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SPECIAL REPORT

A watershed for the Netherlands Journal of Medicine: open internet access

J.P.H. Drenth

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ABSTRACT

As of now, the *Netherlands Journal of Medicine* will become freely accessible online. This represents a turning point for the Journal as open access publishing provides instant and universal availability of published work to any potential reader, worldwide, completely free of subscriptions, passwords and charges. This assures the widest possible dissemination of scientific knowledge from the Journal without boundaries of limited circulation or local availability of a hardcopy. We believe that by opening up we are making research available to a much wider range of readers than our print and subscription model would have been able to achieve.

Now in its 47th year of publication, the Editorial board of the *Netherlands Journal of Medicine* has decided to steer the Journal into the electronic arena. As of now the content of the *Netherlands Journal of Medicine* will be freely accessible online. Immediately after publication of a paper, its abstract will appear on PubMed, (www.pubmed.gov) and from there the full text can immediately, without any obstacles, be viewed and downloaded. This event marks an important point in the history of the Journal and materialises our continuous efforts to make it better.

Over the past three years, the Editorial board has invested a great deal in improving the design and presentation of the Journal.[1,2] For example, probably one of the most tangible enhancements of the Journal is its art cover. We adopted the art cover because we believe that this promotes the visibility of the Journal, and gives the Journal its own personality. On the other hand, we have worked hard to improve its content and have attracted high-quality manuscripts from Dutch and foreign authors alike. As Editorial board we have chosen to adopt an active role and this has resulted in a steady increase in the number of high-calibre manuscripts submitted. For a large part, the Journal's content mirrors the research efforts of members of the Dutch Society of Internal Medicine and as such it provides unique insight into the contemporary biomedical research in our country. The Society has a vested interest in advancing clinical research and has an interest in making information as widely available as possible. The first interest of the Editorial board lies with the reader and accordingly, our mission statement indicates that the Journal also serves as a platform for (Dutch) young clinical investigators. We have instigated a yearly competition with prizes for the best original article, review and case report in the Journal.[3,4] We also started photo quizzes as an illustrative feature of the Journal.[5] We believe that this yearly contest positively contributes to the increasing quality of the Journal. Without any doubt, the hardcopy version of the Journal appeals to many of us. Most readers have indicated that they like the physical appearance and enjoy browsing through the pages to find snippets of knowledge that are useful to them. Although this is important to us, we must recognise that electronic journals offer readers a particular ease of access. Indeed, with open access publishing, many interests seem aligned, because we get what we want: both speed of publication and free access to important new findings for readers. Ideally, an electronic platform allows readers to work across different journals, find exactly where certain ideas are being discussed, or move readily from citation to source.

In order to provide open access the Editorial board went for a two-step strategy. First, we noted that it could take four to six months after the publication of a manuscript in the Journal before its citation was listed on the PubMed website (www.pubmed.gov). This was bothersome because most researchers take PubMed as an entry point for a literature search. Consequently, scientific knowledge from the Journal that was readily available only reached the scientific community some six months after publication. In close collaboration with the publisher of the Journal we have now closed the electronic gap, and papers are now instantly picked up by PubMed.

As an important second step, the *Netherlands Journal of Medicine* has adopted a dual-mode open access model which publishes an immediate and complete edition of its subscription-based print version online. The full text version was already directly available for members at the website of the Dutch Society of Internal Medicine (www.internisten.nl). From this issue onwards, it will be possible to access the full text version directly from the PubMed website. Each abstract on PubMed will be accompanied by the *Netherlands Journal of Medicine* logo and by clicking it, the full text of the paper can be downloaded as an Adobe Portable Document Format version. We believe that by opening up we are making research available to a much wider range of readers than our print and subscription model would have been able to achieve. We are now able to disseminate important clinical knowledge not bound by boundaries of circulation or by local availability of a hardcopy.

For the near future we foresee that open access will bring some benefits. We are convinced that our Journal will become more attractive to authors who want their results to be read and applied widely. We expect that, in line with experiences with other Journals, our efforts will not only result in a upward trend in manuscript submissions, but that these will also have a positive effect on the quality of the submissions. There is evidence available which indicates that free online papers have more impact than those published in conventional print-only journals. Actually, free online articles are cited earlier and more frequently because they are more easily available.[6]

As a final step we envisage the implementation of an online submission system. This tool allows electronic submission, aids in the editorial workflow and permits online peer review. We hope that this will enhance the efficiency of the editorial processing of manuscripts and, more importantly, will decrease time between submission and publication. We think that the future is ours, as publication in the Journal assures wide and immediate dissemination.

Finally, let us thank those of you who read, submit and review the *Netherlands Journal of Medicine* papers. You make the journal what it is today and the *Netherlands Journal of Medicine* is not our journal so much as yours. As we are continuously working to improve accessibility and quality of the Journal, we welcome your advice and encourage you to send us your comments.

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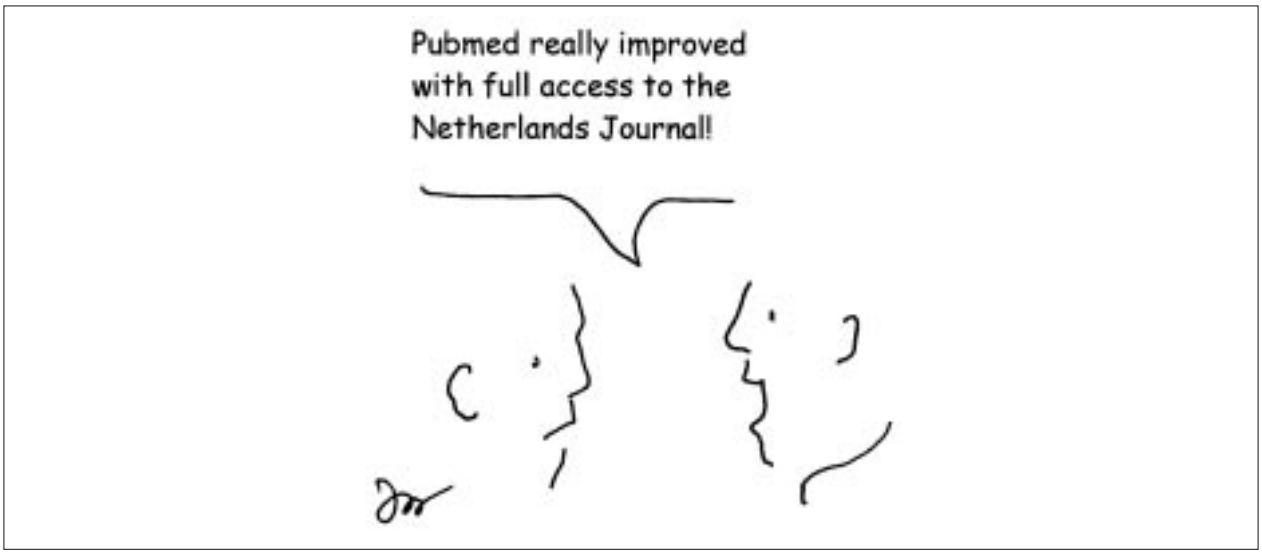

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SPECIAL REPORT

Submit your case as a photo quiz!

Editorial board, the Netherlands Journal of Medicine

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The *Netherlands Journal of Medicine* has a fine tradition in publishing articles for the Photo Quiz series. The quintessence of a good Photo Quiz is the presentation of a case that challenges the reader to make the diagnosis from visual clues in the accompanying image. Sometimes they illustrate an unusual disorder[1-3] or highlight a remarkable symptom or unusual presentation of an otherwise common disorder.[4-6] In either case they should be instructive and entice the reader to think about the possible diagnosis. The question section may state the history of the case and note the findings and the outcome, but it should not divulge the diagnosis. Ideally, this should be accompanied by no more than three high-quality figures with arrows marking their key elements. The question should be written in a single paragraph. The answer should give the diagnosis and outline the key teaching points. We think that these clinical cases in photo quiz form keep the Journal lively and interesting. The Editorial board of the *Netherlands Journal of Medicine* welcomes submission of photographs and material to the Photo Quiz series.

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ERRATUM

The review 'Nonalcoholic steatohepatitis' by P.L.M. Jansen published in Neth J Med 2004;62(7):217-24 has also been inadvertently published in Eur J Gastroenterol Hepatol 2004;16(11):1079-85. The author apologises for this error.

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EDITORIAL

Is this reaction caused by this drug?

W.L. Diemont†

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ABSTRACT

The case report on cotrimoxazole-induced pancreatitis by Versleijen *et al*. deals with the assessment of the probability that cotrimoxazole induced the acute pancreatitis: a causality assessment. In this editorial, we comment on this assessment from a clinical, pharmacological and epidemiological perspective. Moreover, the consequences of the results of the assessment are discussed.

