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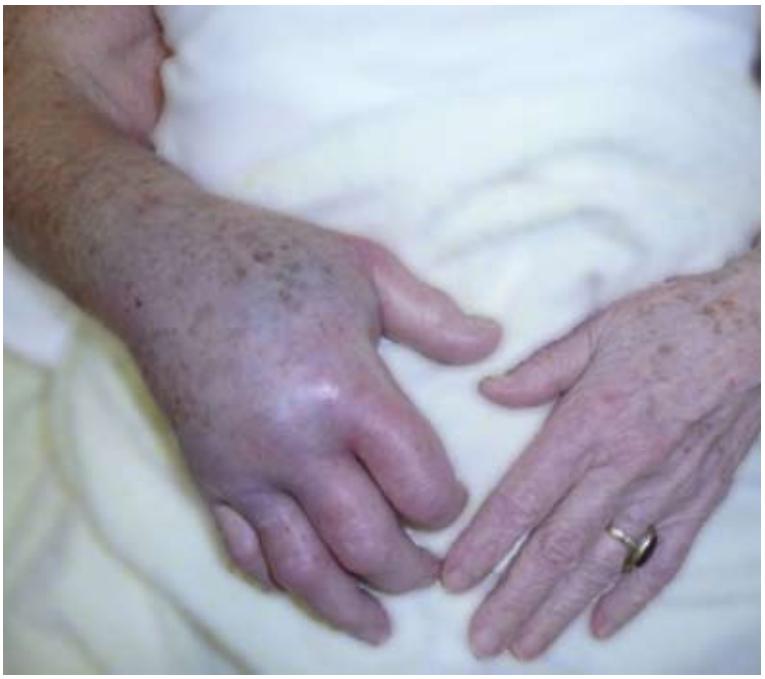

PHOTO QUIZ: A woman with a painful and swollen hand, see page 111

Testing for phaeochromocytoma
•
PPI-triple therapy with levofloxacin for HP eradication
•
Hepatocellular carcinoma
•
Hypoparathyroidism based on a 22q11 deletion

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VAN ZUIDEN COMMUNICATIONS

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Geeralien Derksen-Willemsen
Radboud University Nijmegen Medical Centre
Department of General Internal Medicine 463
PO Box 9101
6500 HB Nijmegen
The Netherlands
Tel.: +31 (0)24-361 04 59
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Contents

EDITORIAL

Is there still a place for pharmacological testing for phaeochromocytoma? 84
H.J.L.M. Timmers, J.W.M. Lenders

REVIEW

Systemic treatment in hepatocellular carcinoma; 'A small step for man...' 86
C.D.M. Witjes, C. Verhoef, H.M.W. Verheul, F.A.L.M. Eskens

ORIGINAL ARTICLES

Evaluation of Endocrine Tests. C: glucagon and clonidine test in phaeochromocytoma 91
P.H. Bisschop, E.P.M. Corssmit, S.J. Baas, M.J. Serlie, E. Endert, W.M. Wiersinga, E. Fliers

Seven-day PPI-triple therapy with levofloxacin is very effective for *Helicobacter pylori* eradication 96
R.W.M. Schrauwen, M.J.R. Janssen, W.A. de Boer

CASE REPORTS

Symptomatic hypoparathyroidism based on a 22q11 deletion first diagnosed in a 43-year-old woman 102
K. van den Berge, K. Diderich, P. Poddighe, A. Berghout

Hypocalcaemia as presenting symptom of velocardiofacial syndrome 105
E.I. van Vliet, B.M. van Ouwerkerk

LETTERS TO THE EDITOR

Anti-TNFα and agranulomatous tuberculous manifestations: more diagnostic dilemma 107
V.H. Chong

Whipple's disease: easily diagnosed, if considered 108
S.M. Sanavro, H.J. Voerman

Autoimmune haemolytic anaemia due to chronic hepatitis C virus infection treated with prednisone, pegylated interferon and ribavirin 109
M.H.P. Dietvorst, P. van Wijngaarden, R.A. de Man

MONTHLY NJM ONLINE HITLIST

For all articles published in December 2008 110

PHOTO QUIZZES

A woman with a painful and swollen hand 111
M.P. Hendriks, M. van Deuren

Ceftriaxone-associated biliary pseudolithiasis 113
M. Rienstra, M.J.M. Bonten, C.A.J.M. Gaillard

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EDITORIAL

Is there still a place for pharmacological testing for phaeochromocytoma?

H.J.L.M. Timmers[1], J.W.M. Lenders[2,3*]

Department of Medicine, [1]Division of Endocrinology and [2]Division of General Internal Medicine, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands, [3]Department of Internal Medicine III, University Hospital Carl Gustav Carus, Dresden, Germany, [*]corresponding author: e-mail: j.lenders@aig.umcn.nl

Phaeochromocytoma and extra-adrenal paragangliomas are rare catecholamine-producing tumours which, if not timely and properly diagnosed, may result in catastrophic consequences.[1] Definite exclusion or confirmation of the tumour is of utmost clinical importance. For this purpose several biochemical tests are available with those measuring metanephrines as the most accurate ones. Even at a very high prior test probability (for instance 75%), the test of plasma free metanephrines has negative and positive predictive values of nearly 95%.[2] In contrast, at a very low prior test probability of, for instance, 1%, the negative predictive value of plasma free metanephrines approaches 100% but the positive predictive value drops to 6%. Thus, both plasma and urinary metanephrines have a high sensitivity but a limited specificity and this means that they are fraught with a rate of false-positive test results of 20 to 30%, and less then 5% false-negative test results.[2] False-positive results are encountered in patients with slight increments of plasma metanephrines (<4 x the upper reference limit) or of urinary metanephrines (<2 x the upper reference limit) but no phaeochromocytoma. Several causes of false-positive test results such as medications and inappropriate sampling conditions can be dealt with appropriately. Another cause of a false-positive test result, which is more difficult to handle, is an increased secretion of catecholamines or metanephrines that is unrelated to phaeochromocytoma. This applies to several other clinical conditions that are associated with an increased secretion of catecholamines due to an increased sympathetic activity. Conversely, because of a rare false-negative test result, an occasional patient with a phaeochromocytoma may be missed with these tests. False-negative test results may occur in the event of a very small phaeochromocytoma or episodic catecholamine secretion by the tumour.

In the last 60 years several pharmacological tests have been developed to unmask the presence of a phaeochromocytoma. These include glucagon, histamine and tyramine provocation tests, and clonidine and pentolinium suppression tests. The glucagon test and the clonidine test are the most widely used. Despite their use in several studies, abnormal responses of plasma catecholamines are not uniformly defined. Most papers traditionally rely only on the response of plasma norepinephrine levels for both glucagon and clonidine test. More importantly, normal responses of plasma catecholamines to these agents have hardly been assessed in healthy asymptomatic subjects. Finally, only a few small populations of mostly symptomatic patients, who appeared to have no phaeochromocytoma, have been studied as reference populations.

In the current issue, Bisschop *et al.* describe their results of the glucagon and clonidine test in 11 patients with a phaeochromocytoma and in 44 patients in whom a phaeochromocytoma was considered to be excluded.[3] For both tests, plasma norepinephrine responses were used as test outcome. Sensitivity and specificity of the glucagon was 30 and 100%, respectively, whereas the clonidine test was not considered to be diagnostic at all. Although the study sample includes only 11 patients with phaeochromocytoma, the authors should be commended for this prospective clinical study. Based on their findings, the authors conclude that both tests are obsolete and should be abandoned. However, in our opinion, the practical implications of their results are not the same for both tests.

The glucagon test was originally designed by Lawrence in 1967 to unmask a phaeochromocytoma in patients with hypertension.[4] In the earlier years, an excessive blood pressure response to glucagon over that to a cold pressor test was considered diagnostic but later the response of plasma catecholamines (particularly plasma norepinephrine) was used. Since this is a provocation test,

one would expect this test to be performed in patients with normal or only slightly elevated plasma catecholamines. Up to now, several small studies have been carried out with maximal sensitivity of 81%.[5] In contrast, in the current study sensitivity was only 30%. This disparity is probably related to different inclusion criteria (such as inclusion of patients with increased or normal baseline plasma norepinephrine levels, patients with or without genetic predisposition) and different diagnostic criteria. Whatever the reason, it is quite clear, also from previous studies, that the glucagon test lacks sufficient sensitivity to rule out phaeochromocytoma. Taking into account the small risk of side effects (10 to 20% of the patients need phentolamine treatment for excessive blood pressure responses) and irrespective of its excellent specificity, the authors are correct that the glucagon test should be abandoned in clinical practice. Finally, the most definite argument to stop using this test is its redundancy because the sensitivity of basal plasma free metanephrines is already almost optimal, being 97% in patients with a genetic predisposition and 99% in patients with an apparently sporadic phaeochromocytoma.[2]

The findings of the clonidine test in the study by Bisschop *et al*. has a different clinical implication. This test, designed by Bravo in 1981, was introduced to distinguish false-positive test results (for instance due to sympathetic activation) from true-positive test results (patients with phaeochromocytoma).[6] Also for this test, plasma norepinephrine responses have been employed in most studies. Similar to the glucagon test, this test has also been performed in patient cohorts with different baseline plasma norepinephrine levels. Even more importantly, several different diagnostic criteria as test parameter have been used. Some studies used a plasma norepinephrine level of >2.96 nmol/l after clonidine as diagnostic criterion while others used this criterion and/or a plasma norepinephrine response of <50% three hours after clonidine administration. The sensitivities reported in previous studies varied between 97 to 99% while we came to a sensitivity of only 67%.[7] The current study, also using plasma norepinephrine responses, found a sensitivity of only 20% with a specificity of 93%, suggesting that this test lacks diagnostic power altogether. These significant differences are probably due to differences in patient inclusion and in the diagnostic criteria used.

This situation is, however, different when plasma normetanephrine instead of plasma norepinephrine is used as test marker. If plasma normetanephrine is used (failure to suppress defined as a decrease of <40% from basal and a persistent increased basal plasma normetanephrine of >0.60 nmol/l), sensitivity and specificity improve to 96 and 100%, respectively.[7] Similar results have been described both in patients with an apparent sporadic phaeochromocytoma and in those with a genetic syndrome. Thus, while lack of suppression of plasma norepinephrine or normetanephrine both provide strong evidence for phaeochromocytoma, only the suppression of normetanephrine provides reliable evidence that a phaeochromocytoma is not present.

Therefore, in contrast to the glucagon test, which indeed should be omitted from our diagnostic armoury, there is still a place for the clonidine test in patients with slightly elevated baseline plasma metanephrines or catecholamines, provided the response of plasma normetanephrine is used instead of plasma norepinephrine. The improved availability of measurements of plasma metanephrines guarantees that this test can still be used in daily clinical practice.[8]

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REVIEW

Systemic treatment in hepatocellular carcinoma; 'A small step for man...'

C.D.M. Witjes[1,2], C. Verhoef[1*], H.M.W. Verheul[3], F.A.L.M. Eskens[4]

[1]Section of Surgical Oncology, Daniel den Hoed Cancer Center, [2]Section of Hepatobiliary and Transplantation Surgery, and [4]Department of Medical Oncology, Erasmus University Medical Center, Rotterdam, the Netherlands, [3]Department of Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)10-704 15 06, e-mail: c.verhoef@erasmusmc.nl

ABSTRACT

Hepatocellular carcinoma (HCC) is the third most common cause of cancer mortality worldwide. In localised disease, orthotopic liver transplantation, surgical resection or local ablations are the mainstay of treatment. In unresectable or metastatic HCC, systemic therapy has unfortunately yielded disappointing results and therefore until recently was generally considered to be ineffective. Most patients with HCC have an underlying liver disease and many drugs may exacerbate the underlying liver disease. Recently, two randomised phase III trials with sorafenib in patients with advanced or metastatic HCC have shown a significant increase in progression free and overall survival of approximately two months, which is an absolute novum for this disease. Sorafenib is therefore now considered a viable treatment option in patients with unresectable or metastatic HCC, a good performance status and Child-Pugh A liver cirrhosis. Despite this very promising result, of major concern is the treatment-related toxicity as observed in these and other trials by sorafenib treatment. However, the important first significant survival benefit by systemic treatment has generated hope for the development of new treatment strategies which will be more efficacious, have favourable toxicity profiles and will further extend survival of this still highly lethal disease.

KEYWORDS

Advanced hepatocellular carcinoma, sorafenib, therapy

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third cause of cancer-related mortality. While the incidence of HCC is high in Asia and parts of Africa, in the Western world its incidence is low although increasing.[1-3] As an example, the yearly incidence of HCC in the Netherlands is an average of 250 patients, and between 1989 and 2000 this figure has not changed.[4]

Cirrhosis is the main risk factor underlying HCC, and there is a clear association between chronic infections with hepatitis C virus, hepatitis B virus, excessive alcohol consumption, cirrhosis and HCC. However, HCC also occurs in a noncirrhotic liver. Surgical resection, local ablation and liver transplantation are the mainstay of treatment of localised HCC. Unfortunately, only 25% of patients will present with localised disease and can receive such a potentially curative therapy.[5,6] In patients unable to receive any of these alternatives, systemic therapy was generally considered to be ineffective.[7] Recently, results of two randomised phase III trials have been published showing efficacy of sorafenib in advanced HCC. In this review we will give a brief overview of the currently applied local treatment options for HCC and will discuss data of the past and present systemic treatment for HCC. As nowadays various different treatment options for patients with HCC can and should be considered, it is the conviction of the authors that patients should be treated in experienced hepatobiliary centres with the availability of a multidisciplinary input.

