxillofacial Pathology, 3rd ed. Saunders/ Elsevier, 2009; p.19-20.
2. Jainkittivong A, Langlais P. Buccal and palatal exostoses: Prevalence and concurrence with tori. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;90:48-53.
3. van der Waal I. Mondziekten, kaak- en aangezichtschirurgie. Bohn Stafleu van Loghum; 2009; p. 134.
4. Fotiadis C, Tsekouras DK, Antonakis P, Sfiniadakis J, Genetzakis M, Zografos GC. Gardner's syndrome: a case report and review of the literature. World J Gastroenterol. 2005;11(34):5408-11.

Netherlands
The Journal of Medicine

Controlled hypothermia and recovery from postanoxic encephalopathy in near-drowning victim

A. Oude Lansink-Hartgring, F. Ismael

Department of Critical Care, University Medical Center Groningen, Groningen, the Netherlands

Dear Sir,

De Pont and colleagues[1] describe a case report of a near-drowning victim who was comatose after resuscitation due to cardiac arrest, who completely recovered from post-anoxic status epilepticus with controlled hypothermia. We highly appreciate the effort and thoroughness of the authors in conducting this well documented case report and it supports the hypothesis that controlled hypothermia may offer neuroprotection in patients with post-anoxic encephalopathy.

We would like to describe our experience with a near-drowning victim and controlled hypothermia with the use of venovenous extracorporeal life support (ECLS).

A 28-year-old woman was submersed in water for 30 minutes after an attempted suicide. She was found pulseless, resuscitation was started, and she was brought to hospital.
On arrival the Glasgow Coma Score was 3 and her core temperature 28.5 °C. Her pupils were dilated, not reactive to light and there were no corneal reflexes.
Laboratory results showed lactic acidosis (pH 6.51, lactate 17.0 mmol/l). She was taken to the operating room to start venoarterial ECLS. However, on arrival in the operating room she had a return of spontaneous circulation and the intended venoarterial ECLS was switched to venovenous ECLS to support oxygenation on suspicion of extreme pulmonary oedema after near-drowning. On arrival on the ICU her body temperature was 28.4 °C. The venovenous ECLS had a blood flow of 3.5 litres/hour. Gradually the patient was rewarmed to 32.5 °C after 24 hours and 35 °C in 48 hours. After three days she was weaned off the venovenous ECLS.
On day 5 she still scored an EMV of 1-1-tube, pupils reactive to light, positive corneal reflexes with rhythmic movement of mouth and extremities. Electro-encephalography showed a severe encephalopathy pattern, there were no indications of status epilepticus. The patient slowly recovered her consciousness, with maximal EMV on day 8. She was extubated on day 10. Five weeks after admission she was discharged without any neurological deficits.

After our experience, we support the hypothesis that controlled hypothermia can be used for near-drowning victims who are comatose after resuscitation for neuroprotection even with concurrent use of venovenous-ECLS.

REFERENCE

1. de Pont AC, de Jager CP, van de Bergh WM, Schultz MJ. Recovery from near drowning and postanoxic status epilepticus with controlled hypothermia. Neth J Med. 2011;69:196-7.

INFORMATION FOR AUTHORS

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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