KEYWORDS

Adverse drug reaction reporting systems, drug effects, pancreatitis, trimethoprim-sulphamethoxazole combination

The case report by Versleijen *et al.* on cotrimoxazole-induced pancreatitis deals with several important issues on drug safety and causality assessment.[1] An adverse drug reaction (ADR) is 'A response to a drug which is noxious and unintended, and which occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function'.[2] The wording 'a response to a drug' raises the question on causality: to what extent is the adverse event caused by the use of the drug? The answer to this question, reflecting causality assessment, requires a multidisciplinary approach from a clinical, pharmacological and epidemiological perspective.

From a clinical point of view, the temporal relationship between the use of sulphamethoxazole and the diagnosis of acute pancreatitis supports a causal relationship. The previous episode with similar epigastric pain during cotrimoxazole therapy, however, challenges causality, since recovery during ongoing use of cotrimoxazole is not very likely.

Another clinical aspect may be that a positive rechallenge strongly supports causality. However, pancreatitis may be a recurrent disease, and the recurrence of pancreatitis may be coincidentally related with the readministration of cotrimoxazole. The reason for the rechallenge, despite the strong suspicion that cotrimoxazole had caused an acute pancreatitis, raises questions. Were the physicians involved not aware of the suspicion, or was it an intended rechallenge due to the lack of an alternative antibiotic therapy?

From a pharmacological point of view, we agree with the authors that no pharmacologically plausible link exists between cotrimoxazole and pancreatitis. This implies that the pancreatitis is facilitated by traits of the patient and not by (known) pharmacological traits of the drug. Such patient-related ADRs are called type B reactions and characterised by the lack of a dose-effect relationship, very low incidence but usually severe in nature, such as anaphylactic reactions.[3] Another pharmacological issue is that trimethoprim and sulphamethoxazole both inhibit cytochrome P450 isoenzymes, such as 2C8 and 2C9.[4,5] If concomitant medication had been involved, such inhibition could have caused an increase in serum levels of co-medications that are metabolised by these enzymes.

From an epidemiological point of view, causality assessment involves the use of (pharmacovigilance) databases. In general the Netherlands Pharmacovigilance Centre Lareb

usually uses the pharmacovigilance database of the WHO collaborating centre in Uppsala, Sweden, involving spontaneous reporting systems of more than 75 countries and with more than 3 million reports. The association between trimethoprim and/or sulphamethoxazole and pancreatitis has not been reported statistically more or less frequently than all the other associations in the entire database (reporting odds ratio 0.88 (CI 95: 0.73-1.02)). This implies that all over the world reporters of ADRs have an 'average' concern about this association as compared with their concern about all other reported associations.
Similar calculations could be made in the database with regard to the suggestion by the authors that ulphonamide hypersensitivity could be involved in the described association. Nevertheless, it should be emphasised that such calculations do not prove causality but express the concerns of reporters of ADRs. Moreover, epidemiological studies in such databases require a proper assessment of quality and completeness of the involved reports, as illustrated by a study on drug-induced acute pancreatitis in the Netherlands.[6] In this series, the time between the first intake of the suspect drug and the onset of acute pancreatitis varies between four hours (jectrolan as contrast agent during an endoscopic retrograde cholangiopancreatography (ERCP)) and two years (captopril), but the median latency time was about 15 days. This challenges the causal relationship in the described case report because of its latency period of many years.

Besides these three aspects, other sources may support causality as well. The presence of pancreatitis in the summary of product characteristics (SPC) of the involved drugs, available via www.cbg-meb.nl > medicines data bank,[7] at least implies that the causality of the association was worth mentioning. The SPCs of trimethoprim does not mention pancreatitis as ADR, in contrast to the SPCs of the combined formulation of trimethoprim with sulfamethoxazole. Moreover, previous publications on associations, as pointed out by the authors, support such an association as well.

Concluding that the reported association between cotrimoxazole and pancreatitis has sufficient body of evidence to be considered as having a 'probable' or even 'certain' causal relationship,[2] what should the consequence be for clinical practice in this patient?
First, it should not be considered a reason to withhold cotrimoxazole to a patient who needs it. Adverse effects of type B seldom occur and are unpredictable. The database of the Netherlands Pharmacovigilance Centre contains one report (out of more than 50,000 reports received between 1987 and 2005) of acute pancreatitis in association with trimethoprim and none in association with cotrimoxazole. The number of patients per year with a prescription with a combination of sulphonamide and trimethoprim between 2000 and 2004 is about 179,556.[8] Although, due to underreporting, no incidence figures should be calculated from a spontaneous reporting system, one may conclude, also based on the sparse reports in literature, that the described association is rare.
Second, the purpose of a published case report is to point to the existence of a possible association between a drug and an ADR. Its effect is not only limited to the association that has been described. It is a constant reminder to the physician that the existence of a possible ADR should be part of every differential diagnosis. Patients, physicians, dentists and pharmacists should be informed about this type B ADR, since unintended re-exposure may have a dramatic sequel. An intended re-exposure, e.g. due to the lack of alternative medications with a vital indication, should be done only under close medical supervision under well-equipped conditions.
Finally, the case report is a trigger to remind physicians treating a patient with acute pancreatitis that drugs may be involved in the aetiology and should be withhold as much as possible.

NOTE

Willem L. Diemont unexpectedly died on 25 June 2005 in Berlin while on his way to a clinical pharmacology congress in Poznan, Poland. Willem Diemont was a specialist in internal medicine and head of the unit Adverse Event Notifications of the Netherlands Pharmacovigilance Centre Lareb, which he joined in 1997.
Correspondence according to this editorial can be mailed to E.P. van Puijenbroek, e-mail: e.vanpuijenbroek@lareb.nl.

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REVIEW

Mastocytosis and adverse reactions to biogenic amines and histamine-releasing foods: what is the evidence?

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ABSTRACT

Background: It has been suggested that normal concentrations of biogenic amines and 'histamine-releasing foods' may exacerbate symptoms in mastocytosis. The purpose of this study was to look for scientific evidence in the literature on diets restricted in biogenic amines and histamine-releasing foods in the treatment of mastocytosis.
Methods: Medline (1966 to 2004), Cinahl (1982 to 2004) and the Cochraine Library were searched for double-blind placebo-controlled food challenge (DBPCFC) studies with biogenic amines and/or histamine-releasing foods in mastocytosis.
Results: No studies employing DBPCFC with dietary biogenic amines or histamine-releasing foods in mastocytosis were found. Only a few *in vitro* studies in other diseases, animal studies and studies in humans in which histamine-releasing agents were incubated directly with duodenal tissues were found. One case was reported of severe adverse reactions to alcohol in mastocytosis, objectified by an open challenge.
Conclusion: Despite the widespread belief that biogenic amines and histamine-releasing foods may cause allergy-like, non-IgE-mediated symptoms in certain patients, the role of diets restricted in biogenic amines and histamine-releasing foods in the treatment of mastosytosis remains hypothetical but worthy of further investigation. There is some evidence for adverse reactions to alcohol in mastocytosis.

KEYWORDS

Adverse reactions, biogenic amines, double-blind placebo-controlled food challenge, histamine-releasing foods, mastocytosis

INTRODUCTION

Mastocytosis is an uncommon disorder characterised by the accumulation of mast cells in various tissues including bone marrow in the systemic form of the disease. The essential role of mast cells in allergic diseases was recognised a long time ago but the physiological role of mast cells is still unclear. However, there is substantial evidence that mast cells may play a key role in acquired and innate immune responses in host defences against micro-organisms[1,2] and in wound healing and angiogenesis.[3] Besides the well-known triggering of degranulation of mast cell cells by crosslinking of the FcεRI receptor by specific allergens, several other stimuli such as neuropeptides (substance P), complement factors (C3a, C5a), lipoproteins, adenosine and superoxides may activate mast cells.[3]
It has been suggested that foods containing high but nontoxic levels of biogenic amines and the 'histamine-releasing foods' may exacerbate symptoms in mastocytosis. Alcohol is also considered to be a triggering factor in mast cell degranulation.[4] Therefore, it has been suggested that diets restricted in biogenic amines or histamine-releasing foods may be beneficial for some patients with systemic mastocytosis. However, many authorities do not mention diet intervention as a common therapeutic measure,[5] whereas others suggest certain foods to be a possible trigger, but only in certain patients.[4]

The purpose of this study was to verify in the literature if there is any evidence for the putative beneficial effects of elimination diets restricted in biogenic amines or histamine-releasing foods.

Biogenic amines

Biogenic amines are normal constituents of many foods. These low-molecular-weight organic bases are formed in plants and animals, and also endogenously in the human body. Biogenic amines arise in foods by enzymatic decarboxylation of free amino acids.[6] Biogenic amines are divided into monoamines, such as tyramine, serotonin, phenylethylamine, dopamine, epinephrine and norepinephrine, and diamines, such as histamine, cadaverine and putrescine. Adverse reactions to foods are most frequently reported in foods containing high levels of tyramine and histamine, which is referred to as histamine poisoning or scrombroid poisoning.[7] This intoxication is caused by high levels of histamine, for example in spoiled scrombroid fish, such as tuna and mackerel. Tyramine and histamine are formed by decarboxylation of tyrosine and histidine respectively, both amino acids.[6,7]

Endogenously synthesised biogenic amines fulfil important metabolic functions in the body. Histamine plays a role in normal and abnormal biological processes, including vasodilatation, gastric acid secretion and allergic reactions. In contrast to histamine, tyramine is vasoconstrictive and may cause an increase in blood pressure.