TREATMENT OF LOCALISED HCC

Partial liver resection

Partial liver resection is the treatment of choice for HCC in a noncirrhotic liver. Irrespective of the usual large tumour size (8 to 10 cm), five-year survival rates exceeding 50% have been described.[8] Among patients with underlying cirrhosis, strict selection criteria are required to avoid treatment-related complications. While in the 1970s a cirrhotic liver was considered a contraindication for resection, partial hepatic resection is now a safe and viable option with a perioperative mortality of less than 5%. Currently only the presence of extrahepatic disease, lack of sufficient hepatic functional reserve (Child-Pugh B or C cirrhosis), multi-focal hepatic disease, and main portal vein involvement and of course severe comorbidity are still considered contraindications for partial liver resection. Studies of surgical resection in HCC over the past ten years have demonstrated five-year survival rates of 25 to 92%.[5,9] This wide range is primarily explained by differences in patient selection. Severity of underlying liver disease, number and size of HCC nodules and the presence of portal hypertension are important prognostic factors. Careful selection of patients with a single HCC <5 cm, with a preserved liver function without portal hypertension yields five-year survival rates of 70%.[7]

Orthotopic liver transplantation

Thomas E. Starzl performed the first liver transplantation in 1963. Since then, orthotopic liver transplantation (OLT) has become the worldwide mainstay for patients with resectable HCC. In theory, total hepatectomy followed by OLT is the optimal curative approach, as this procedure removes the 'precancerous' liver and all microscopic disease at the time of resection. Worldwide, the so-called 'Milan criteria' are the most accepted selection criteria for OLT in HCC.[10] In the decisive study by Mazzaferro *et al.*, patients with a relatively limited HCC (1 nodule <5 cm, 3 nodules < 3 cm) had a comparable outcome to transplanted patients not suffering from HCC (four-year survival of 75%). Some centres consider these criteria to be too restrictive. Yao *et al.* reported the outcome of 70 patients with HCC undergoing OLT and found that patients with a single lesion ≤6.5 cm, two to three nodules with the largest ≤4.5 cm or a total tumour diameter ≤8 cm had a 75% five-year survival.[11]

Recent studies have confirmed the outcome of these expanded criteria, the University of California, San Francisco (UCSF) criteria; comparing the Milan and UCSF criteria, no statistical difference in five-year post-transplant survival was found.[12] Future studies will have to prove feasibility and acceptability of these expanded criteria.

Microscopic vascular tumour invasion is the main prognostic factor. Unfortunately, this cannot always be assessed reliably in the preoperative situation. For patients with HCC in decompensated cirrhosis, OLT remains the only curative option. It is obvious that the presence of extrahepatic disease is an absolute contraindication for OLT.

Local ablative therapies

Local ablation techniques are accepted alternative therapies for unresectable HCC. Interstitial laser coagulation, cryotherapy, microwave ablation, percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) are the most frequently used approaches. Five-year survival rates up to 70% have been described.[13] Underlying liver disease and number and size of HCC lesions are the main prognostic factors. An adequately performed RFA is now considered the preferred local ablative treatment. When tumours are located close to bile ducts or large vessels, PEI remains a valuable option. Recent studies comparing the efficacy of surgery and local ablative therapies in small HCC have clearly demonstrated that a well-performed local ablation yields similar survival rates and less morbidity compared with surgery.[14-16] Based upon these reported success rates, RFA and PEI should be classified as potentially curative. In the future, local ablative therapies will probably become the mainstay of treatment of small tumours (3 cm).

Transarterial chemoembolisation

Transarterial chemoembolisation (TACE) is an accepted alternative therapy for unresectable HCC without extraheptical spread. Meta-analysis reported a survival benefit after TACE especially in patients with a decompensated liver function. Therefore, TACE might be therapy of choice in careful selected patients.[13]

TREATMENT OF ADVANCED HCC

As mentioned before, until recently no proven or standard systemic therapy for advanced HCC could be defined. Numerous small phase II studies with hormonal treatment, immunotherapy or cytotoxic chemotherapy all yielded disappointing response rates and no significant effects on disease-free and overall survival. Based upon the successes of so-called 'targeted' therapies in other solid tumours, renewed interest in possibilities for such systemic therapy in HCC has emerged.

Several preclinical studies have suggested that some small molecule tyrosine kinase inhibitors (TKI) as well as monoclonal antibodies inhibit important signalling pathways in tumour cells and can inhibit angiogenesis in HCC. Using this evidence, a number of nonrandomised phase II trials have been performed to investigate these agents, either alone or in combination with chemotherapy in patients with HCC (*table 1*).[17-22]

Table 1. *Phase II trials with targeted agents in hepatocellular carcinoma*

Author (year)	n	Agent/dose	PR (%)	TTP (months)	MS (months)
Philip (2005)[17]	38	Erlotinib 150 mg once daily	8	3.2	13
Abou-Alfa (2006)[18]	137	Sorafenib 400 mg twice daily	2.2	4.2	9.2
Zhu (2006)[19]	33	Gemcitabine/oxaliplatin + bevacizumab	20	5.3	9.6
Louafi (2007)[20]	44	GEMOX + cetuximab	23	6.3	11.5
Zhu (2008)[21]	34	Sunitinib 37.5 mg once daily	3	4	9.9
Siegel (2008)[22]	46	Bevacizumab 10/5	13	6.5	12.4

PR = partial response; TTP = time to progression; MS = median overall survival.

Sorafenib is such a small molecule tyrosine kinase inhibitor. It inhibits signalling pathways relevant for both tumour cell proliferation (C-RAF, B-RAF, V600E B-RAF, c-KIT and FLT-3) and angiogenesis (C-RAF, VEGFR-2, VEGFR-3 and PDGFR-β). In preclinical studies sorafenib was able to inhibit tumour growth in several human tumour xenograft models.[23] Based upon results obtained in smaller phase I and II trials, two large randomised phase III trials have been performed (*table 2*).

RANDOMISED PHASE III TRIAL OF SORAFENIB IN ADVANCED HCC

The largest randomised phase III trial in HCC (SHARP trial) was performed in a Western population.[24] Patients were randomly assigned to receive continuous oral treatment with 400 mg sorafenib twice daily or matching placebo in combination with best supportive care. Treatment was continued until disease progression or presence of unacceptable drug-related adverse effects.
With respect to demographic characteristics, no significant differences between the two groups were observed, whereas approximately half of the patients had not been previously treated, and locoregional therapy (TACE, PEI and/or RFA) had failed in the remaining patients. Important outcomes for clinical evaluation are summarised in *table 3*. Although no significant difference in tumour response was observed, both progression-free survival and overall survival increased significantly following exposure to sorafenib. The percentage of patients who discontinued dosing was 13 in the placebo group and 32 in the sorafenib group. In the placebo group 101 patients (33%) and in the sorafenib group 154 patients (52%) interrupted the treatment because of drug-related adverse effects. Median duration of treatment was 23 weeks in the sorafenib group and 19 weeks in the placebo group. Drug-related adverse events (all grades) were reported in 80% of the patients in the sorafenib group and in 52% of patients in the placebo group. Primary drug-related adverse events reported were dermatological (constitutional and hand-foot skin reaction) and gastrointestinal (diarrhoea, nausea). A second randomised phase III trial was performed in Asian-Pacific patients. In this study, patients were in a 2:1 setting assigned to receive either sorafenib 400 mg twice daily or matching placebo.[25] Treatment was continued until disease progression or the

Table 2. *Randomised phase III trials of sorafenib and hepatocellular carcinoma*

Patient characteristics	Llovet et al.[24]		Cheng et al.[25]	
	Sorafenib n=299	Placebo n=303	Sorafenib n=150	Placebo n=76
Median age (years)	64.9±11.2	66.3±10.2	51 (23-86)	52 (25-79)
Child-Pugh class A	95%	98 %	97 %	97 %
ECOG-PS:				
0	54%	54%	25%	28%
1	38%	39%	69%	67%
2	8%	7%	5%	5%
Macroscopic vascular invasion	36%	41%	64%	66%
Extrahepatic spread	53%	50%	36%	34%
Macroscopic vascular invasion, extrahepatic spread, or both	70%	70%	-	-
Underlying hepatitis B	19%	18%	71%	78%
Underlying hepatitis C	29%	27%	11%	4%
Alcoholic cirrhosis	26%	26%	-	-

ECOG-PS = Eastern Cooperative Oncology Group - performance status.

Table 3. *Outcome randomised phase III trials of sorafenib and hepatocellular carcinoma*

Outcome	Llovet et al.[24]		Cheng et al.[25]	
	Sorafenib n=226	Placebo n=242	Sorafenib n=150	Placebo n=76
Progression-free survival (months)*	5.2	2.8	2.8	1.4
Overall survival (months)*	10.7	7.9	6.5	4.2
Response rate (%)	2	1	3	1
Drug-related adverse events grade 3/4 (%)	35	15	26	2

***Median.**

occurrence of unacceptable drug-related adverse effects. With respect to the demographic characteristics no relevant differences between the two study groups were found. When comparing the population enrolled in this trial with that enrolled in the SHARP trial, the most striking differences are the predominant cause of underlying liver disease (predominantly hepatitis B infection in the Asian-Pacific population as opposed to hepatitis C infection in the Western population) and the median age, which is much lower in the Asian-Pacific population.

Important outcomes of this trial are summarised in *table 3*. Comparable with the results obtained in the SHARP trial, progression-free survival almost doubled and overall survival increased significantly. In both studies, these favourable results were obtained while the response rate was remarkably low.

Primary drug-related adverse events reported were dermatological (constitutional and hand-foot skin reaction) and gastrointestinal, and also these observations were strikingly remarkable in the two studies reviewed. The toxicity of sorafenib is a serious problem. Approximately 50% of patients have to interrupt or stop treatment because of sorafenib-induced toxicity. Toxicity of this type of agent was initially not expected and is of concern for combination treatment strategies.[26] Optimal clinical management of these side effects is of high priority to optimise treatment intensity for patients and thereby treatment outcome.

UNRESECTABLE OR METASTATIC HCC; CURRENT CLINICAL PRACTICE.

For patients diagnosed with advanced or metastatic HCC, sorafenib is currently the only treatment option that has demonstrated survival benefit in randomised controlled trials. Of note here is that these trials almost exclusively enrolled patients in a favourable clinical and biochemical condition, (Child-Pugh liver function class A and ECOG-PS 0 or 1). In the limited number of patients enrolled in both trials suffering from more severe underlying liver disease, e.g. Child-Pugh B, the response rate to sorafenib seemed to be comparable to that observed in patients with Child-Pugh A which, as mentioned before, was very low. The effects of sorafenib on progression free or overall survival of patients in Child-Pugh B was not reported separately in the two trials and future studies must therefore address this issue in more detail, and until results are known, patients with advanced or metastatic HCC and Child-Pugh B (and especially C) liver cirrhosis should not be treated systemically with either sorafenib or any other agent outside the setting of clinical studies. Fortunately, a large number of these clinical studies are ongoing or will be initiated in the near future, giving patients an increasing opportunity to become exposed to new and potentially effective antitumour agents.

TACE is a worldwide-accepted treatment for unresectable HCC without extrahepatic spread. Survival seems to increase after TACE, especially in patients with a compensated liver function.[13] At the moment TACE is the standard treatment for patients with intermediate BCLC staging (patients without extra-hepatic disease and limited HCCs in the liver).[27] Whether the addition of sorafenib to TACE could be beneficial to these patients is currently explored in clinical trials.

SORAFENIB AND HCC: THE FUTURE

The positive results of sorafenib in advanced or metastatic HCC open new avenues for this agent in less advanced stages of HCC. Trials exploring the role of sorafenib as adjuvant treatment following such curative treatment options as resection and RFA are currently ongoing.[28]

As the response rate of HCC to sorafenib is only very low (2 to 3%), it is very unlikely that sorafenib could turn out to be effective as induction treatment in an attempt to render an unresectable HCC to a resectable disease. It cannot be excluded that this obviously disappointing response rate depends on drug dose, and therefore increasing the dose might increase the response rate in HCC; of note here is that these observations have been made in advanced renal cell carcinoma.[29] Current trials are comparing sorafenib with chemotherapies or other targeted agents in order to improve outcome of unresectable HCC.[30,31] Trials combining sorafenib with RFA or TACE will be initiated soon, partly based on promising data on such combinations (sorafenib and RFA) in mice.[32]

CONCLUSION

HCC is a complex disease that merits a multidisciplinary approach. In resectable and irresectable and/or metastatic disease progress has been made with the treatment by the introduction of new treatment modalities. Based upon these results, efforts to improve outcome even further are currently underway, and hopefully the breakthrough that has been observed in recent years will turn out to be the beginning of new era where HCC is considered a treatable and increasingly curable disease.

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ORIGINAL ARTICLE

Evaluation of Endocrine Tests. C: glucagon and clonidine test in phaeochromocytoma

P.H. Bisschop[1*], E.P.M. Corssmit[2], S.J. Baas[1], M.J. Serlie[1], E. Endert[3], W.M. Wiersinga[1], E. Fliers[1]

Departments of [1]Endocrinology and Metabolism, [3]Clinical Chemistry, Laboratory of Endocrinology and Radiochemistry, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands, [2]Department of Endocrinology, Leiden University Medical Centre, Leiden, the Netherlands, [*]corresponding author: tel.: +31 (0)20-566 60 71, fax: +31 (0)20-691 76 82, e-mail: p.h.bisschop@amc.uva.nl

ABSTRACT

Background: The diagnosis of phaeochromocytoma is based on the demonstration of catecholamine excess. Urine and plasma metanephrine measurements are highly sensitive tests for the diagnosis of phaeochromocytoma, but moderate elevations in metanephrines lack optimal specificity.

In this study we aimed to evaluate the diagnostic value of additional tests, i.e. glucagon stimulation and clonidine suppression test, in patients with moderately elevated catecholamines and/or metanephrines.