Generally, monoamine oxidases (MAO A and MAO B) and diamine oxidase (DAO) play a major role in the catabolism of monoamines and diamines, respectively. These enzymes are located in the gastrointestinal tract, and also in the liver, lung, platelets, spleen and kidneys. These enzymes prevent the absorption of unmetabolised biogenic amines into the circulation. The metabolism of histamine is depicted in *figure 1*. There are two main enzymatic pathways:

- Histamine is converted by histamine methyltransferase (HMT) to methylhistamine (MH), and subsequently metabolised by MAO to methylimidazole acetic acid (MIMA) or
- histamine is deaminated by DAO to imidazole acetic acid (IAA).[8]

Dietary biogenic amines are especially found in aged and fermented food, and in foods containing relatively high amounts of free amino acids.[9] Most notably, ripened cheese, fermented meat, smoked and tinned fish, sauerkraut and fermented yeast and fermented soy products usually contain significant amounts of histamine. Concentrations of histamine may vary considerably and a daily dietary intake of histamine of 100 to 200 mg can easily be reached, depending on the foods chosen. In *tables 1* and *2*, the

Table 1 *Foods rich in histamine*

Food categories	Mg histamine/100 gram	Mg histamine/serving
Cheese: Gouda, Cheddar, Danish Bleu, Emmenthaler, goats cheese, Gorgonzola, Mascarpone, Parmesan	3.3-171	0.7-35 (20 gram)
Meat: fermented meat, hare, (dry) sausage, raw ham	3.0- 27	0.6-5.5 (20 gram)
Fish: herring, smoked mackerel, tinned fish (sardines), tuna fish,	0.8-16.5	0.6-11.5 (70 gram)
anchovy products	95-344	9.5-34 (10 gram)
Vegetables: egg plant, spinach, sauerkraut	2.5-11.5	5-23 (200 gram)
Alcoholics: beer and wine	0.6-1.6	0.6-1.6 (100 ml)
		1.2-2.2 (200 ml)
Fermented foods: Tamari, marmite, trassi, tempe	8.3-212	0.8-21 (10 gram)

Table 2 *Foods rich in tyramine*

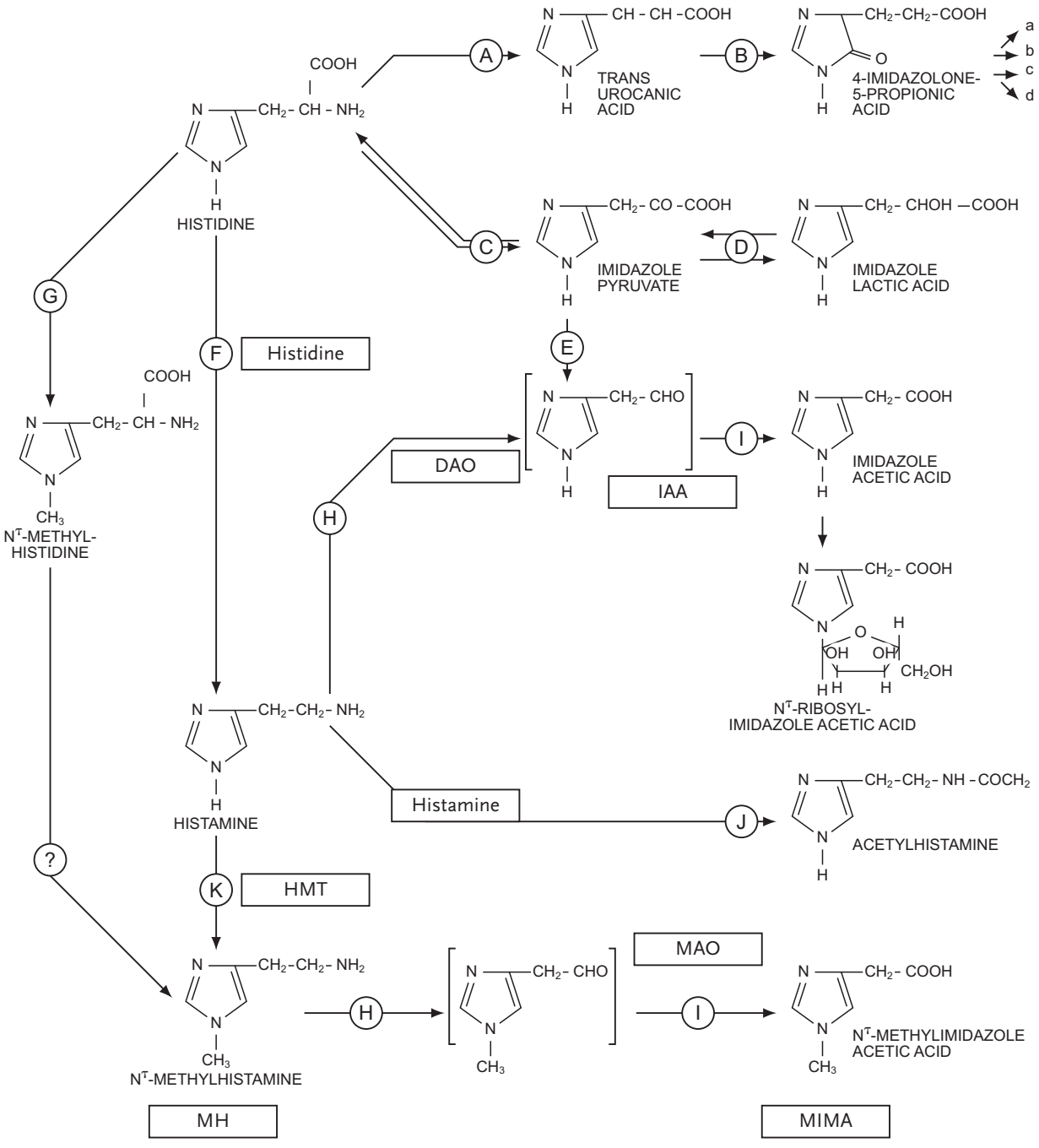
Food categories	Mg tyramine/100 gram	Mg tyramine/serving
Cheese: most cheeses	5.0-152	1.0-31 (20 gram)
Chocolate	0.8	0.2 (25 gram)
Meat: fermented meat, hare, (dry) sausage, raw ham	8.5-56	1.7-11 (20 gram)
Fish: smoked fish, tinned fish (sardines, tuna), shrimps	2.5-12.9	1.8-9 (70 gram)
anchovy products	6.4-13	0.6-1.3 (10 gram)
Vegetables: egg plant, spinach, sauerkraut	6.1-16.5	12.2-33 (200 gram)
Alcoholics: beer and wine	0.5-3.6	0.5-3.6 (100 ml)
		1.0-7.2 (200 ml)
Fermented foods: soy sauce, marmite, trassi, tempe	15-178	1.5-18 (10 gram)

respective amounts of histamine and tyramine in mg per serving in food categories rich in biogenic amines are shown. In *table 3* some foods low in histamine in these food categories are shown.

Histamine-releasing foods

Many foods or food components are considered to have the capacity to release histamine directly from tissue mast cells in the body.[9] These foods are listed in *table 4*.

Figure 1 *Metabolism of histidine and histamine*[8]



DAO = diamine oxidase (or histaminase); IAA = imidazole acetic acid; HMT = histamine methyltransferase; MAO = monoamine oxidase; MH = methylhistamine; MIMA = methylimidiazole acetic acid.

Table 3 *Exceptions: Foods low in histamine*

Food categories	Mg histamine/100 gram
Cheese: Edam, Brie, Camembert, Feta, Mozzarella	0.0-0.3
Fish: fresh/frozen fish (codfish, salmon, sole, whiting, place, crab, mussels)	0.0-0.1
Chocolate	0.1
Fermented foods: tofu	0.1

Table 4 *Foods with suggested histamine-releasing capacities*

Additives	Alcohol	Chocolate
Citrus fruit	Crustaceans	Egg white
Fish	Liquorice	Nuts
Papaya	Peanuts	Pineapple
Pork	Spices	Spinach
Strawberries	Tomatoes	

MATERIALS AND METHODS

Medline (1966-2004), Cinahl (1982-2004) and the Cochraine Library were searched in the English and German language for clinical studies on adverse reactions to biogenic amines and histamine-releasing foods in mastocytosis. Clinical studies were defined as studies employing dietary elimination followed by DBPCFC studies with biogenic amines or histamine-releasing foods in human beings with suspected or confirmed mastocytosis. For studies on biogenic amines the keywords (biogenic amin* or histamin* or methylhistamin*) were combined with (mastocytosis or systemic mastocytosis or mast cell disease or urticaria pigmentosa) and (food or diet or hypersens* or adverse or intolerant or intolerance or allerg* or toxicity or nonIgE). For studies on histamine-releasing effects of foods the keywords (histamine releas* or releas*) were combined with (mastocytosis or systemic mastocytosis or mast cell disease or urticaria pigmentosa) and (food or diet or hypersens* or adverse or intolerant or intolerance or allerg* or toxicity or nonIgE).

RESULTS

No clinical studies employing DBPCFC were found on (adverse) effects of dietary biogenic amines in mastocytosis, or on (adverse) effects to histamine-releasing foods. Only one study employing an open food challenge with histamine rich foods was found.[10] In this study Vidal and others described a patient with a rare form of cutaneous mastocytosis (telangiectasia macularis eruptiva perstans) suspected to suffer from adverse reaction to fish, but in whom open food challenges with fresh crayfish, shrimp and tuna were negative.

Studies in human beings on the putative histamine-releasing effects of foods are limited to reports of such effects on *ex vivo* duodenal tissue performed by Moneret-Vautrin and coworkers.[11] They showed that incubating duodenal biopsy material with various histamine-releasing pharmacological agents, such as compound 48/80, Concanavalin A, the calcium ionophore A 23187, and anti-IgE resulted in increased nonspecific release of histamine by duodenal mast cells. They demonstrated that in subjects with non-IgE-mediated adverse reactions to food, duodenal mucosal biopsies show massively degranulated cells in sharp contrast to those seen in controls.

According to these authors, these findings support the hypothesis that the digestive mucosal mast cells may be abnormally sensitive to histamine liberation in some subjects.[12] However, they showed no *in vivo* data to support the relevance of these findings. In addition, we found no clinical studies using oral challenge tests to support the hypothesis for the histamine-releasing capacity of foods. Only a few *in vitro* studies and animal studies in other diseases have demonstrated histamine-releasing effects of foods or food components, but no literature on this subject was found dating from the last two decades. Schachter and Talesnik found in 1952 that egg white releases histamine in nonsensitised animals when injected intravenously.[13] In this article the authors refer to unpublished data by Schachter on histamine-releasing effects of strawberries and to a publication on histamine liberation by shellfish, published by WDM Paton in 1954 in J Physiol 1954;123:58P, and by E. Urbach in Allergy 1946, Heineman, London. We were not able to obtain a copy of these old articles to verify their conclusions. In some studies, it was demonstrated *in vitro* that in both chronic urticaria patients and controls, food additives were able to release histamine from leucocytes.[14,15] However, no differences between patients and controls were found. Baenkler and coworkers conducted *in vitro* tests with wheat, egg, milk and fish on the mucosa of patients suffering from several intestinal diseases, including patients suffering from food intolerance and inflammatory diseases.[16] In all these patients skin tests with these foods were negative. Significant differences were found in histamine-release between patient groups. Surprisingly, the smallest amount of histamine release was found in the food-intolerant patients, the largest amount in the inflammatory group. We could not find any study on histamine-releasing effects of most of the foods suggested of having histamine-releasing capacities, as shown in *table 4*.

In one case, adverse effects to alcohol in mastocytosis were reported. Bandmann *et al.* described a patient with anaphylactic-like reactions to alcohol, objectified by open challenge.[17] This patient reacted to 20 ml of cognac with flush, tachycardia, severe headache and diarrhoea.

DISCUSSION

Despite the widespread belief that biogenic amines and histamine-releasing foods may cause allergy-like, non-IgE-mediated symptoms in certain patients, our literature search failed to identify any clinical study in patients with mastocytosis employing DBPCFC to demonstrate putative adverse effects of these foods and food components.
Several hypotheses have been formulated to explain such putative adverse reactions to normal amounts of biogenic amines, although these hypotheses have not been put forward in relation to mastocytosis. Firstly, a decreased level of DAO is said to be a cause of intolerance to biogenic amines. Some studies showed a decreased level of DAO in patients with atopic dermatitis and chronic urticaria.[18-21] Secondly, inhibiting effects of substances on DAO, MAO or HMT might be other causes for intolerance to biogenic amines. Many inhibitors of MAO, DAO or HMT in food have been identified.[7] For example, other biogenic amines such as tyramine might inhibit these enzymes and could theoretically potentiate histamine intoxication. Furthermore, there have been numerous reports on hypertensive crises in patients using MAO-inhibiting drugs[22] in combination with foods rich in tyramine. Remarkably, there are no data available on the hazardous effect of MAO-inhibiting drugs and histamine release in patients with mastocytosis. Thirdly, the barrier disruptive hypothesis has been described by Taylor.[7] This hypothesis holds that several potentiators might interfere with the protective function of intestinal mucin. These potentiators, such as cadaverine and putrescine (other biogenic amines), would bind to mucine and in this way facilitate an increased absorption of histamine. There is a lack of evidence supporting these putative mechanisms and no clinical studies suggesting that these mechanisms might be operative.
A recent literature survey from 1966 to 2001 by Jansen *et al.* on adverse reactions to biogenic amines showed no supportive evidence for the relation to migraine, headache or wine intolerance. In relation to chronic urticaria no methodologically sound studies were found.[23] In one DBPCFC study in healthy volunteers,[24] symptoms such as headache, dizziness and discomfort in 11 out of 54 challenges were found after 5 mg phenylethylamine, but not after 25 mg histamine or 25 mg tyramine.
There is no convincing evidence that patients with mastocytosis have increased releasability of mast cells as compared with normal mast cells. It is generally thought that symptoms resulting from mediator release are due to high mast cell load,[4,5] rather than to increased releasability. Furthermore, there are no DBPCFC studies in human beings supporting the widely held belief that foods should have histamine-releasing capacity. The hypothesis that foods may have a histamine-releasing capacity is based on several older *in vitro* studies, animal studies in other diseases demonstrating histamine-releasing effects of foods, and on studies in which pharmacological substances were incubated directly with digestive tract mucosal tissues. Thus, the normal digestive influences on foods are eliminated and the significance of these findings is doubtful. The Committee of Adverse Reactions to Foods of the American Academy of Allergy and Immunology[25] concluded in 1984 that the effects of such foods are 'unproven'. Taken together, the theories on adverse reactions to normal amounts of biogenic amines and the histamine-releasing capacities of foods in patients with mastocytosis are unproven. However, the persistence of these theories on adverse reactions to biogenic amines and the histamine-releasing capacity of foods suggest that further investigation is necessary to confirm or reject the putative effects of these food substances.
Adverse reactions to alcohol may be relevant in some patients with mastocytosis, although the prevalence of this phenomenon is unknown.[17] The explanation for the documented anaphylactic reaction to alcohol in mastocytosis can be found in similar metabolic pathways for alcohol and histamine;[5,26] alcohol and acetaldehyde inhibit DAO, which results in elevated histamine levels. Furthermore, acetaldehyde has shown to have a degranulating and histamine-releasing effect on mast cells.[26-28]
Elevated concentrations of histamine metabolites are found in patients with mastocytosis. Although measurement of serum tryptase is currently being used in diagnosing mastocytosis,[4,5] in some centres the measurement of urinary excretion of the histamine metabolites MIMA and MH is used as an additional biochemical marker for mastocytosis. These metabolites are formed endogenously, but also dietary intake enhances the urinary excretion of these amines.[6-8,29] Furthermore, Keyzer and others found that a diet high in protein (138 g) with exclusion of foods that may be rich in histamine significantly enhances the amount of MH.[29] This is caused by high levels of tyramine in diets rich in protein. Therefore, dietary restrictions in histamine and protein intake before urinary sampling of histamine metabolites are justified.
In conclusion, a review of the literature provided no sound scientific support for beneficial effects of diets restricted in biogenic amines and histamine-releasing foods in patients with mastocytosis. Therefore, the role of these diets in the treatment of mastocytosis remains hypothetical. However, systematic studies have not been carried out and further investigation is warranted. Such studies should

confirm putative adverse effects of these foods and food components by DBPCFC. Although there is little evidence incriminating alcohol as a cause of adverse reactions in patients with systemic mastocytosis, caution is probably warranted until more studies have been done.

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NOTE

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ORIGINAL ARTICLE

Anti-inflammatory effects of troglitazone in nondiabetic obese subjects independent of changes in insulin sensitivity

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ABSTRACT

Background: Obesity is characterised by insulin resistance and by elevated levels of proinflammatory markers. We investigated whether, in the absence of changes in glucose, thiazolidinediones (TZDs) have anti-inflammatory effects and whether improvement of insulin sensitivity correlates with suppression of inflammatory markers.