Methods: Patients with suspected phaeochromocytoma with moderately elevated catecholamines and/or metanephrines in plasma or urine were subjected to the glucagon stimulation and clonidine suppression test. The presence of phaeochromocytoma was confirmed by histology and the absence by a disease-free extended follow-up.

Results: Fifty-five patients were included. Phaeochromocytoma was diagnosed in 11 patients. The follow-up period in patients without phaeochromocytoma was 56 (19 to 154) months. The sensitivity of the glucagon test was 30% and the specificity 100%. The clonidine test had no discriminative power, because the area under the ROC curve was not significantly different from 0.5.

Conclusion: The clonidine suppression test without normetanephrine measurements and the glucagon stimulation test are not sensitive enough to safely exclude phaeochromocytoma in patients with mildly elevated plasma or urine catecholamines.

KEYWORDS

Clonidine, glucagons, phaeochromocytoma

INTRODUCTION

The early diagnosis of phaeochromocytoma is important, because unrecognised phaeochromocytoma is a potentially lethal condition. The diagnosis, however, poses a challenge for every physician. A relatively large number of patients may present with only minor signs and symptoms. In a Swedish study, the diagnosis was made only at autopsy in 40% of 439 patients with phaeochromocytoma, whereas phaeochromocytoma was an incidental finding in 14%.[1] On the other hand, in a series of patients clinically suspected of phaeochromocytoma (on the basis of signs and symptoms), the diagnosis was established in only one of 300.[2] Of patients with hypertension, phaeochromocytoma may be found in only ~0.1%.[3] Since missing the diagnosis could have serious consequences, diagnostic testing demands a high degree of sensitivity. In daily practice, biochemical testing aimed at the demonstration of excessive catecholamine production is performed. Biochemical tests include plasma epinephrine, norepinephrine and/or metanephrines, and 24-hour urinary excretion of epinephrine, norepinephrine and their O-methylated metabolites metanephrine and normetanephrine.[4] The demonstration of increased levels of plasma or urinary catecholamines and their metabolites should suffice to make a diagnosis of phaeochromocytoma likely. However, mildly elevated concentrations of catecholamines and their metabolites may be aspecific and could provide a dilemma as to further management given the low prevalence of phaeochromocytoma. To address this issue we prospectively analysed the value of additional dynamic tests for phaeochromocytoma in patients showing a mild catecholamine excess at initial screening. Either a provocative test with intravenous glucagon and/or a suppressive test with oral clonidine can be performed.

The current literature is not conclusive about the relative merits of these dynamic tests due to small patient series, differently defined control groups and differences in analytical assays.[3,5,6] Reported sensitivities and specificities for the glucagon provocation test were 60 to 81% and 100%, respectively, and for the clonidine suppression test 97% and 67 to 99%, respectively.[3,5,6] Grossman *et al.* reported a sensitivity of 100% and a specificity of 79% for the combination of the glucagon and clonidine test.[5] The present study was designed to prospectively evaluate the diagnostic accuracy of the glucagon provocation test and the clonidine suppression test for diagnosing phaeochromocytoma in groups of patients frequently encountered in an outpatient clinic of Internal Medicine/ Endocrinology, namely patients with clinical suspicion of phaeochromocytoma, an adrenal incidentaloma or genetic predisposition to phaeochromocytoma combined with a positive initial biochemical screening.

MATERIALS AND METHODS

Subjects

Since 1993 biochemical testing for phaeochromocytoma at the Academic Medical Center (University of Amsterdam) has been carried out by a stepwise approach. Initial screening consists of measurement of plasma epinephrine and norepinephrine, combined with measurement of 24-hour urinary excretion of epinephrine and norepinephrine and their metabolites metanephrine and normetanephrine. Fasting plasma catecholamines were collected from an indwelling venous catheter 30 and 45 minutes after insertion of the venous catheter while patients were in a supine position. Two consecutive 24-hour urine samples were collected while patients refrained from coffee, nuts, bananas and alcohol. If any of the measured concentrations were above the institutional reference value (plasma: epinephrine >0.55 nmol/l, norepinephrine >3.25 nmol/l; urine: epinephrine >275 nmol/24 hours, norepinephrine >890 nmol/24 hours, metanephrine>0.80 µmol/24 hours, normetanephrine>2.00 µmol/24 hours) an additional glucagon stimulation and clonidine suppression test were performed. Only in patients with plasma catecholamine concentrations exceeding 11.1 nmol/l were the glucagon and clonidine tests skipped and imaging of the adrenals was performed.[7] For this study we included all patients between 1993 and 2005 with a positive initial screening who underwent subsequent glucagon and clonidine testing. These patients had been screened for phaeochromocytoma because of 1) symptoms and signs that could fit the diagnosis phaeochromocytoma 2) an adrenal incidentaloma, 3) genetic predisposition for phaeochromocytoma (multiple endocrine neoplasia type 2 or succinate dehydrogenase complex subunit D mutation. 4) a history of paraganglioma/phaeochromocytoma. Exclusion criteria were parental drug abuse, alcohol abuse and pregnancy. Antihypertensive drugs or any other medication interfering with the tests was stopped or switched to doxazosin at least five days, but preferably two weeks, before the tests. Additional exclusion criteria were RR >160/100 mmHg to carry out the glucagon stimulation test and RR <100/60 mmHg to carry out the clonidine suppression test. In case of a positive glucagon and/or clonidine test a CT scan or MRI was performed, followed by MIBG scanning when indicated. Each diagnosis of phaeochromocytoma was confirmed by histology. The absence of a phaeochromocytoma in the nonoperated patients was ascertained by a disease-free extended follow-up. We checked the medical charts and/or asked the general practitioner about the patient's health condition with special attention to signs or symptoms suggestive of phaeochromocytoma.

Glucagon stimulation test

After three baseline samples were drawn at 15-minute intervals (-30, -15 and 0 min), 1 mg of glucagon was injected intravenously and its effect on plasma epinephrine and norepinephrine concentrations was measured in blood samples taken one, two and three minutes after injection. Baseline epinephrine and norepinephrine concentrations were calculated as the mean of three baseline samples. Heart rate and blood pressure were recorded every minute with an automated sphygmomanometer until ten minutes after administration of glucagon.

Clonidine suppression test

At least one hour after injection of glucagon, a baseline blood sample was drawn followed 15 minutes later by a second sample and then 0.3 mg clonidine was administered orally. Baseline norepinephrine concentrations were calculated as the mean of the two baseline samples. Blood pressure and heart rate were recorded every 15 minutes with an automated sphygmomanometer until 180 minutes after clonidine administration. A blood sample for determination of norepinephrine concentrations was drawn 180 minutes after intake of clonidine.

Analytical methods

Plasma epinephrine and norepinephrine were assayed by RP-HPLC with fluorimetric detection after solvent extraction and derivatisation with 1,2-diphenylethylenediamine.[8] The inter-assay CV was 6 to 11%. Detection limits were 0.05 nmol/l for plasma epinephrine and norepinephrine.

Statistical methods

Values below the detection limits of the assays were included as having the value of 50% of the detection

limit. The glucagon test was considered positive if plasma norepinephrine was >11.83 nmol/l or if the increase in plasma norepinephrine after glucagon was more than three times the basal values.[5] The clonidine test was considered positive if there was less than 50% reduction in plasma norepinephrine and plasma norepinephrine was >2.95 nmol/l three hours after clonidine administration.[5,9] Data are reported as median (minimum – maximum). Area under curve of the receiver-operator-characteristic (ROC) curves were analysed with SPSS 14.0. P values below 0.05 were considered statistically significant.

RESULTS

Patient characteristics

We included 55 patients of whom 11 (20 %) had a phaeochromocytoma. Patient characteristics are shown in *table 1*. The follow-up period in patients without phaeochromocytoma was 56 (19 to 154) months. Eight patients were on doxazosin during the tests, but none of these patients proved to have a phaeochromocytoma.

Glucagon stimulation test

The glucagon test was not performed in one patient because of hypertension (blood pressure >160/100 mmHg). The results of the glucagon test are shown in *figure 1*. The sensitivity of the glucagon test was 30% and the specificity 100% using cut-off values of 11.83 nmol/l for the norepinephrine peak and a threefold increase (*table 2*). The epinephrine response was highly variable and did not discriminate between patients with and without phaeochromocytoma (*figure 1*). Areas under the ROC curve were 0.691 (95% confidence interval (CI) 0.465 to 0.917; p=0.061) and 0.848 (95% CI 0.696 to 1.000; p=0.001) for the norepinephrine peak and fold-increase, respectively (*figure 2*).

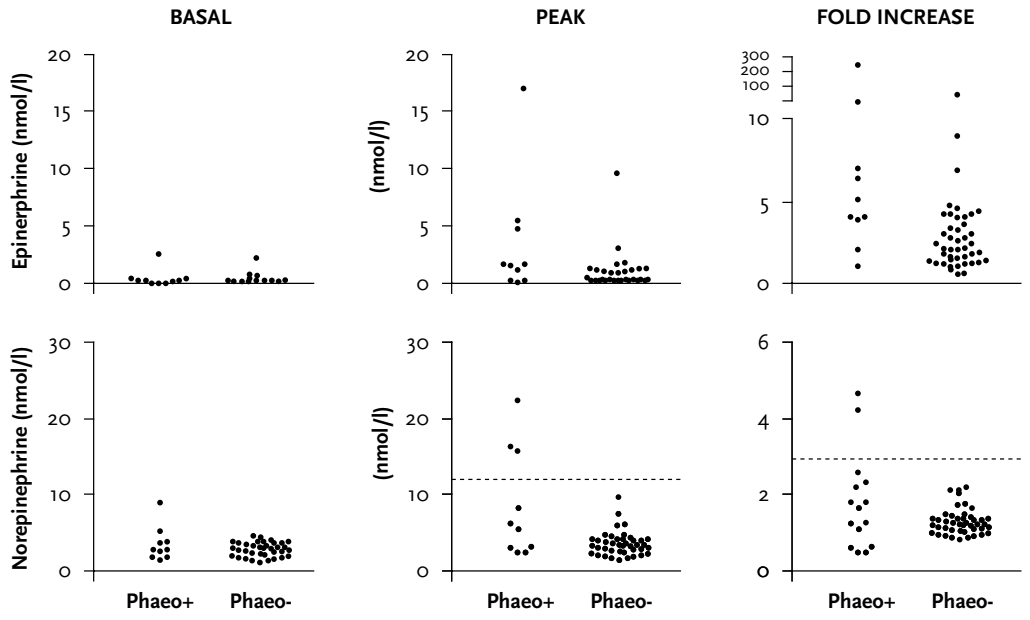
Table 1. *Patient characteristics*

Indication for testing	Male/ female (n)	Age (years)	Phaeochromocytoma n(%)
Clinical suspicion	13/19	47 (19-76)	4 (13)
Genetic predisposition	3/2	32 (19-65)	3 (60)
Adrenal incidentaloma	7/9	57 (43-72)	3 (19)
Recurrence	0/1	69 (69-69)	1 (100)
Total	*23/32*		*11 (20)*

Table 2. *Glucagon stimulation test*

Glucagon test result (n)	Phaeochromocytoma		
	Yes	No	Total
Positive	3	0	3
Negative	7	44	51
Total	*10*	*44*	*54*

Figure 1. *Glucagon stimulation test: Epinephrine and norepinephrine at baseline and after administration of glucagons*



Dotted lines indicate reference values obtained from the literature.[5,6]

Figure 2. *Glucagon stimulation test: Receiver-operator curve for peak (dotted line) and fold-increase (solid line) of norepinephrine*

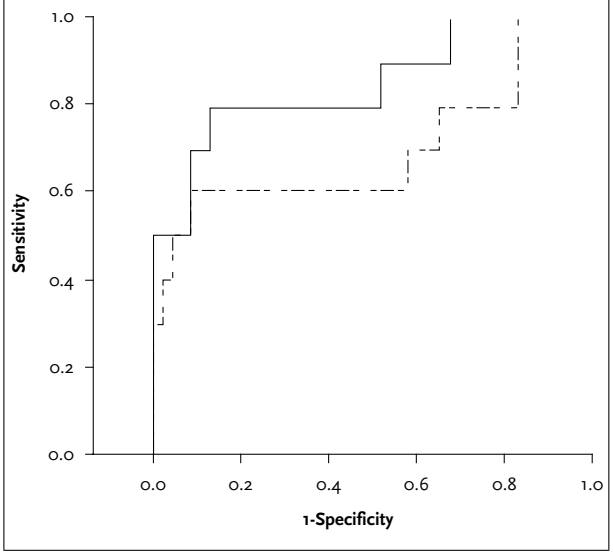
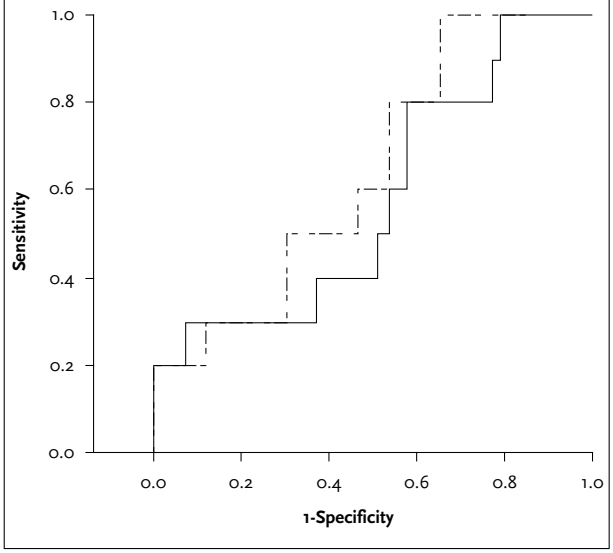


Table 3. *Clonidine suppression test*

Clonidine test result	Phaeochromocytoma		
	Yes	No	Total
Positive	2	3	5
Negative	8	40	48
Total	*10*	*43*	*53*

Figure 4. *Clonidine suppression test: Receiver-operator curve for nadir (dotted line) and relative decrease (solid line) of norepinephrine*



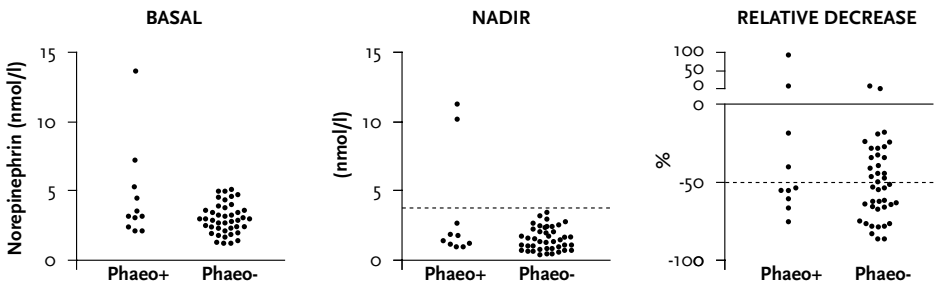
Clonidine suppression test

The clonidine test was not performed in two patients because of hypotension (blood pressure <100/60 mmHg). The results of the clonidine test are shown in *figure 3*. The sensitivity of the clonidine suppression test was 20% and the specificity 93% using the following cut-off values: 50% reduction in plasma norepinephrine after clonidine and plasma norepinephrine >2.95 nmol/l after three hours (*table 3*).[5,9] The areas under the ROC curve were 0.644 (95% CI 0.468 to 0.820; p=0.159) and 0.579 (95% CI 0.380 to 0.778; p=0.440) for the relative norepinephrine decrease and the absolute plasma concentrations after clonidine, respectively (*figure 4*).