Methods: We performed a randomised double-blind placebo-controlled crossover study with troglitazone (400 mg daily for eight weeks) in 15 normoglycaemic obese subjects. We measured plasma high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), leptin, tissue-type plasminogen activator (tPA), plasminogen activator inhibitor-1 (PAI-1) and tumour necrosis factor-α (TNF-α) after each of the two treatment periods and in 13 age- and sex-matched lean individuals.

Results: Obese subjects were insulin resistant (decreased glucose infusion rate (GIR) during euglycaemic hyperinsulinaemic clamp) and had higher plasma levels of hsCRP, IL-6, leptin, tPA, and PAI-1 compared with lean subjects. TNF-α also tended to be higher. Troglitazone improved insulin sensitivity (mean increase in whole body glucose uptake 23.1 ± 10.5% (p=0.047)) and normalised plasma concentrations of hsCRP, tPA and TNF-α, whereas it did not significantly change IL-6, leptin and PAI-1. Changes in GIR did not correlate with changes in inflammatory markers.

Conclusion: Troglitazone induces suppression of some of the inflammatory markers that are elevated in normoglycaemic obese subjects. The suppression of inflammatory markers, however, does not correlate with improvement in insulin sensitivity, suggesting involvement of partially differential mechanisms in these effects of TZDs.

KEYWORDS

Inflammation, insulin sensitivity, obesity, thiazolidinediones, trial

INTRODUCTION

Insulin resistance, which is a common feature of obesity, appears to be central to the pathogenesis of type 2 diabetes.[1-3] Insulin resistance is hypothesised to develop in obesity mainly as a result of adverse effects of elevated plasma levels of free fatty acids (FFA).[4,5] FFA or their metabolites can impair insulin action and inhibit glucose transport activity by stimulation of protein kinase C isoforms. Furthermore, by increasing the level of oxidative stress, FFA activate stress-sensitive signalling pathways such as the nuclear transcription factor κB (NFκB) pathway, which plays an important role in inflammation.[6] In addition to FFA, chemical messengers synthesised and secreted by adipocytes, such as tumour necrosis factor-α (TNF-α) and leptin, may influence insulin sensitivity.[1,4]

Recent studies have indeed confirmed a role for inflammation in the pathogenesis of insulin resistance. Plasma concentrations of the inflammatory mediators interleukin-6 (IL-6), TNF-α and TNF-receptor and plasminogen activator inhibitor-1 (PAI-1) were elevated in the obese.[7-12] Moreover, some markers of subclinical vascular inflammation, in particular high-sensitivity C-reactive protein (hsCRP) and IL-6, correlated with insulin sensitivity[7,8,13,14] and were shown to be powerful independent predictors of development of type 2 diabetes.[15,16]

**P. Smits and A.F.H. Stalenhoef were not involved in the handling and review process of this paper.

Thiazolidinediones (TZDs), peroxisome proliferator-activated receptor-gamma (PPAR-γ) agonists, improve insulin sensitivity.[17-19] PPAR-γ regulates the transcription and expression of specific genes that affect fat in adipose tissue, skeletal muscle and the liver.[17] TZDs induce a redistribution of fat out of skeletal muscle and liver into peripheral adipocytes[20-22] resulting in a sustained decrease in plasma FFA[23,24] and hence an improvement in insulin sensitivity and a reduced activation of stress-sensitive signalling pathways. Activation of PPAR-γ receptors may also directly contribute to reduction of chronic subclinical inflammation.[25,26] Several studies have shown that TZDs reduce NFκB activation and decrease elevated levels of PAI-1 and hsCRP, but most of these studies were performed in subjects with diabetes.[27-31] However, it is uncertain whether a decrease in plasma glucose levels contributes to the decreases in inflammatory parameters. Therefore, we investigated effects of troglitazone on plasma levels of hsCRP, IL-6, leptin, tissue-type plasminogen activator (tPA), PAI-1 and TNF-α in nondiabetic obese subjects characterised by insulin resistance.

MATERIALS AND METHODS

Subjects

The study groups consisted of 15 obese and 13 lean normotensive, healthy volunteers on no medication. Inclusion criteria were age between 25 and 50 years, nonsmokers, normal fasting glucose concentration, stable body weight, and a body mass index (BMI) between 27 and 36 kg/m^2 for the obese and between 19 and 25 kg/m^2 for the lean. All gave written informed consent. The experimental protocol was approved by the hospital ethics committee.

Protocol

After inclusion, the obese subjects received either troglitazone (2x200 mg daily) or placebo for eight weeks in a randomised double-blind crossover design. Participants were strictly advised to maintain their weight and not to change their diet. At the end of each of the two treatment periods and after an overnight fast, 30 ml of blood was collected in EDTA-containing tubes and plasma was isolated by centrifugation at 4°C and stored in aliquots at -80°C. Subsequently, a euglycaemic-hyperinsulinaemic clamp (insulin [Actrapid; Novo-Nordisc], infusion rate 430 pmol x m^{2}x min^{-1} [60 mU/dl/min]) was performed for 120 minutes. Body weight, waist-hip measurements, ECG, fat skinfold thickness, possible side effects, and serum chemical and haematological profiles were determined as a safety precaution. Between the treatment periods there was a two-week washout phase. Compliance was monitored by pill counts and amounted to over 90%.

Analytical measurements

Plasma glucose was measured by the glucose oxidation method (Beckman Glucose Analyser 2; Beckman Instruments, Fullerton, CA, USA). Plasma insulin was measured with a double antibody radioimmunoassay (interassay coefficient of variation 6.2%). FFAs (nonesterified fatty acids, NEFAs) were analysed with an enzymatic method (ACS-ACOD, NEFA C-kit; Waco, Neuss, Germany). Cholesterol and triglycerides were determined by enzymatic methods (Boehringer-Mannheim, Mannheim, Germany) on a Hitachi 747 analyser (Hitachi, Tokyo, Japan). HsCRP[32] was measured by an ELISA from Kordia (Leiden, the Netherlands); serum leptin by a RIA kit from Linco Research Inc. (St. Louis, MO, USA); IL6[33] by the Pelikine Compact human IL6 ELISA kit from CLB (Amsterdam, the Netherlands); and TNFα, tPA and PAI-1 were determined by specific ELISAs as described elsewhere.[34]

Statistical analysis

Data are presented as mean ± SE and evaluated by using Student's t-test for paired and unpaired data. Linear regression analysis was performed to examine the relationship between (changes in) variables. Significance was set at a p value of less than 0.05. The computer programme ASTUTE (Microsoft Ink, Redmond, WA, USA) was used for the analysis.

RESULTS

Characteristics of the two study groups are presented in *table 1*. The groups were properly matched for age and sex. Fat percentage, waist-hip ratio, fasting plasma insulin, triglycerides and NEFAs, but not fasting glucose and cholesterol, were significantly higher in the obese than in the lean control subjects. The obese had higher diastolic blood pressure than the lean; no difference was seen for systolic blood pressure. Compared with the values for insulin sensitivity of lean subjects, the obese were clearly insulin resistant: whole-body glucose uptake during euglycaemic hyperinsulinaemic clamp was significantly lower in the obese than in the lean control subjects. Insulin sensitivity (i.e. glucose infusion rate (GIR) during euglycaemic hyperinsulinaemic clamp) correlated strongly with BMI (r=-0.67, n=26, p=0.0002).

Plasma levels of hsCRP, IL-6, leptin, tPA, and PAI-1 were significantly higher in the obese compared with the lean group (*table 2*). Plasma level of TNF-α tended to be higher in the obese subjects, but this difference did not attain statistical significance (*table 2*). HsCRP, IL-6 and leptin were strongly associated with BMI (r=0.53, 0.48, and 0.70, respectively) and with GIR (r=-0.51, -0.55, and -0.57, respectively; *figure 1*). TPA was weakly associated with

Table 1 *Characteristics of study groups*

	Lean	Obese	P value
Number (m/f)	13 (7/6)	15 (9/6)	
Age (year)	38.3 ± 2.1	37.4 ± 1.2	NS
BMI (kg/m²)	21.9 ± 0.6	31.7 ± 0.8	<0.001
Fat (%)	22.6 ± 1.7	35.8 ± 1.8	<0.001
Waist/hip	0.86 ± 0.02	1.01 ± 0.02	<0.001
Total cholesterol (mmol/l)	4.61 ± 0.27	4.91 ± 0.26	NS
Triglycerides (mmol/l)	0.82 ± 0.10	2.11 ± 0.59	0.05
NEFAs (mmol/l)	0.38 ± 0.05	0.65 ± 0.08	0.01
Glucose (mmol/l)	5.2 ± 0.1	5.5 ± 0.1	NS
Insulin (pmol/l)	40.9 ± 1.4	85.6 ± 2.1	<0.001
GIR (μmol/kg/min)	53.9 ± 4.3	27.0 ± 2.9	<0.001
Systolic BP (mmHg)	118 ± 3	123 ± 3	NS
Diastolic BP (mmHg)	73 ± 4	82 ± 2	<0.05

BMI = body mass index; NEFAs = nonesterified fatty acids; GIR = glucose infusion rate; BP = blood pressure; NS = not significant.

Table 2 *Plasma concentrations of markers of inflammation in lean subjects and in obese subjects treated with placebo or troglitazone*

	Lean (n=13)	Obese (n=15)	
		Placebo*	Troglitazone*/†
HsCRP (mg/l)	1.06 ± 0.32	4.53 ± 1.28‡	2.23 ± 0.61ns/‡
TNF-α (ng/l)	3.37 ± 0.39	4.54 ± 0.51ns	3.67 ± 0.49ns/§
IL-6 (ng/l)	1.08 ± 0.15	1.60 ± 0.13‡	1.57 ± 0.19‡/ns
Leptin (μg/l)	6.0 ± 1.4	17.2 ± 3.2§	16.5 ± 3.3§/ns
tPA (μg/l)	1.02 ± 0.10	1.58 ± 0.13§	1.15 ± 0.12ns/§
PAI-1 (μg/l)	9.2 ± 1.0	19.5 ± 3.0§	17.2 ± 2.3§/ns

HsCRP = high-sensitivity C-reactive protein; TNF-α = tumour necrosis factor-α; IL-6 = interleukin 6; tPA = tissue-type plasminogen activator; PAI-1 = plasminogen activator inhibitor-1. *T-test *vs* values of lean control subjects; †paired t-test *vs* values of placebo-treated obese subjects; ‡p<0.05; §p<0.01; ns = not significant.

GIR (r=0.44) but not with BMI. TNF-α was not associated with GIR but correlated weakly with BMI (r=0.40). PAI-1 was not associated with GIR or BMI.

Troglitazone was well tolerated. No changes were observed for body weight, plasma triglycerides, total cholesterol, and fasting plasma glucose concentration (data not shown). A nonsignificant decrease was observed for plasma NEFAs (from 0.65 ± 0.08 to 0.48 ± 0.06 mmol/l, p=0.08). Fasting insulin concentrations tended to decrease but remained elevated compared with those in the lean control subjects (data not shown). Insulin sensitivity improved, as evidenced by increased whole-body glucose uptake during euglycaemic hyperinsulinaemic clamp: mean percentage increase in whole body glucose uptake amounted to 23.1 ± 10.5% (p=0.047).

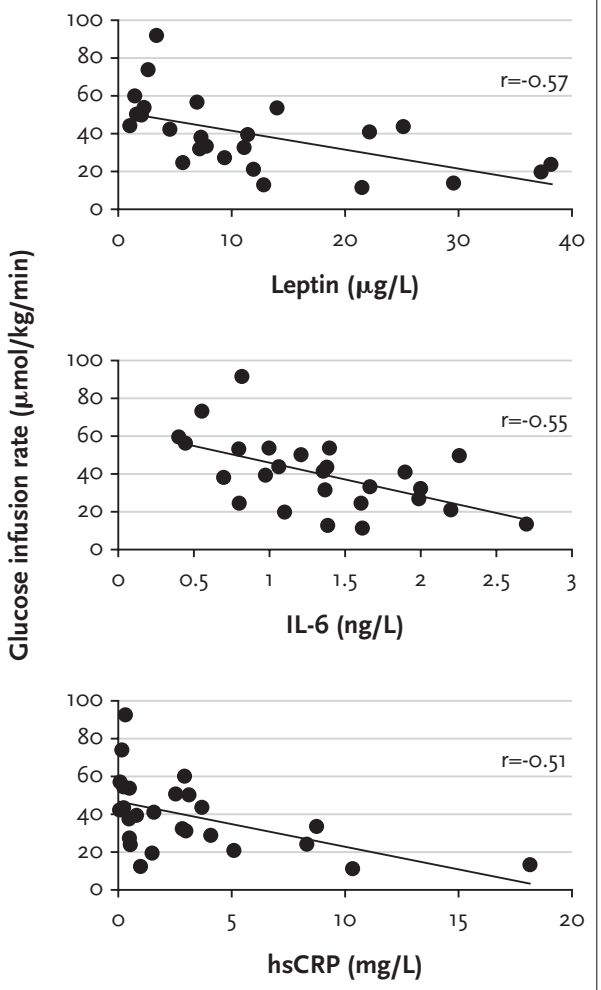
During troglitazone treatment, plasma concentrations of hsCRP, TNF-α and tPA of the obese decreased significantly (*table 2*). Consequently, after treatment these values were not different from those of the lean control subjects. Plasma levels of IL-6, leptin and PAI-1 in the obese did not significantly change during troglitazone treatment and remained elevated compared with the lean subjects.

Troglitazone-induced changes in hsCRP, TNF-α and tPA did not correlate with changes in GIR (data not shown).

DISCUSSION

In line with previous reports, we confirmed the presence of a proinflammatory state in insulin-resistant obese subjects,[15,16,35] as levels of hsCRP, IL-6, leptin, tPA, and PAI-1 were significantly higher in the obese than in the lean. Moreover, levels of hsCRP, IL-6, and leptin inversely correlated with insulin sensitivity. This is consistent with

Figure 1 *Association of insulin sensitivity with plasma levels of leptin, interleukin-6 (IL-6) and high-sensitivity C-reactive protein (hsCRP)*



the hypothesis that chronic subclinical inflammation is involved in the pathogenesis of insulin resistance.[16] The eight-week treatment with troglitazone normalised plasma levels of hsCRP, TNF-α and tPA in obesity but did not affect levels of IL-6, leptin and PAI-1. Since the obese were insulin resistant but did not have diabetes, the observed troglitazone-induced changes in inflammatory markers cannot be caused by changes in blood glucose metabolism.[36]

Plasma TNF-α was not significantly elevated in our obese, insulin-resistant study group. Earlier reports on plasma concentrations of TNF-α in obesity are not uniform. Whereas Hauner *et al.* observed normal plasma levels of TNF-α in obesity,[10] others reported markedly elevated plasma TNF-α concentrations in obese subjects when compared with lean controls.[12] The latter suggested that circulating TNF-α in obesity reflects the level of expression of TNF-α message and protein synthesis in adipose tissue.[12] However, recently, Kern *et al.* demonstrated that despite markedly increased TNF-α secretion from adipose tissue in obesity, plasma levels of TNF-α need not deviate from normal.[8] In their study it was the adipose-secreted form of TNF-α that displayed the strongest relationships with obesity and insulin resistance. They and others suggested that adipose tissue-derived TNF-α and leptin (a messenger molecule produced solely by adipose tissue) induce the production of IL-6 and hsCRP, thus contributing to the development of a proinflammatory state with increasing body weight in obesity.[37,38] The strong relationships that we observed between BMI and insulin sensitivity and plasma hsCRP, IL-6 and leptin but not plasma TNF-α, are in agreement with this hypothesis.

It was previously shown that in prediabetics, troglitazone reduced plasma concentrations of hsCRP[39] and rosiglitazone suppressed the generation of reactive oxygen species by mononuclear cells *ex vivo* and reduced plasma hsCRP and MCP-1.[40] In both studies only nondiabetic obese subjects (7 for troglitazone and 11 for rosiglitazone) and no healthy lean controls were included. More recently, other studies have also shown that rosiglitazone decreased markers of inflammation and endothelial activation (CRP, PAI-1 and von Willebrand factor) in nondiabetic subjects with stable coronary artery disease,[41] hypertension,[42] or metabolic syndrome.[43] It is unclear whether these decreases correlated with an improvement in insulin sensitivity. Our study included 15 obese well-characterised insulin-resistant subjects and 13 healthy lean controls and had a double-blind placebo-controlled crossover design. We have demonstrated that plasma levels of all studied inflammatory parameters are elevated in obesity and that some of them normalise during troglitazone treatment. Thus, there was a distinct though selective beneficial effect of troglitazone on the inflammatory status. To our knowledge, effects of TZDs on tPA and leptin have not been investigated before and IL-6 has not been previously studied in prediabetics. In our study, as with rosiglitazone in patients with type 2 diabetes,[27] troglitazone did not significantly change plasma levels of IL-6. Furthermore, the observed decreases in the levels of the inflammatory proteins hsCRP, TNF-α and tPA did not correlate with troglitazone-induced improvement in insulin sensitivity. In addition, despite some improvement in insulin sensitivity by troglitazone, the obese group remained quite insulin resistant as compared with the lean group. In contrast, troglitazone almost normalised the levels of the proinflammatory cytokines. Together, these observations suggest that troglitazone exerts its action, at least partially, via different pathways. Troglitazone may affect inflammation directly by activation of PPAR-γ present on monocytes,

monocyte-derived macrophages, vascular endothelial cells, and vascular smooth muscle cells,[25] or, via antioxidant properties of the α-tocopherol structure contained in troglitazone, reduce oxidative stress and inhibit activation of NFκB.