DISCUSSION

This is the first study to prospectively evaluate the diagnostic accuracy of two dynamic biochemical tests for the diagnosis of phaeochromocytoma in a group of patients frequently encountered in an outpatient clinic, namely those who had been tested because of suspicious symptoms and signs or predisposing conditions (adrenal incidentaloma or genetic predisposition) and who showed mildly elevated plasma or urine catecholamine levels. The sensitivity and specificity of these tests using reference values from the literature were 30 and 100% for the glucagon test and 20 and 93% for the clonidine test, respectively.

Figure 3. *Clonidine suppression test: Norepinephrine at baseline and after administration of clonidine*



Dotted lines indicate reference values obtained from the literature.[5]

Although the literature reports additional value of the glucagon stimulation test and/or the clonidine suppression test in equivocal cases, our study shows that even in cases with a positive screening test, sensitivity of both tests is low. By lowering the cut-off values for the glucagon test, sensitivity increased from 30 to 90%, but specificity was reduced from 100 to 48%. Given the serious consequences of failure to diagnose a phaeochromocytoma, the glucagon test thus provides no additional value over the measurement of plasma and urine catecholamines and metanephrines in these patients.

The results of the clonidine test in this study differ from some previously published studies on the clonidine test. ROC curve analysis for the clonidine test showed an area under the curve that was not different from 0.5, which indicates that the clonidine test cannot discriminate between patients with and without phaeochromocytoma, whereas others showed a sensitivity of 66 to 100% and a specificity of 93 to 100%.[10,11] Our contrasting findings are probably related to different patient selection criteria. We did not include patients with baseline norepinephrine concentrations above 11.5 nmol/l since this degree of norepinephrine excess is considered pathognomic for phaeochromocytoma. Instead, these patients were not subjected to the clonidine suppression test, but imaging was performed straightaway. Consequently, only four out of ten patients with phaeochromocytoma had baseline norepinephrine concentrations that were above the institutional reference value. In contrast, in the study by Eisenhofer *et al.* 44 out of 48 patients with phaeochromocytoma had increased baseline norepinephrine concentrations and a significant proportion had norepinephrine concentrations above 11.5 nmol/l.[11] Still 16 out of 48 patients with phaeochromocytoma were not detected by a conventional clonidine suppression test. However, with the introduction of plasma normetanephrine measurements during the clonidine test 46 out of 48 patients could be detected.[11] Our observations as well as those of others indicate that the clonidine suppression test without measurement of plasma normetanephrine is not a suitable test for phaeochromocytoma, especially when baseline norepinephrine concentrations are normal or only marginally increased.[5,9,12]

CONCLUSION

The clonidine suppression test without normetanephrine measurements and the glucagon stimulation test are not sensitive enough to safely exclude phaeochromocytoma in patients with mildly elevated plasma or urine catecholamines.

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ORIGINAL ARTICLE

Seven-day PPI-triple therapy with levofloxacin is very effective for *Helicobacter pylori* eradication

R.W.M. Schrauwen, M.J.R. Janssen, W.A. de Boer*

Department of Internal Medicine and Gastroenterology, Bernhoven Hospital, Oss, the Netherlands,
*corresponding author: tel.: +31 (0)412-62 14 23, e-mail: w.deboer@berhoven.nl

ABSTRACT

Background: *Helicobacter pylori* infection causes lifelong gastritis and is associated with the development of peptic ulcer disease, MALT lymphoma and gastric cancer. Many patients benefit from *H. pylori* eradication therapy. PPI-triple therapy is recommended as initial therapy. Quadruple therapy, with a PPI, bismuth, and two antibiotics, used to be recommended as second-line therapy, but can no longer be prescribed because bismuth is no longer available. Therefore, there is an urgent need for new effective rescue therapies. Levofloxacin-based therapies were suggested as an alternative to quadruple therapy. The aim of this study is to examine the efficacy and tolerability of such a one-week therapy with levofloxacin and esomeprazole combined with either amoxicillin or clarithromycin in a Dutch population.

Methods: Between February 2005 and November 2006, 123 consecutive *H. pylori* positive patients were enrolled in this study. The first 59 patients were treated with esomeprazole, amoxicillin and levofloxacin (group I). The next 64 patients were treated with esomeprazole, clarithromycin, and levofloxacin (group II). Both therapies were compared for efficacy and tolerability.

Results: In group I the overall (ITT) cure rate was 96% and in group II it was 93%. Minor side effects occurred in 29% of patients in group I and in 41% of patients in group II. Major side effects that warranted discontinuation of therapy occurred in two patients in group II.

Conclusion: Seven-day triple therapy with esomeprazole, levofloxacin and either amoxicillin or clarithromycin for seven days is very effective and safe for *H. pylori* eradication. The combination with amoxicillin seems to be better tolerated than the combination with clarithromycin.

KEYWORDS

Helicobacter pylori, levofloxacin, therapy

INTRODUCTION

Helicobacter pylori infection causes life-long gastritis and is associated with the development of peptic ulcer disease, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer.[1] Some dyspeptic patients and even asymptomatic persons benefit from *H. pylori* eradication therapy. Most experts believe that eradication of *H. pylori* may possibly prevent gastric cancer.[2,3] There are several well-investigated eradication therapies available. The ideal therapy for this common infection should be simple, safe, cheap, well-tolerated and of short duration, and above all it should reach a high cure rate.

Most guidelines, including the Dutch Institute for Healthcare Improvement (CBO)/Dutch College of General Practitioners (NHG) guideline 'Gastric complaints',[4] advise triple therapy with a proton pomp inhibitor (PPI) and two different antibiotics for an initial attempt at *H. pylori* eradication. After a first antibiotic treatment, a urea breath test to test for cure is usually recommended. In case of persisting infection, the guidelines recommend the use of quadruple therapy, which contains a PPI, bismuth subcitrate and two different antibiotics.[5-11] Although this therapy has the disadvantage of a complicated dosing regimen, it is very effective and is even considered to be a first-line treatment in some countries.

However, unfortunately, in the Netherlands and many other European countries, bismuth is no longer available and quadruple therapy can therefore no longer be prescribed. A possible solution could be the use of a new single multi-drug anti-*Helicobacter* capsule that contains bismuth, tetracycline and metronidazole, but even though this drug has now been approved by the FDA it has not yet received approval from the European authorities to enter the market.[12] Hence, there is an urgent need for new effective therapies in the Netherlands, especially for backup after failure of a first-line PPI-triple therapy.

Levofloxacin is a fluoroquinolone antibacterial agent with a broad spectrum of activity against both Gram-positive

and Gram-negative bacteria as well as atypical respiratory pathogens.[13] Several studies demonstrated the efficacy of levofloxacin in the treatment of infection of the respiratory tract, genitourinary tract, skin and skin structures.[13] The antibacterial activity coupled with its excellent bioavailability and ease of dosing make levofloxacin an attractive antibiotic for treatment of *H. pylori* infection. Recently, some studies have reported excellent results with the use of levofloxacin in *H. pylori* eradication, not only as first-line therapy but also as second line and as rescue therapy.[14-29] In association with other antibiotics, levofloxacin reached high *H. pylori* eradication rates (often over 90%) with a low incidence of side effects. All eradication studies with levofloxacin were performed in other countries than the Netherlands and these data need confirmation in our country.[30,31] Therefore, the aim of the present study is to examine the efficacy and tolerability of a one-week therapy with levofloxacin, esomeprazole and either amoxicillin or clarithromycin in a Dutch population.

PATIENTS AND METHODS

Between February 2005 and November 2006, 123 consecutive *H. pylori* positive patients who visited Bernhoven Hospital (Oss) due to dyspepsia, with or without ulcer disease, were enrolled in this open-label study. Bernhoven Hospital is a non-academic community hospital in the southeast of the Netherlands. In all patients, *H. pylori* status was determined by analysis of gastric biopsies or through a well-validated ^{13}C urea breath test (BreathID™, Oridion Systems).[32] The first 59 patients were treated with esomeprazole 40 mg (Nexium®), amoxicillin 1000 mg and levofloxacin 500 mg (Tavanic®), all given twice daily for seven days (Group I). The next 64 patients were treated with esomeprazole 40 mg (Nexium®), clarithromycin 500 mg, and levofloxacin 500 mg (Tavanic®), all given twice daily for seven days (Group II). Patients were instructed to take their treatment precisely as prescribed and were informed about possible side effects. Furthermore, patients were asked whether they had undergone previous attempts to eradicate *H. pylori* or whether they had received pretreatment with a PPI. At least five weeks after finishing therapy, *H. pylori* status was tested again with either biopsy-based tests if endoscopy was clinically indicated or otherwise with the ^{13}C urea breath test. Side effects were recorded by the treating physician at the end of the treatment and graded on a five-point, internationally accepted scale, which we have described before. This scale contains five categories, ranging from 'no adverse effects' (category A), 'slight discomfort not interfering with daily activities' (category B), 'moderate adverse effects interfering with daily activities' (category C), 'severe adverse effects, work not possible' (category D), to 'severe adverse affects, discontinuation of treatment' (category E).[33] The two therapies were compared for efficacy and tolerability.

Upper gastrointestinal endoscopy

A subgroup of patients underwent upper gastrointestinal endoscopy. During endoscopy seven biopsies were taken. Four (2 antrum and 2 corpus) for histology, two (1 antrum and 1 corpus) for two separate CLO-tests® and one (1 antrum) for culture. Patients were considered *H. pylori* positive if one of these three different tests on either antrum or corpus biopsies was positive.

^{13}Urea breath test

Another subgroup of patients had a *H. pylori* urea breath test (BreathID™ from Oridion Systems, a commercially available and well-validated near-patient ^{13}C-urea breath test), instead of endoscopy.[32] Care was taken to ensure that patients had not taken any PPIs for at least one week before the urea breath test. Breath samples were recorded as positive if the $^{13}CO_2/^{12}CO_2$ ratio was above 5‰. Values below the 5‰ cut-off were considered to be *H. pylori* negative. This test provides excellent sensitivity and specificity in diagnosing *H. pylori* infection, both before and after *H. pylori* eradication.

Statistical analysis

Baseline characteristics of both patient groups were compared using the Student's t-test or χ^2 square test where appropriate. *H. pylori* eradication rates with 95% confidence intervals were calculated and compared using the χ^2 test. This analysis was repeated for the subgroup of patients with a prior (failed) attempt to eradicate *H. pylori*. For analyses the SAS® statistical software package (SAS Institute Inc., USA) was used. Statistical significance was defined as a p value <0.05. Missing values were excluded from analyses.

RESULTS

Population

A total of 123 consecutive *H. pylori* positive patients (mean age 53 (SD 16), 44% male,) were included between February 2005 and November 2006. Twenty-seven of these patients had a diagnosis of peptic ulcer disease (22%). The first 59 patients were treated with esomeprazole, amoxicillin and levofloxacin (group I), the following 64 patients with esomeprazole, clarithromycin and levofloxacin (group II). *Table 1* shows that, although not randomised, both patient groups had similar baseline characteristics. However, there were more patients with a history of failed *H. pylori* eradication in group I.

Table 1. *Baseline characteristics*

	Levofloxacin, esomeprazole and amoxicillin (n=59)	Levofloxacin, esomeprazole and clarithromycin (n=64)	P value for difference
Mean age	55 years (SD 15)	52 years (SD 16)	0.26
Male gender	26 (44%)	28 (44%)	0.97
Smoking	17 (29%)	20 (31%)	0.77
PPI-pretreatment	18 (31%)	23 (36%)	0.53
Previous therapy failure	14 (24%)	5 (8%)	0.01
Body mass index	25.7 (SD 3.9)	26.1 (SD 4.5)	0.62

PPI = proton pomp inhibitor.