Although troglitazone has been withdrawn from most markets because of liver toxicity,[44] details on its effects and mechanisms of action are of interest with respect to the other members of the TZDs, as rosiglitazone and pioglitazone.

Previously we reported an increase in the ratio of large buoyant to small dense LDL, a decrease in LDL *in vitro* oxidisability, and an increase in plasma Lp(a) concentration in obese subjects treated with troglitazone.[45,46] We now add to this the selective anti-inflammatory effects of troglitazone in obesity: plasma levels of hsCRP, IL-6, leptin, tPA, PAI-1 and TNF-α (borderline significant) were elevated in the obese compared with those in lean controls, but troglitazone only reduced CRP, TNF-α and tPA. Considering the fact that low-grade inflammation is increasingly recognised as a key process in the aetiology and pathogenesis of insulin resistance and cardiovascular disease, the anti-inflammatory effects of TZDs may contribute to primary prevention of diabetes and result in improved cardiovascular outcomes. Long-term prospective studies with clinical endpoints, such as the ongoing DREAM trial that evaluates the ability of rosiglitazone to delay progression of type 2 diabetes in a nondiabetic population with impaired glucose tolerance, and RECORD (Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycaemia in Diabetes), will hopefully answer this question.

In conclusion, our data confirm the association between increased inflammation/fibrinolysis and insulin resistance, and show that TZDs selectively suppress plasma markers of inflammation, independent of changes in glucose control and, at least in part, independent of their effect on insulin sensitivity.

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ORIGINAL ARTICLE

HbA1c in healthy, pregnant women

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ABSTRACT

Aim: Congenital malformations and macrosomia in infants of women with type 1 diabetes mellitus (DM1) still occur, even if diabetic control is considered 'good' (i.e. HbA1c below the nonpregnant upper reference value of 6.3%). We, therefore, measured HbA1c in healthy, pregnant women to determine whether the upper reference value for pregnant women should be lower than the nonpregnant value.
Methods: We investigated HbA1c, measured by high-performance liquid chromatography (HPLC), in two groups of healthy primigravid women. Group 1 (n=30; 30.0 ± 5.3 (mean ± sd) years; body mass index (BMI) before pregnancy 21.7 ± 5.3 kg/m^2) had a gestational age of <18 weeks (14.5 ± 2.1). Group 2 (n=32; 30.7 ± 4.9 years; BMI before pregnancy 23.2 ± 4.6 kg/m^2) were >30 weeks (34.6 ± 2.5) pregnant. None of the women had diabetes in the family in the first and/or second degree.
Results: Group 1 had an HbA1c of 4.3 ± 0.3% (range 3.9-5.0) and in group 2 the HbA1c was 4.7 ± 0.4% (range 3.6-5.9) (p<0.001). No relation was found between HbA1c and BMI *vs* birth weight, corrected for gestational age, within the groups.
Conclusions: Healthy, pregnant women had a low HbA1c, particularly in the first trimester of pregnancy. This might implicate that for prevention of congenital malformations and macrosomia in pregnant DM1 women HbA1c should be below 5% in the first trimester of pregnancy and below 6% in the third trimester.

KEYWORDS

Congenital malformations, HbA1c, healthy pregnant women, macrosomia, type 1 diabetes mellitus

INTRODUCTION

Shortly after the introduction of insulin treatment in type 1 diabetes mellitus (DM1) in 1922, maternal mortality in pregnancy nearly decreased to the general population level, but perinatal mortality and other complications of diabetic pregnancies only diminished when diabetes control improved in the course of the years.[1]
Greene *et al.* found an increase in major congenital malformations of 4 to 39% in diabetic pregnancies with HbA1c values above 12.7% (which is approximately equivalent to an HbA1c value of 10%) during the first trimester.[2] Recently the Diabetes and Pregnancy Group, France, reported results from the French multicentric survey of the outcome of pregnancy in women with pregestational diabetes showing an increase in major congenital malformations of 4.4% (twice that of the general population) in the women with an HbA1c above 8%.[3] However, Evers *et al.* who performed a nationwide prospective study on the risk of complications of pregnancy in women with type 1 diabetes in the Netherlands found that the incidence of all congenital malformations was already increased in pregnancies with 'excellent or good' first trimester HbA1c (<7%) to 6.3% (twice that of the general population), although the incidence was twice as high (12.9%) as that of those with nonoptimal HbA1c (>7%).[4] So these authors concluded that near-optimal maternal glycaemic control (HbA1c < 7%) is apparently not good enough.
In an other report from this study in the Netherlands, the same authors reported that despite apparently 'good' glycaemic control (HbA1c <7%) in type 1 diabetic pregnant women, the incidence of macrosomia was still very high.[5] The issue of optimal glycaemic control in diabetic pregnancy is thus still not solved. It is known that HbA1c is lower in

healthy, pregnant women, compared with the nonpregnant state, but there is discrepancy with respect to the course of HbA1c in nondiabetic pregnancy. Worth *et al.* found an increase;[6] Parentoni *et al.*,[7] Hartland *et al.*,[8] O'Kane *et al.*[9] and Nielsen *et al.*[10] found no significant change; Lind *et al.*,[11] Hanson *et al.*[12] and Günter *et al.*[13] found a decrease.
We therefore measured HbA1c in healthy women in the first and third trimester of pregnancy in order to determine whether the upper reference value for pregnant women should be lower than the nonpregnant value and whether it may change during pregnancy.

MATERIALS AND METHODS

We investigated HbA1c in two groups of healthy, primigravid women who visited the Department of Obstetrics of Leiden University Medical Centre for antenatal care. Group 1 (n=30) was less than 18 weeks pregnant and group 2 (n=32) more than 30 weeks. Age, body mass index (BMI) before pregnancy and gestational age of the women in the groups are shown in *table 1*. None of the women had diabetes in the family in the first and/or second degree. The birth weight of the 62 children, corrected for gestational age, was normal.[14]
HbA1c was measured by an automated determination with a high-performance liquid chromatography (HPLC) analyser.[15] Standard procedures were used for statistical calculations: mean ± sd, student's t-test for between-group comparisons, and linear regression analysis for within-group comparisons (HbA1c and BMI *vs* birth weight, corrected for gestational age). The study was conducted according to the Declaration of Helsinki principles. The Medical Ethical Committee of Leiden University Medical Centre approved the study. The participants of the study gave their informed consent.

RESULTS

In group 1 the mean HbA1c (± sd) was 4.3 (± 0.3)% with a range of 3.9 to 5.0%. Group 2 had a mean HbA1c (± sd) of 4.7 (± 0.4)% with a range of 3.6 to 5.9% (*table 1* and *figure 1*). The difference in HbA1c between the groups was highly significant (p<0.001). No relation was found between HbA1c and BMI vs birth weight, corrected for gestational age, within the groups (group 1: HbA1c *vs* birth weight: r=0.142, p=0.45 and BMI vs birth weight: r=-0.349. p=0.07; group 2: HbA1c *vs* birth weight: r=0.266, p=0.14 and BMI vs birth weight: r=0.318, p=0.08).

DISCUSSION

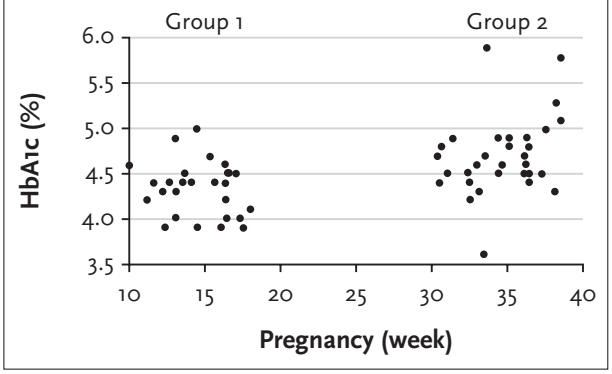
We found a low upper HbA1c range level of 5 % in the first trimester of pregnancy, compared with the nonpregnant upper HbA1c reference value of 6.3% in our hospital, and a higher upper HbA1c range level of 5.9% in the third trimester of pregnancy.
The low level of HbA1c in the first trimester of pregnancy is caused by the low mean preprandial and postprandial blood glucose values[16] and by the increase in young erythrocytes which diminishes the percentage of glycosylated haemoglobin.[17] The increase in HbA1c in the third trimester of pregnancy is caused by the increase in the mean postprandial blood glucose value.[16] This is in agreement with the findings of Monnier *et al.* who reported that in type 2 diabetic patients the relative contribution of postprandial glucose excursions to HbA1c is predominant in fairly well-controlled patients, whereas the contribution of fasting hyperglycaemia increases gradually with a worsening of the diabetes.[18]
Our findings might implicate that for prevention of congenital malformations and macrosomia in diabetic pregnancies, HbA1c should be below 5.0% in the first trimester of pregnancy and below 6.0% in the third trimester. With respect to macrosomia, the recommendation of a low HbA1c in the first trimester is supported by the data of Gold *et al.* who showed that birth weight, corrected for gestational age, is best correlated with the HbA1c of 0 to 12 weeks of gestational age in women with type 1 diabetes.[19] So our study suggests that in order to prevent congenital malformations and macrosomia, HbA1c in the first trimester of diabetic pregnancy should be below 5.0%. However, Evers *et al.* found self-reported severe hypoglycaemia in 41% of 264 pregnant diabetic women during the first

Table 1 *Data (mean ± sd) of age, body mass index (BMI) before pregnancy, gestational age and HbA1c of the groups*

	Age (years)	BMI (kg/m²) before pregnancy	Gestational age (weeks)	HbA1c (%) (range)
Group 1 (n=30)	30.0 ± 5.3	21.7 ± 5.3	14.5 ± 2.1	4.3 ± 0.3* (3.9-5.0)
Group 2 (n=32)	30.7 ± 4.9	23.2 ± 4.6	34.6 ± 2.5	4.7 ± 0.4* (3.6-5.9)

* p<0.001.