Table 2. *Effectiveness of Helicobacter pylori eradication*

	Levofloxacin, esomeprazole and amoxicillin (n=59)	Levofloxacin, esomeprazole and clarithromycin (n=64)	P value for difference
Helicobacter pylori eradicated (ITT)	57 (97%)	59 (92%)	0.29
Helicobacter pylori eradicated (PP)	57 (97%)	59 (95%)	0.69
Side effects	17 (29%)	26 (41%)	0.17
Cessation due to side effects	0 (0%)	2 (3%)	0.17
2nd/3rd line therapy (ITT)	14/14 (100%)	4/5 (80%)	0.09
First-line therapy (ITT)	43/45 (96%)	55/59 (93%)	0.61

ITT = intention to treat; PP = per protocol.

Effectiveness of H. pylori eradication

Of the 59 patients treated with esomeprazole, amoxicillin and levofloxacin, 57 were cured of their *H. pylori* infection, yielding a 97% cure rate (95% CI 92 to 100%), both for intention-to-treat (ITT) and per protocol (PP) analysis. Of the 64 patients treated with esomeprazole, clarithromycin and levofloxacin, 59 had successful *H. pylori* eradication, yielding an ITT cure rate of 92% (95% CI 86 to 99%) and a PP cure rate of 95% (95% CI 90 to 100%) (p value for difference ITT 0.29; PP 0.69))(*table 2*).

If only patients with first-line therapy were included ITT cure rates were 43/45 (96%, 95% CI 91 to 100%) for patients treated with esomeprazole, amoxicillin and levofloxacin and 55/59 (93%, 95%CI 87 to 100%) for patients treated with esomeprazole, clarithromycin and levofloxacin (p value for difference: 0.61).

If only patients with second/third-line therapy were included ITT cure rates were 14/14 (100%) for group I and 4/5 (80%) for group II (p value for difference 0.09).

Side effects

Both regimens were well tolerated, although in the regimen with clarithromycin discontinuation of treatment occurred in two patients. Reasons for discontinuation were nausea in one and rupture of an Achilles tendon in the other patient. When tested, neither of these patients were cured of their infection. Minor side effects occurred in 29% of all patients in group I and in 41% of all patients in group II (p=0.17). Diarrhoea was the most common side effect.

DISCUSSION

The results of this study show that levofloxacin can be used safely for eradication of *H. pylori*. They also show that its use leads to high cure rates in Dutch patients. A limitation of the present study is that it is not a blinded randomised clinical trial. However, treatment allocation was determined only by the date of inclusion, not by patient characteristics. This is confirmed by the comparable baseline characteristics of both groups. Furthermore, cure of *H. pylori* infection is a solid, well-defined endpoint and open studies like ours are well accepted in this field in order to identify new treatment options.

The triple therapy with esomeprazole, amoxicillin and levofloxacin reached a 96% (43/45) per protocol cure rate when used as initial therapy. With the identical therapeutic regimen in primary treatment Antos *et al*. cured 28/30 of patients (93.3%) in France.[22] Retreatment after failure of an initial anti-*Helicobacter* therapy is generally considered to be more difficult than initial therapy, but when we employed this triple drug therapy as second-line therapy in eight patients and as third-line therapy in six patients it was successful in all these patients. Most studies in the literature also deal with the use of levofloxacin in second- or third-line therapy.

Several studies have directly compared different levofloxacin-based combinations with several different quadruple combinations in retreatment. Two systematic reviews and meta-analyses have summarised these studies,

and the data suggest that the levofloxacin-based regimens were a good alternative to quadruple therapy.[16,20] In fact, levofloxacin containing treatments had superior cure rates as compared with the standard quadruple regimens.[16,20,34] The cure rates achieved with levofloxacin in these studies, however, were somewhat lower then the >95% we have achieved in our study.[16,20] Furthermore, one study suggested that the levofloxacin-based triple therapy was also superior to rifabutin-based triple therapy, which is another established rescue therapy.[23] Our excellent result with esomeprazole, amoxicillin and levofloxacin for retreatment in patients who failed to be cured with their previous therapy therefore supports this large body of data from the literature.[14-20,22-29,35] The triple therapy with esomeprazole, clarithromycin and levofloxacin reached a 96% (55/57) per protocol cure rate when used as initial therapy. At present, triple therapy with a PPI, amoxicillin and clarithromycin is usually our primary therapy. After failure of this regimen bacteria resistant to clarithromycin are usually encountered, whereas resistance to amoxicillin is extremely rare.[30,31] Amoxicillin can therefore be used in retreatment, but microbiologically it is not logical to use clarithromycin again in retreatment. Nevertheless, we cured four out of five patients who had previously failed a PPI, amoxicillin and clarithromycin combination with regimen II. Few studies are available regarding the combination of levofloxacin with clarithromycin or another macrolide.[24,28] Randomised head-to-head studies of regimen I *vs* regimen II are unavailable. However, theoretically, primary clarithromycin resistance might have a negative impact on the cure rates of the regimen we used in group II.

At the moment it is not clear whether levofloxacin should be used in seven or ten-day regimens. Some authors have consistently used ten-day therapies and meta-analysis showed this to be superior to seven-day therapy.[16,20] Our results with seven days of treatment in Dutch patients are already >95% and therefore we do not promote increasing the length of treatment from seven to ten days for the Dutch situation. Most Italian ten-day studies used a lower dose of levofloxacin, either 500 mg once daily or 250 mg twice daily. Others and we chose a higher dose of 500 mg twice daily for seven days and this has been shown to be a well-tolerated and safe dosage schedule.[21] Therefore, we would at present not recommend using a lower dose.[20]

A possible threat to the efficacy of levofloxacin is the presence of antimicrobial resistance to fluoroquinolones.[31] The prevalence of this resistance has been determined in only a limited number of studies. The only Dutch data date back to 1999, when Debets-Ossenkopp reported a primary resistance rate of trovafloxacin of 4.7%.[36] In France, resistance to fluoroquinolones increased from 3.3% in 1999 to 17.5% in 2003.[37] In 2006, it was reported to be 16.8% in Belgium.[38] The high rate of resistance in these countries probably mirrors the increasing use of quinolones to treat various common infections.[39] A well-documented much lower use of quinolones in the Netherlands[40] as compared with France and Belgium probably results in lower levels of fluoroquinolone resistance in Dutch patients. This might also explain why our cure rates are at the top of what is reported in the literature. The higher dose of levofloxacin, however, is another possible explanation for our relatively high cure rate.

Levofloxacin as an antibiotic is generally well tolerated.[13] Overall in our study adverse effects were reported by 35% of the patients, but these were most often mild. Twenty-nine percent of patients in group I reported side effects, but they did not lead to discontinuation in any of these patients. Forty-one percent of patients in group II, a higher number in comparison with group I, reported side effects. In two patients, treatment was discontinued, because of extreme nausea and an Achilles tendon rupture. The latter is a known, but rare and severe complication, but in our patient, a 70-year-old man, it healed and had no long-term consequences. According to an investigation in the United States tendon rupture occurs in less than four per million prescriptions of levofloxacin,[41] and it should not therefore be a reason to fear the use of this antibiotic. Based on our data it appears that the combination of levofloxacin with amoxicillin is better tolerated and causes fewer side effects then the combination of levofloxacin with clarithromycin.

Then, based on our own experience and our review of the literature, what should be the place of levofloxacin in *Helicobacter* therapy in the Netherlands? According to the Dutch CBO/NHG consensus a triple therapy with a PPI, amoxicillin and clarithromycin should be the initial regimen for treating *H. pylori* infection in the Netherlands. Janssen *et al.* recently reported the prevalence of *H. pylori* antibiotic resistance in the east of the Netherlands. This study shows that primary metronidazole resistance was stable throughout the study period (1997-2002) with a mean prevalence of 14%. The prevalence of primary clarithromycin resistance was still very low (mean prevalence 1%).[42] Therefore, a regimen with amoxicillin and clarithromycin is a logical and microbiologically sound choice for an initial anti-*Helicobacter* therapy in the Netherlands and it should probably remain our initial therapy. It leads to high cure rates and with these high cure rates it is almost impossible to demonstrate superiority with another therapy. An Italian study showed superiority of the regimen we used in group II over standard PPI-triple therapy, but resistance against clarithromycin is much higher in Italy than in the Netherlands.[21] Levofloxacin-based therapies are an alternative for primary treatment in the Netherlands. However, we would not yet recommend them as initial therapy, especially since data on primary fluoroquinolone resistance of *H. pylori* in the Netherlands are not yet available.

According to the guidelines, all patients who received initial therapy should be tested for cure if urea breath testing is available. Patients also have a desire to know whether or not they are truly cured.[8,4,25,43] Quadruple therapy is recommended in the Dutch guideline as back-up therapy[4] but bismuth (De-Nol® or Ranitidine Bismuth Subcitrate (Pylorid®)) is no longer available. In this situation it seems logical to accept a levofloxacin-based regimen as a universal second-line therapy. Those who are not cured after an initial attempt can be retreated with the esomeprazole, amoxicillin, and levofloxacin regimen that we employed in group I. In case of penicillin allergy it is advisable to initially use a seven-day combination of a PPI, clarithromycin 250 mg or 500 mg, and metronidazole 500 mg, all twice daily. If this fails, the levofloxacin regimen we used in group II can be used as rescue therapy. A triple therapy of a PPI with levofloxacin and tinidazole (500 mg twice daily) might be an alternative in this setting.[29]

CONCLUSION

Seven-day triple therapy with esomeprazole, levofloxacin and either amoxicillin or clarithromycin is very effective and safe for *H. pylori* eradication in the Netherlands. These regimens can be used as first-line therapy and as second-line therapy. The combination with amoxicillin appears to cause fewer side effects. Regimens incorporating levofloxacin provide us with new and long awaited possibilities to treat *H. pylori*. Although it may be too early to promote their use as initial therapy we can, based on our data as well as the literature, already advise the use of these regimens for back up after failed initial therapy.

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NOTE

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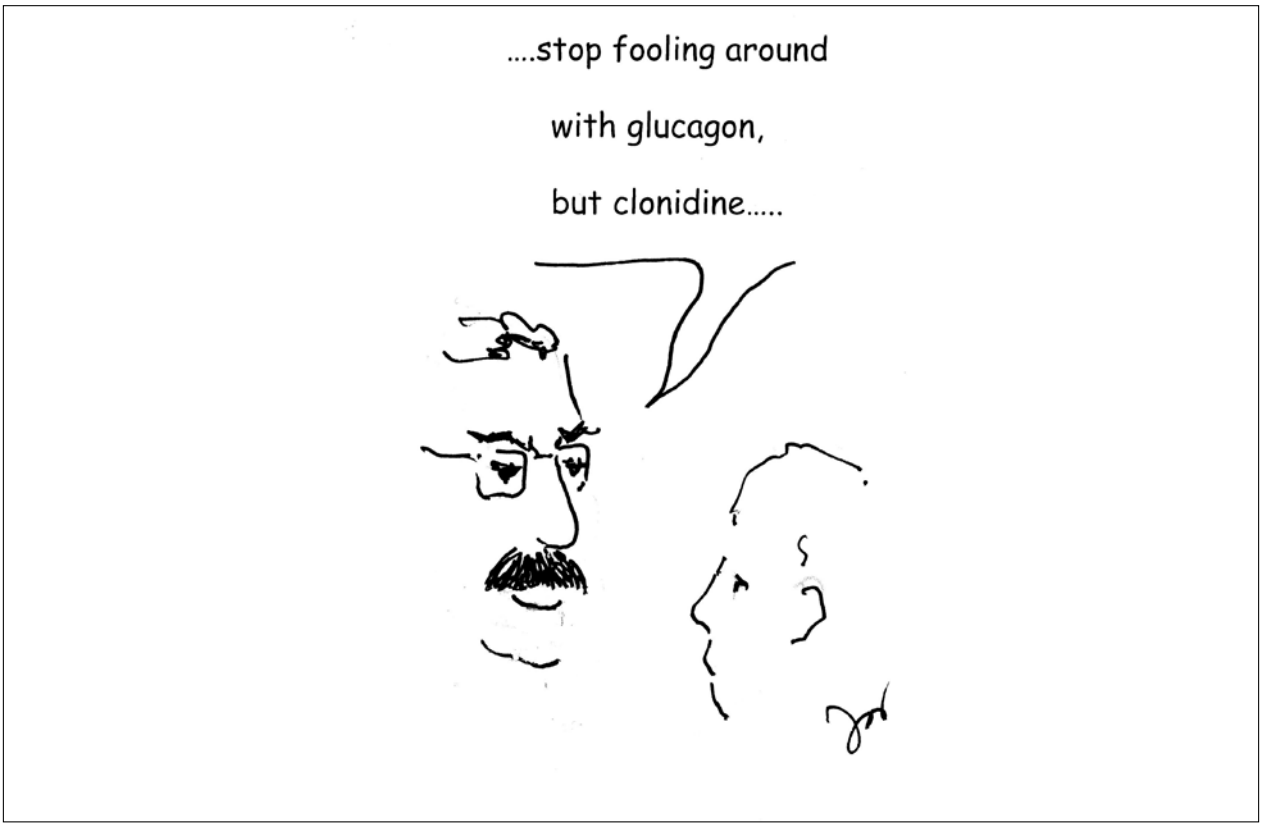

Schrauwen, et al. Seven-day PPI-triple therapy with levofloxacin for *Helicobacter pylori* eradication.

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CASE REPORT

Symptomatic hypoparathyroidism based on a 22q11 deletion first diagnosed in a 43-year-old woman

K. van den Berge[1], K. Diderich[2], P. Poddighe[2], A. Berghout[1*]

[1]Department of Medicine, Medical Centre Rijnmond Zuid, Rotterdam, the Netherlands, [2]Department of Clinical Genetics, Erasmus Medical Centre – Erasmus University Rotterdam, the Netherlands, [*]corresponding author: e-mail: Berghouta@mcrz.nl

ABSTRACT

Congenital hypoparathyroidism usually manifests in early childhood with hypocalcaemia with or without clinical characteristics. This report describes a Caucasian woman who, at the age of 43 years, was diagnosed with dysgenesis of the parathyroid glands due to a *de novo* microdeletion in chromosome 22q11 or DiGeorge syndrome. This syndrome is characterised by a considerable variability in clinical symptoms, including heart defects, thymic hypoplasia and mental retardation. Our patient presented with generalised convulsions due to extreme, symptomatic hypocalcaemia. The convulsions had been apparent for 18 months at the time of the diagnosis. Remarkably, whereas parathyroid hormone levels were undetectable, the 1,25-dihydroxy vitamin D level was normal. Chromosome 22q11 deletion was confirmed by fluorescence *in situ* hybridisation analysis.

KEYWORDS

22q11, DiGeorge, hypocalcaemia, hypoparathyroidism

CASE REPORT

A 43-year-old Caucasian woman was evaluated for thrombocytopenia. She had been suffering from convulsions for 18 months before presentation, for which antiepileptic agents were prescribed with insufficient response. At the time of presentation of the seizures bilateral calcifications were seen in the basal ganglia and cerebellum on CT scanning of the brain. The patient's history also included bronchitis, bilateral surgery for cataract and a significant learning disability. Her medication consisted of levetiracetam and salbutamol.

At physical examination we saw a woman of short stature who was noted to have subtle facial dysmorphisms (*figure 1*), i.e. a high forehead, a long philtrum, mild ptosis and

Figure 1. *Close up photograph of the patient with hypoparathyroidism, showing the high forehead, a long philtrum, mild ptosis and upslanting palpebral fissures*

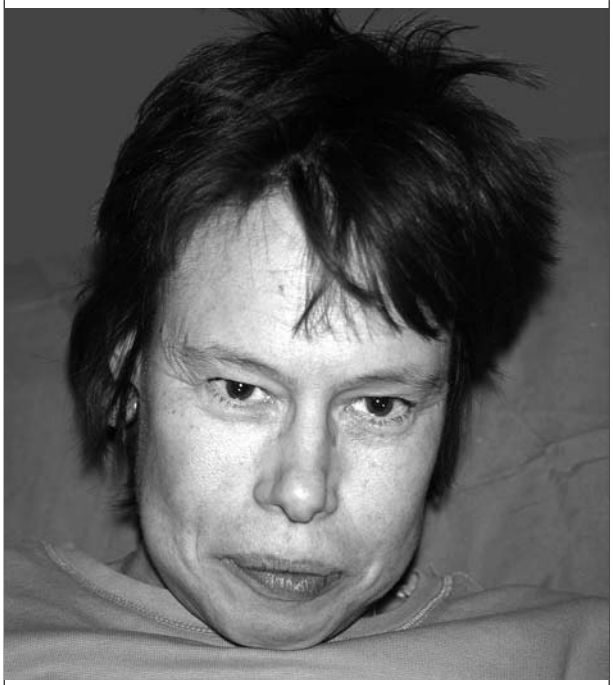


Photo with permission from patient.

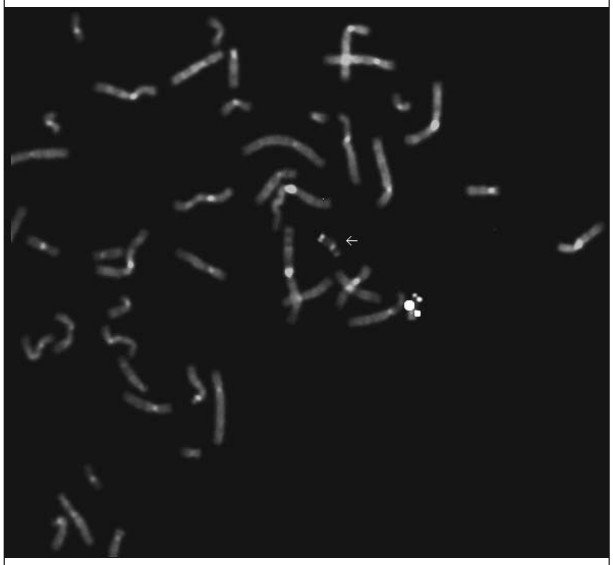
upslanting palpebral fissures. Examination of the heart and lungs was without abnormal findings but there was a remarkable hypoplasia of the breasts. Abdomen and external genitals were normal and inspection of the extremities showed no abnormalities. The patient gave oral consent for publication of this case report.

Blood tests showed a marked thrombocytopenia with a mildly lowered red cell count and a normal white cell count with normal differential. Kidney function was slightly impaired, but potassium and sodium were normal. There was an extreme hypocalcaemia of 1.27 mmol/l (ionised calcium 0.58 mmol/l) at a normal albumin level. Serum magnesium was low at 0.47 mmol/l. A spot urinary calcium was 0.54 mmol/l, which was considered to be elevated as related to the serum calcium concentration as the patient was unable to collect a 24-hour urinary sample. Hormonal measurements included a normal thyroid-stimulating hormone, mildly lowered 25-hydroxy vitamin D level, a normal 1,25 dihydroxy vitamin D level and an undetectable concentration of the parathyroid hormone (PTH). PTH levels remained undetectable after correction of hypomagnesiaemia. The diagnosis of hypoparathyroidism with extreme hypocalcaemia, resulting in seizures was made. The differential diagnosis comprised both acquired and congenital hypoparathyroidism. There was no evidence for acquired hypoparathyroidism, i.e. surgery, infections, or storage diseases. Congenital causes of hypoparathyroidism can be differentiated into several rare disorders. For example, it can result from destruction of the parathyroid glands due to an autoimmune process. Congenital hypoparathyroidism can be found in association with other endocrinopathies or specific developmental and mitochondrial disorders.[1]

Based on the hypoparathyroidism and the dysmorphic phenotype, the possibility of chromosome 22q11 deletion syndrome was suspected. Standard chromosome analysis showed a normal karyotype. Fluorescence *in-situ* hybridisation analysis was performed according to previously established methods[2] with biotine-labelled cosmid probes 122B5 and M51, demonstrating a deletion for chromosome 22q11.2 (DiGeorge/Shprintzen region) (*figure 2*).

Electrocardiography showed a mildly prolonged QT interval and a transthoracic echocardiogram revealed normal cardiac chambers and normal outflow tracts. To analyse the patient's thrombocytopenia, a bone biopsy was performed. Pathological examination revealed a mild marrow fibrosis with normal maturation and count of all haematopoietic cell lines. These findings are consistent with excess platelet turnover as is sometimes seen in levetiracetam use, but have also been mentioned as a part of the 22q11 deletion syndrome.[3] Conventional X-rays of the hands turned out to be normal and bone mineral densitometry was performed, also showing values in the normal range.

Figure 2. *Fluorescent in-situ hybridisation (FISH) using the cosmid probe 122B5 (white), shows a deletion of the DiGeorge/Shprintzen region on one chromosome 22q11.2*



The BAC probe CTD-3018K1 (red) is a subtelomeric probe and is used as a control probe. This probe shows hybridisation signals on both chromosomes 22.

Levetiracetam was stopped and the patient was initially treated with 1 µg calcitriol, which was tapered off, 1 g calcium, 1448 mg magnesium hydroxyde and 25 mg hydrochlorothiazide daily. The convulsions disappeared as the serum calcium concentration rose to normal at 2.05 mmol/l.

DISCUSSION

Hypoparathyroidism due to congenital agenesis or hypoplasia of the glands typically becomes apparent in early childhood and is a rare finding in the adult patient. This case report describes a 43-year-old patient with complete hypoparathyroidism due to chromosome 22q11 deletion syndrome, a congenital condition that results in hypoplasia of the parathyroid glands and is usually diagnosed in early life. In addition to the biochemical findings, some clinical characteristics, such as subtle facial dysmorphisms, short stature and mental impairment, pointed to this syndrome. It was only when the patient developed thrombocytopenia, probably due to the use of anticonvulsant drugs, that the serum calcium levels were evaluated and a severe hypocalcaemia, which had probably been present for years, was found. This eventually resulted in the diagnosis of 22q11 microdeletion syndrome.

Cerebral calcifications are relatively common in hypoparathyroidism and its aetiology has not been completely elucidated. It may be related to the duration of

hypocalcaemia and hyperphosphataemia more than to the lack of parathyroid hormone itself.[4]

The 22q11 deletion syndrome, also known as the DiGeorge syndrome, Shprintzen syndrome or velo-cardio-facial syndrome, was first described in 1965 in children with the triad of hypoparathyroidism, recurrent infections and thymic hypoplasia.[5] As a consequence of the microdeletion, there is a congenital failure in the development of the derivatives of the various pharyngeal arches and pouches.[6] Approximately 1/7500 live births are affected by this deletion, which typically occurs *de novo*, making it the most common contiguous gene deletion syndrome in humans.[3]

In addition to the originally described triad, a wide variety of clinical findings may accompany the 22q11 deletion syndrome. Most patients, in contrast to our patient, have structural heart disease[3,7] and more or less obvious dysmorphisms of the face and are therefore diagnosed in early childhood. A striking feature of the patient in this case report is the time of diagnosis, at the age of 43. She became symptomatic at age 41, when she developed generalised seizures. To the best of our knowledge, our patient is the eldest person in whom the syndrome has been diagnosed. A similar case was described in a 32-year-old patient a few years ago.[8] A plausible explanation for the late-onset symptomatic hypocalcaemia is inadequate parathyroid reserve, in which PTH secretion may be sufficient to maintain normocalcaemia in normal conditions, but is unable to create an adequate response in the situation of hypocalcaemic stress, which for example can occur during ageing, surgery, infection or pregnancy.[9] Symptomatic hypocalcaemia, however, rarely develops because of the compensatory increase in the PTH secretion, which is defective in our patient.

Of interest is the absence of structural changes in the patient's bone. Most patients with chronic depletion of PTH develop abnormalities in bone mineralisation, i.e. increased bone mineral density,[10] though skeletal abnormalities are only prevalent in 17 to 19% of patients with DiGeorge syndrome.[11] We do not have a plausible explanation for this finding, but suppose that in our patient parathyroid function deteriorated over time by mechanisms that are unknown. On the other hand, it can be envisaged that she could maintain low calcium levels by the extrarenal hydroxylation of 25-hydroxy vitamin D. This is supported by the finding of low normal 1,25-dihydroxy vitamin D levels in our patient.[12]

CONCLUSION

Hypoparathyroidism first diagnosed in adulthood may, in rare cases, be due to parathyroid gland hypoplasia, secondary to the chromosome 22q11 deletion syndrome. The diagnosis could be missed due to either the unfamiliarity of physicians with the syndrome or the variable and sometimes subtle phenotype. Because chromosome 22q11 deletion is relatively common, the diagnosis should be considered in patients with idiopathic hypoparathyroidism because of the potential benefit that can be derived from genetic counselling.

ACKNOWLEDGEMENTS

We thank P. Lips, MD, PhD, for his comments on the article.

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Netherlands
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CASE REPORT

Hypocalcaemia as presenting symptom of velocardiofacial syndrome

E.I. van Vliet[1], B.M. van Ouwerkerk[2*]

Departments of [1]Internal Medicine and [2]Endocrinology, Albert Schweitzer Hospital, Dordrecht, the Netherlands, [*]corresponding author: tel.: +31 (0)78-654 11 11

ABSTRACT

Hypocalcaemia due to hypoparathyroidism is a rare finding in adults. The coexistence of cardiac abnormalities may be suggestive of a hereditary syndrome. We describe a case of velocardiofacial syndrome in a woman without a family history of this disorder. The hypocalcaemia was treated with calcium and vitamin D supplementation.

KEYWORDS

Genetics, hypocalcaemia, velocardiofacial syndrome

INTRODUCTION

Velocardiofacial syndrome (VCF) was first described by Shprintzen *et al.* in 1978 and is characterised by cleft palate or velopharyngeal insufficiency, cardiac abnormalities, characteristic facies and learning disabilities.[1] It is associated with 22q11.2 microdeletion. This deletion occurs in one in 4000 births. The deletion is sporadic in most cases, but an autosomal dominant form has been described. Another clinical syndrome that results from the same developmental field defect as VCF is the DiGeorge syndrome.

Diagnosis is usually established in early childhood. Affected individuals often die young, mainly due to congenital heart disease. It is less common that VCF is diagnosed in adulthood. Three cases with DiGeorge syndrome which presented with hypocalcaemia in adulthood have been reported.[2,3] We report another patient presenting in adulthood. This case is of a woman presenting with hypocalcaemia on routine blood examination.

CASE REPORT

A 43-year-old woman presented to the outpatient clinic of Internal Medicine with complaints of diarrhoea for four weeks, abdominal pain and fever. Her medical history revealed surgery for a ventricular septal defect and surgery for a cleft palate in early childhood, epilepsy, absent kidney, scoliosis, recurrent pelvic inflammatory disease and depressive disorder which required treatment with antidepressants. Her family history was insignificant and she had conceived no children. She had been working in an administrative function. There was no history of learning disabilities.

On examination she had a round face with hypertelorism (*figure 1*). She was of short stature. Signs of Chvostek and Trousseau were negative.

Figure 1. *Face of patient described*

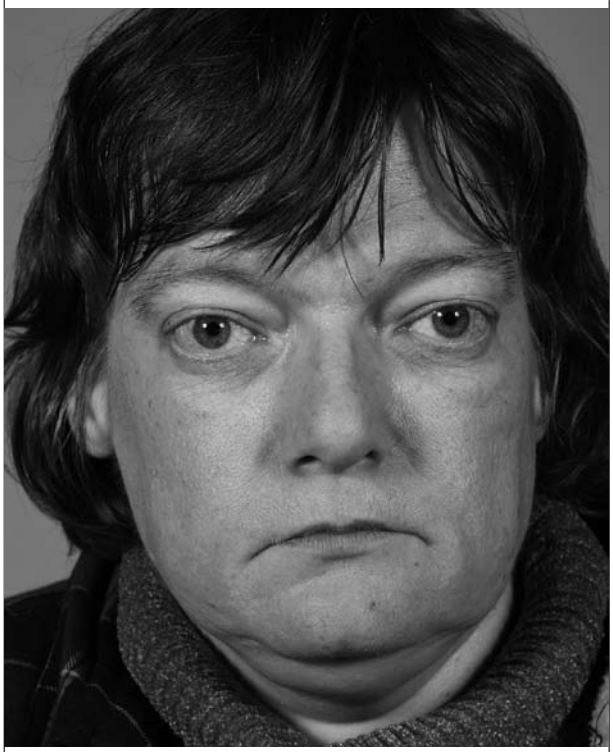

Photo with permission from patient.

Laboratory examination revealed a white blood cell count of 24.8 10^9/l (4.3 to 10.0), CRP 296 mg/l (0 to 10), calcium 1.14 mmol/l (2.20 to 2.65) with an ionised serum calcium level of 0.64 mmol/l (1.12 to 1.32), phosphorus 1.86 mmol/l (0.80 to 1.40) and magnesium 0.65 mmol/l (0.70 to 1.05). The intact parathyroid hormone level was 2.2 (0 to 6.0 pmol/l), 25-hydroxy vitamin D was 57 nmol/l (25 to 150) and 1,25-dihydroxy vitamin D was 80 pmol/l (48 to 161).
ECG showed a prolongation of the QT interval (corrected QT time of 505 milliseconds).
During admission she developed spontaneous movements of her arms and legs.
She was given calcium glubionate (1080 mg calcium/24 hours) intravenously and vitamin D (alfacalcidol 0.25 µg per day), after which her calcium levels returned to normal.
A CT scan of the abdomen was performed because of persistent abdominal pain and revealed a tubo-ovarian abscess, which has been removed surgically.
After discharge she continued with calcium chewing tablets (1000 mg) and vitamin D capsules (Etalpha 2 µg). On follow-up she remained normocalcaemic.
Because of the combination of hypocalcaemia, surgery for ventricular septal defect and for a cleft palate and her facial appearance, we suspected her of having VCF syndrome. Chromosomal analysis (fluorescence *in situ* hybridisation) revealed a submicroscopic deletion of the 22q11 chromosome, consistent with the velocardiofacial syndrome.

DISCUSSION

The clinical spectrum of the VCF is very variable. Originally, VCF was characterised by cleft palate, cardiac abnormalities, characteristic facies and learning disabilities.[1] Other clinical features include short stature, thymic hypoplasia, psychiatric disorders, hypocalcaemia and renal disease.[4] Diagnosis is usually made in early childhood. Patients with VCF diagnosed in adulthood are generally family members of previously diagnosed VCF patients (e.g. parents of children with diagnosed VCF syndrome), but rarely present with hypocalcaemia.[5]
Hypocalcaemia is relatively common in children with VCF with incidence rates varying from 17 to 60%.[4,6,7] Especially young infants have a high incidence of hypocalcaemia.
A couple of case reports have been published on patients with VCF presenting with hypocalcaemia in adulthood. Kar *et al.* describe two patients.[2] The first patient was a 24-year-old woman who had a generalised seizure; blood investigation revealed hypocalcaemia. She was suspected of a genetic syndrome because of the postnatal death of her young child. The second patient was a 52-year-old woman, diagnosed with hypocalcaemia on routine blood examination. She had a history of a congenital heart condition (patent ductus arteriosus). Chromosomal analysis of both patients revealed a submicroscopic deletion in the 22q11 region.
Van den Bosch *et al.* describe a 29-year-old woman, with tetany due to hypocalcaemia.[3] Her medical history included a cleft palate at birth, recurrent pulmonary infections during childhood, growth retardation, an atrial septum defect and a right descending aorta. Chromosomal analysis revealed a chromosome 22q11.2 deletion.
Frequent causes of hypocalcaemia are hereditary or acquired hypoparathyroidism, hypomagnesaemia, chronic renal failure, lacking or ineffective active vitamin D, pseudohypoparathyroidism and severe, acute hyperphosphataemia due to tumour lysis syndrome or acute renal failure.[8] These should always be included in the differential diagnosis of hypocalcaemia.

CONCLUSION

In a patient with hypocalcaemia and a complex medical history, the velocardiofacial syndrome should be considered, as this has great implications for future family planning and genetic counselling.

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LETTER TO THE EDITOR

Anti-TNFα and agranulomatous tuberculous manifestations: more diagnostic dilemma

Dear Editor,

I read with interest the case report by Verhave *et al.* and the accompanying editorial on the use of antitumour necrosis factor α (anti-TNFα) and resultant tuberculosis published in a recent issue of the journal.[1,2] Among its many other functions, anti-TNFα inhibits the action of TNFα, which is required for macrophages to phagocytose *Mycobacterium* and stimulate granuloma formation to control the infection. Tuberculous manifestations without granuloma formations have been shown to be common among reported cases of patients treated with infliximab.[3] Such manifestation is certainly a new aspect of the already varied manifestations of tuberculosis (TB) infections. For clinicians working in regions where TB remains common, this will certainly pose a major challenge and cause for concern for those who are unaware of this association. In our local setting, we are so used to seeing granulomatous manifestations and we rely heavily on their presence, with or without caseation, to make a diagnosis of TB infection, at times even just based on the presence of granuloma alone. In our experience, the yield of isolating the *Mycobacterium* on histology is only achieved at best in 50% and tissue cultures are not usually done due to initial lack of suspicion at the time of investigations, compounded by nonviability of tissue after being placed in formalin.[4,5] With the increasing use anti-TNFα agents as in our setting, these cases certainly highlight the importance of clinical suspicion in patients using anti-TNFα and the performance of tissue culture. It also highlights the dawn of a new type of manifestation that further compounds to the dilemma of diagnosing TB infections.

V.H. Chong
Gastroenterology Unit, Department of Medicine, Raja Isteri Pengiran Anak Saleha (RIPAS) Hospital, Bandar Seri Begawan BA 1710, Brunei Darussalam, e-mail: chongvuih@yahoo.co.uk

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LETTER TO THE EDITOR

Whipple's disease: easily diagnosed, if considered

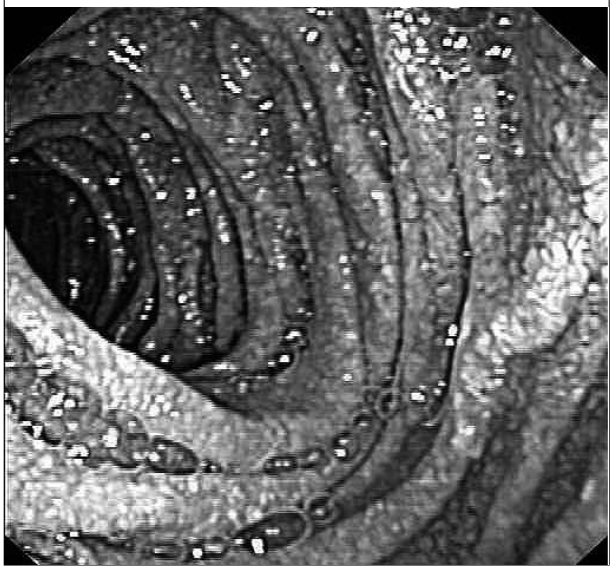
Dear Editor,

We read with interest the article by Schijf *et al.* 'Whipple's disease: easily diagnosed, if considered'.[1] The diagnosis of Wipple's disease can be difficult if not considered and may even include a diagnostic laparotomy for suspicion of retroperitoneal lymphoma.[2] But even if the diagnosis is considered it may not be easy. We describe a patient in whom duodenal biopsies showed only one granuloma on microscopic examination, but in whom biopsies of jejunal mucosa showed typical changes consistent with Wipple's disease.

In 2006, a 36-year-old male presented to the outpatient clinic with arthralgia, diarrhoea, weight loss, iron deficiency anaemia and retroperitoneal lymphadenopathy. Gastroduodenoscopy and colonoscopy were performed. Both showed a completely normal appearance of the duodenal and colonic mucosa. Histological examination of the duodenal biopsy showed one granuloma. Tuberculosis, HIV, *Treponema pallidum* haemagglutination assay, Brucellosis, Q-fever, cytomegalovirus and toxoplasmosis were excluded. Sarcoidosis, and autoimmune disorders were all considered but could not be diagnosed. As sarcoidosis of the stomach and duodenum is described as a separate entity, corticosteroid treatment was initiated but without clinical improvement. The patient's anaemia worsened and his weight decreased further.

Then we considered Wipple's disease. As proximal enteroscopy can be misleading we performed jejunoscopy. The duodenal mucosa was normal but the jejunal mucosa was abnormal, showing a whitish reticular pattern consistent with dilated lymphatic vessels (*figure 1*). Microscopic examination showed foam cells in the villi and lamina propria, consistent with Whipple's disease. Polymerase chain reaction for *Thropheryma whipplei* was positive. Antibiotic treatment sulfamethoxazole-trimethoprim twice daily resulted in a full recovery. After one year, treatment was discontinued and to date no signs of relapse have occurred.

Figure 1. *Jejunal mucosa showing a whitish reticular pattern consistent with dilated lymphatic vessels*



When suspecting a diagnosis of Whipple's disease proximal endoscopy can be misleading. Jejunal biopsies should always be performed.

S.M. Sanavro*, **H.J. Voerman**
Amstelland Hospital, Amstelveen, the Netherlands,
**corresponding author: tel.: +31 (0)20-347 47 47, e-mail: sannesanarro@hotmail.com*

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LETTER TO THE EDITOR

Autoimmune haemolytic anaemia due to chronic hepatitis C virus infection treated with prednisone, pegylated interferon and ribavirin

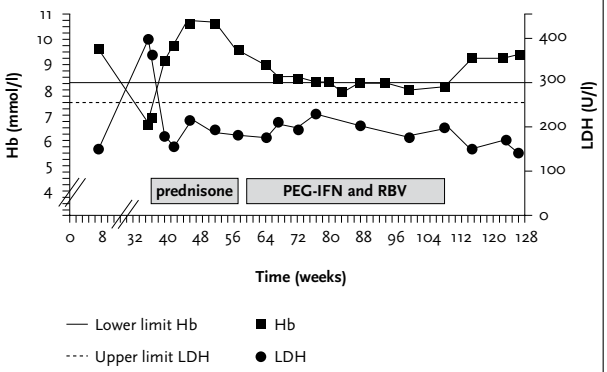
Dear Editor,

Chronic hepatitis C virus (HCV) infection has been associated with various extrahepatic manifestations, including autoimmune cytopenias.[1] Primary autoimmune haemolytic anaemia (AIHA) has been reported as an unusual extrahepatic manifestation.[2]
Combination therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV) is the current treatment of choice for chronic HCV infection.[3] However, this therapeutic regimen can exacerbate underlying autoimmune disorders and may result in a significant anaemia.[4] Though extrahepatic manifestations usually necessitate HCV treatment, the patient with AIHA as an extrahepatic manifestation of HCV infection represents a therapeutic dilemma.

We saw a 29-year-old man of Afghanistan origin with chronic HCV infection, with HCV RNA of 1.3 x 10^6 copies/ml, genotype 1b. Laboratory evaluation revealed moderately increased transaminases (ASAT 63 U/l, ALAT 155 U/l). Test results for HIV and hepatitis B were negative. A percutaneous liver biopsy showed mild chronic inflammation, without signs of fibrosis.
A few months after initial assessment, the patient developed jaundice. Blood tests showed a marked drop in haemoglobin level (from 9.6 to 6.6 mmol/l), macrocytosis (MCV 99 fL) and reticulocytosis (398 x 10^9/l), associated with an elevated indirect bilirubin (34 µmol/l) and lactate dehydrogenase (393 U/l) and a low haptoglobin (<0.20 g/l). A direct Coombs test was positive for IgG. Based on these findings, a diagnosis of Coombs-positive AIHA was made.
The patient received folic acid and prednisone (60 mg/day), which was gradually tapered over a period of five months. Within three weeks his anaemia improved (Hb 9.2 mmol/l) and there was no recurrence of AIHA after discontinuation of prednisone.

Directly after prednisone therapy, the patient started HCV treatment with PEG-IFN alfa-2a (180 µg/week) plus RBV (1000 mg/day) for 48 weeks. During this period the patient's lowest haemoglobin level was 7.9 mmol/l and dose reduction was not required. The patient successfully achieved a sustained virological response. When last seen, the patient was well and had neither signs of HCV infection, nor of AIHA.

Figure 1. *Chronological changes in haemoglobin levels (Hb) and lactate dehydrogenase (LDH) in relation to treatment with prednisone ans pegylated interferon (PEG-IFN) plus ribavirin (RBV)*



HCV infection is a common cause of progressive liver disease. In addition, chronic HCV infection has been associated with a variety of extrahepatic manifestations, including autoimmune disorders. During the past decade, several authors have described the relationship between chronic HCV infection and autoimmune cytopenias.[1] Primary AIHA has been reported as an unusual, but recognised extrahepatic manifestation.[2]
The current best treatment of choice for chronic HCV infection is PEG-IFN plus RBV. However, this treatment combination has a high incidence of haematological side effects. RBV causes a dose-dependent reversible haemolytic anaemia. PEG-IFN may contribute to anaemia by suppressing haematopoiesis.[4] Recently, a few case reports have described the occurrence of severe AIHA induced by PEG-IFN-RBV combination therapy.[5,6] In addition, PEG-IFN can exacerbate pre-existing autoimmune disorders. As a consequence cytopenias and autoimmune diseases are relative contraindications to HCV therapy.

We considered the AIHA in our patient to be an extrahepatic manifestation of chronic HCV infection. We first initiated prednisone therapy, which led to complete remission of AIHA. Subsequent antiviral therapy led to sustained virological response, without recurrence of AIHA. In conclusion, AIHA due to chronic HCV infection can successfully be treated with prednisone therapy and does not form a contraindication to antiviral treatment.

M.H.P. Dietvorst[1], P. van Wijngaarden[1*], R.A. de Man[2]
[1]Department of Internal Medicine, Amphia Hospital, Breda, the Netherlands, [2]Department of Gastroenterology and Hepatology, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands, [*]corresponding author: tel.: +31 (0)76-595 24 04, fax: +31 (0)76-595 33 27, e-mail: PvanWijngaarden@amphia.nl

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MONTHLY NJM ONLINE HITLIST

The table lists online hits for all articles published in the December issue of *the Netherlands Journal of Medicine* 2008 (available online on PubMed since 15 December 2008).

Article	Hits
EDITORIALS	
The *Netherlands Journal of Medicine*: seven years editorial office in Nijmegen, entering a new era	76
Blood pressure measurement in the year 2008: revival of oscillometry?	111
REVIEWS	
Hodgkin's lymphoma: news from an old disease	93
Cardiovascular risk is more related to drinking pattern than to the type of alcoholic drinks	87
ORIGINAL ARTICLES	
Oscillometric blood pressure measurements: differences between measured and calculated mean arterial pressure	127
Reintroduction of Riva-Rocci measurements to determine systolic blood pressure?	94
Thyroid function in patients with proteinuria	98
CASE REPORTS	
Intestinal ischaemia caused by mesenteric inflammatory veno-occlusive disease	111
Anakinra suppresses familial Mediterranean fever crises in a colchicine-resistant patient	76
PHOTO QUIZZES	
Endoscopy for obstructive jaundice	80
An adult with lower abdominal pain	109
MONTHLY NJM ONLINE HITLIST	
For all articles published in September 2008	43
Total	**1105**

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PHOTO QUIZ

A woman with a painful and swollen hand

M.P. Hendriks*, M. van Deuren

Department of Internal Medicine, 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: m.hendriks@aig.umcn.nl

CASE REPORT

An 86-year-old woman came to the emergency room complaining of a painful swollen right hand for the last 18 hours without a history of trauma (*figure 1*). At physical examination her blood pressure was 75/40 mmHg, heart rate 60 beats/min and body temperature 37.3°C. Sensibility of the digits was absent. Laboratory investigation showed leukocytosis, raised C-reactive protein, hyperlactataemia and renal insufficiency (serum creatinine 168 µmol/l). Creatine phosphokinase was still normal. During the first hours in the ER, the swelling progressed to the volar side of the right arm with purple colouring and formation of a blister (*figure 2*).

Figure 1. *Swelling of the right hand*

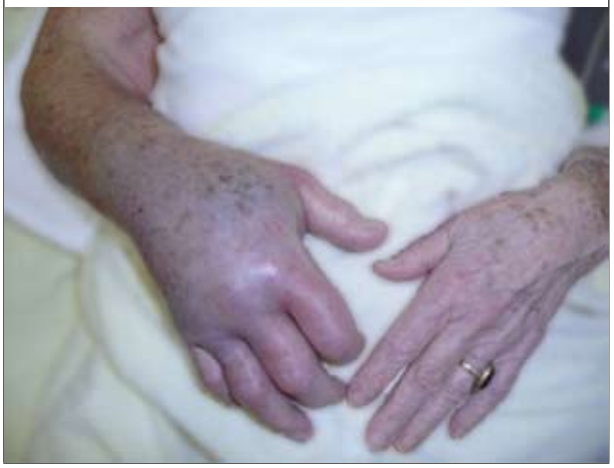
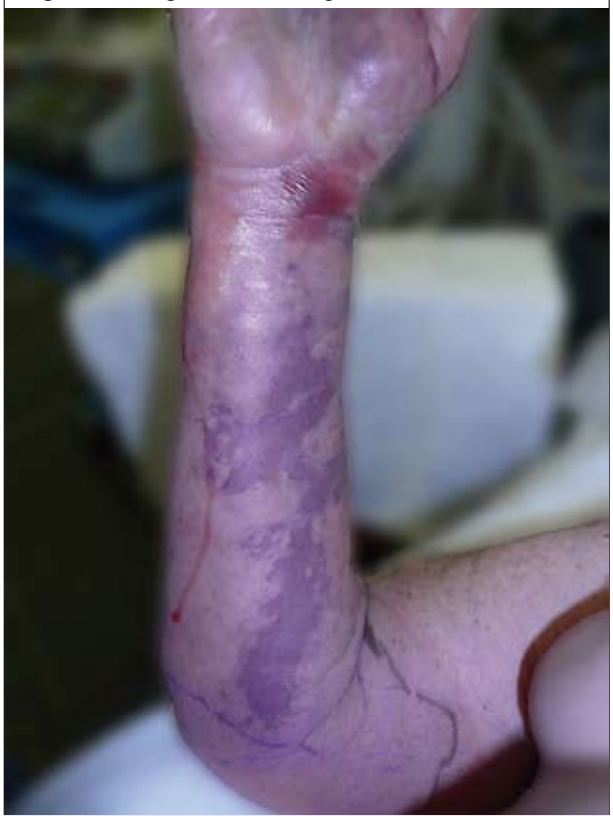


Figure 2. *Progressive swelling*



WHAT IS YOUR DIAGNOSIS?

See page 112 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 111)

A WOMAN WITH A PAINFUL AND SWOLLEN HAND

DIAGNOSIS

The combination of acute severe pain, evident progressive swelling within a few hours, purple colouring of the right hand and forearm and the typical blister at the wrist suspicious for luminescent necrosis (*figure 2*) was suspected to be caused by necrotising fasciitis. Therefore, penicillin and clindamycin intravenously were added to the initially administered ceftriaxone. Although a small surgical incision at admission had not revealed necrosis, a more extensive exploration five hours later showed evident necrosis of the deep muscle loges of the forearm. Debridement was carried out. However, guillotine amputation appeared inevitable nine hours later. The patient died 27 hours after admission from intractable shock. Cultures from the fascia grew haemolytic streptococcus group A, which is the most commonly cultured pathogenic microorganism in necrotising fasciitis after *Staphylococcus aureus*.[1]

Necrotising soft-tissue infections (NSTI) include necrotising forms of cellulites and fasciitis. NSTIs can be divided in three types. Type I mixed infection is caused by aerobic and anaerobic bacteria occurring most commonly after surgical procedures. Type II is caused by group A β-haemolytic *streptococci*, primarily affecting the extremities. Type III is associated with *Vibrio vulnificus*, which enters the subcutaneous tissue via puncture wounds from fish or marine insects. NSTI is a rare infection of the subcutaneous tissue and fascia with a high mortality rate of approximately 20 to 60%.

Establishing the diagnosis is not easy. Clinical findings suggestive for necrotising fasciitis include rapidly spreading oedema, numbness of overlying skin, severe pain out of proportion to skin findings, blister or bullae formation (which is rare in cellulitis and erysipelas), signs of toxic shock syndrome, mental status changes, and the presence of subcutaneous air, if caused by gas-producing organisms.[2] A necrotising infection of an extremity is usually the result of an injury, however in some cases like our patient, no primary cause can be found.[3]

Streptococci secrete exotoxins promoting the immune system's production of TNFα, and interleukins 1 and 6. Beta-haemolytic *streptococci* produce superantigens which activate CD4 T cells, which then activate complement and clotting factors, leading to shock and multiple organ failure.[2] Broad-spectrum antimicrobial therapy should be administered empirically as soon as possible, and should cover Gram-positive, Gram-negative and anaerobic organisms. When a group A streptococcal infection is suspected, addition of clindamycin is highly recommended because it is believed to inhibit exotoxin production. Intravenous immunoglobulins may be a useful additional treatment in type II group A streptococcal NSTI complicated by toxic shock syndrome. Definite treatment involves early and complete debridement of the infected tissue.[3] Delayed recognition, with consequent massive soft tissue loss and sepsis, remains a deadly pitfall in the management of necrotising fasciitis.

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PHOTO QUIZ

Ceftriaxone-associated biliary pseudolithiasis

M. Rienstra[1*], M.J.M. Bonten[2], C.A.J.M. Gaillard[1]

[1]Department of Internal Medicine, Meander Medical Centre, Amersfoort, the Netherlands,
[2]Department of Medical Microbiology, University Medical Centre, Utrecht, the Netherlands,
[*]corresponding author: tel.: +31 (0)33-850 50 50, fax: +31 (0)33-850 26 95, e-mail: M.Rienstra@meandermc.nl

INTRODUCTION

Ceftriaxone is a commonly used third-generation cephalosporin that has antimicrobial activity against many Gram-positive and Gram-negative organisms. Generally, ceftriaxone is a safe antibiotic. We describe a case of bilairy pseudolithiasis due to intravenous ceftriaxone therapy.

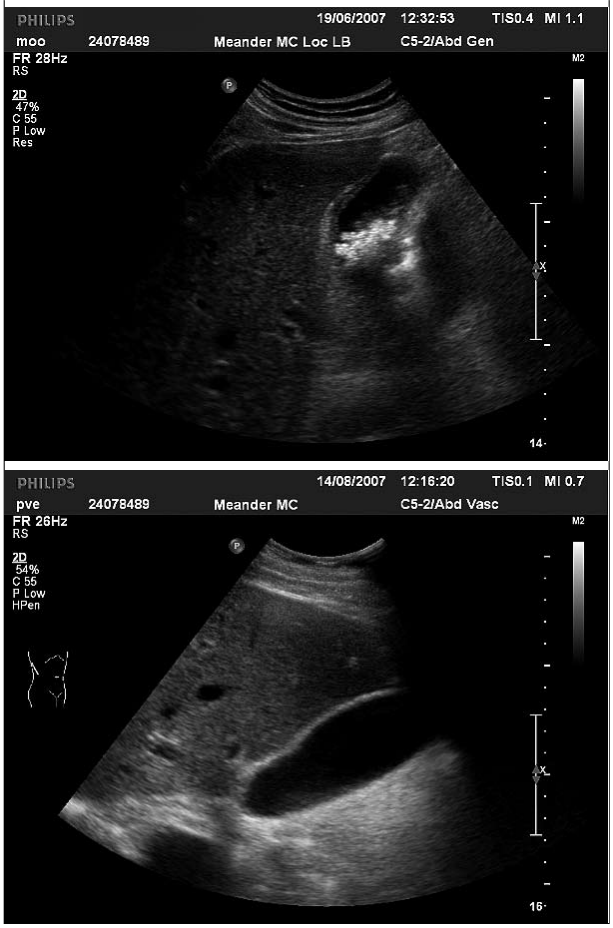
CASE REPORT

A 64-year-old man was admitted with relapsing fever after six weeks of antibiotic treatment for enterococcal aortitis. Eight months before, he underwent aortic root repair surgery for thoracic aorta aneurysm type A, i.e. aortic valve replacement with a bioprosthesis and supra-coronary aorta ascendens replacement.

Because of 1) failure of previous therapy, 2) high-level of aminoglycoside resistance, together with 3) high risk for nephrotoxicity with long-term aminoglycoside use, long-term (three months) therapy with amoxicillin, 2 g every 4 hours, plus ceftriaxone, 2 g every 12 hours was given through a peripherally inserted central catheter. After initiation, antibiotic therapy was continued on an outpatient basis. Every two weeks, the patient was seen at the outpatient clinic.

After three weeks, he developed acute abdominal pain, with elevated bilirubin and liver enzyme levels. Abdominal ultrasound showed multiple, low-echo concrements, but no signs of cholecystitis or choledocholithiasis (*figure 1A*). Previous imaging had been normal. After reduction of the dose and infusion rate of ceftriaxone (to 1 g every 24 hours) the abdominal complaints disappeared and subsequent abdominal ultrasound monitoring failed to demonstrated gall sludge or stones (*figure 1B*).

Figure 1. *Abdominal ultrasound imaging of the gallbladder, showing biliary pseudolithiasis during ceftriaxone treatment (A) and normal gallbladder after discontinuation of ceftriaxone treatment (B)*



WHAT IS YOUR DIAGNOSIS?

See page 114 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 113)

CEFTRIAXONE-ASSOCIATED BILIARY PSEUDOLITHIASIS

DIAGNOSIS

Recently, Gavalda *et al.* reported on the effective and safe combination of ampicillin plus ceftriaxone as treatment of enterococcal endocarditis.[1] In our patient, unfortunately, a side effect of ceftriaxone occurred, i.e. biliary pseudolithiasis, which diminished after reducing the dose. Ceftriaxone is mainly eliminated by the kidney, although 10 to 20% of the drug is eliminated in the bile. Ceftriaxone salt precipitates occur in 0.1 to 1% of all cases. *In vitro* analysis of the biliary precipitates induced by this agent showed the calcium salt of ceftriaxone. At doses ≥2 g, precipitation of ceftriaxone could occur.[2] Furthermore, a high infusion rate of ceftriaxone and impaired gallbladder emptying may also lead to high concentrations of ceftriaxone in the human bile and predispose to biliary pseudolithiasis. In rare cases, most of which have involved children, biliary pseudolithiasis led to abdominal pain, and resolved when ceftriaxone was discontinued.[3,4]

Although bilairy pseudolithiasis is a relatively rare complication of ceftriaxone therapy, clinicians need to be aware of this complication, since adequate monitoring of biliary pseudolithiasis and hyperbilirubinaemia is necessary.

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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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