Figure 1 *HbA1c values in the first (group 1) and third trimester (group 2) of pregnancy*



trimester and in 17% during the third trimester; these women had a mean HbA1c of 6.4 vs 6.7% in women who did not experience hypoglycaemia. In their study HbA1c during the first trimester was ≤6.0% in 32% of the women and 6.1 to 7.0% in 43%.[4] For the whole pregnancy, 41 and 43% of the women had an HbA1c ≤6.0% and 6.1 to 7.0%, respectively.

So it may be difficult to improve diabetic control during pregnancy with the current therapeutic measures without increasing the incidence of severe hypoglycaemia. Evers *et al.* reported that women on the most sophisticated therapeutic method in practice these days, the continuous subcutaneous insulin infusion, had significantly more macrosomic infants than women on treatment with multiple (≥3/day) daily insulin injections.[5] The authors argued that, in general, the reason for this could be that women with a macrosomic infant had a higher HbA1c and less episodes of severe hypoglycaemia compared with women with a nonmacrosomic infant.

Other factors could also play a role in causing complications in diabetic pregnancy. It may be possible that wide blood glucose fluctuations, which occur in diabetic pregnancy as clearly shown by the continuous glucose monitoring system,[20] have a deleterious effect on their own, independent of the mean blood glucose level, as reflected by the HbA1c. This is in agreement with the data of Derr *et al.* who reported that HbA1c is not affected by glycaemic instability.[21]

Unplanned pregnancies also showed more complications, particularly congenital malformations; and pregestational hypertension and/or diabetic nephropathy are a risk for gestational hypertension and (pre)eclampsia.[3,4]

In conclusion, healthy, pregnant women had a low HbA1c, particularly in the first trimester of pregnancy. This might implicate that for prevention of congenital malformations and macrosomia in pregnant, type 1 diabetic women HbA1c should be below 5% in the first trimester of pregnancy and below 6% in the third trimester.

However, with the current therapeutic measures it is difficult to improve diabetic control during pregnancy to the desired level without increasing the incidence of severe hypoglycaemia. For the moment, the best treatment still seems to be multiple daily insulin injections. The search for more optimal treatment modalities of diabetic, pregnant women should have a high priority.

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ORIGINAL ARTICLE

Effect of etanercept and anakinra on inflammatory attacks in the hyper-IgD syndrome: introducing a vaccination provocation model

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ABSTRACT

Background: Hyper-IgD and periodic fever syndrome (HIDS) is an hereditary autoinflammatory syndrome, characterised by recurrent inflammatory attacks. Treatment of HIDS is difficult, although simvastatin is beneficial and etanercept might be effective. Studying the treatment of a rare periodic syndrome is complicated by the varying frequency and severity of symptoms and low prevalence. Our aim was to develop a system of clinical observations to evaluate effectiveness of treatment-on-demand.
Methods: Seven fever episodes in three HIDS patients were monitored, with and without administration of etanercept or anakinra. We developed a clinical score, which includes 12 symptoms. In one patient, inflammatory attacks were provoked by vaccination.
Results and conclusions: At the onset of an attack, all patients reported a clinical score between 20 and 25. The score was used to quantify severity and define the end of an attack. Reproducible monitoring of inflammatory episodes was difficult, even in this pilot study. The effect of early administration of etanercept was variable. In one patient, a fever episode could be readily provoked within 12 to 24 hours by vaccination. In this patient, the IL-1ra analogue anakinra was more successful in aborting the inflammatory attack than etanercept. We propose that this vaccination model will allow evaluation of treatment-on-demand in a controlled setting.

KEYWORDS

Anakinra, etanercept, hyper-IgD syndrome, interleukin-1, periodic fever, TNF, vaccination

INTRODUCTION

The hyper-IgD and periodic fever syndrome (HIDS, MIM#260920) is an autosomal recessively inherited autoinflammatory syndrome, caused by deficient enzyme activity of mevalonate kinase, an enzyme in the isoprenoid pathway. Patients present with a long history of recurrent fever attacks, lasting three to seven days, accompanied by chills, headache, generalised lymphadenopathy, arthralgia, skin lesions, abdominal pain and diarrhoea. Laboratory analysis reveals an intense acute-phase response during fever attack; *ex-vivo* production of tumour necrosis factor (TNF)-α and interleukin (IL)-1β by monocytes and macrophages is significantly higher at the time of attack.[1] An attack is usually preceded by a well-recognisable prodromal phase of malaise, headache and musculoskeletal symptoms.[2] Sometimes, a (trivial) stimulus can be identified as a trigger of the attack, and most HIDS patients experience a severe inflammatory episode after any vaccination. In between two attacks patients are asymptomatic, although the acute-phase response may sometimes persist.
Until now, treatment of HIDS patients is largely supportive and very difficult. Various standard anti-inflammatory drugs (including colchicine, NSAIDS, steroids and thalidomide) have failed to suppress the attacks.[2,3] We have already shown that simvastatin, an inhibitor of HMG-CoA reductase, can be beneficial in reducing the number of days of illness

**J.P.H. Drenth was not involved in the handling and review process of this paper.

when taken continuously.[4] Reports on the response to etanercept, an inhibitor of TNFα, in HIDS have been mixed: favourable in two children,[5] uncertain in one,[6] and no effect in another child.[7]
Studying the effectiveness of a treatment for a rare periodic syndrome is complicated by its variability of frequency and severity of symptoms and its low prevalence. We have tried to develop a system of rigorous clinical observations to evaluate the effectiveness of treatment-on-demand.

METHODS

Three female Dutch HIDS patients gave written informed consent for participation in this study; the study was approved by the local medical ethical committee. The patients were not on any medication apart from that mentioned in the case observations. They were instructed to contact us at the first indication of a fever attack. When that happened, the patient was admitted to hospital for close monitoring, which included regular measurement of body temperature and blood sampling for C-reactive protein (CRP).
We asked the patients to complete a daily symptom score card, rating the absence or presence of 12 different symptoms and their severity on a scale from 0 to 10. These 12 symptoms were lymphadenopathy, nausea, myalgia, arthralgia, aphthous ulcers, abdominal pain, skin lesions, headache, sore throat, tiredness, diarrhoea and nasal congestion. This was used to develop a clinical scoring system to help better delineate the duration and severity of a fever episode. By adding the scores on the individual symptoms, a daily score could thus range from 0 to 120. At the height of a fever episode, patients experienced between 7 and 12 of these 12 symptoms, while the maximal documented score ranged from 45 to 88 points. Despite an anticipated variability in scores between patients and between separate attacks in the same patient, all patients reported a total score between 20 and 25 at the time of presentation at the start of a fever attack. Thus, a score of 20 or higher was taken to represent the presence of a fever attack, while the time point of the first score below 20 was taken as the end of the attack.

CASE OBSERVATIONS

Patient 1

A 35-year-old woman had experienced characteristic febrile attacks since the age of 3 months. At age 33, HIDS was finally diagnosed, confirmed by mevalonate kinase mutation analysis (*table 1*). During childhood, vaccinations used to trigger febrile attacks but the administration of hepatitis A immunoglobulin at the age of 34 years did not precipitate any symptoms. She was closely monitored during an attack which started with a sore throat, myalgias, fatigue, nausea and headache (*figure 1A*). Her body temperature was 36.6°C at presentation. Over the next few days symptoms increased and her body temperature quickly rose to 39.5°C, with a maximum CRP of 102 mg/l. The symptoms of the attack lasted four days although serum CRP concentration was still elevated at seven days (*figure 1A*).
The next time she was admitted at the start of a fever attack, two doses of etanercept (25 mg) were administered subcutaneously at 12 and 36 hours after presentation (*figure 1B*). There was no noticeable difference in her clinical symptoms as expressed in the clinical score, body temperature or decrease in CRP concentration between this fever episode and the previous, untreated one (*figure 1*).

Patient 2

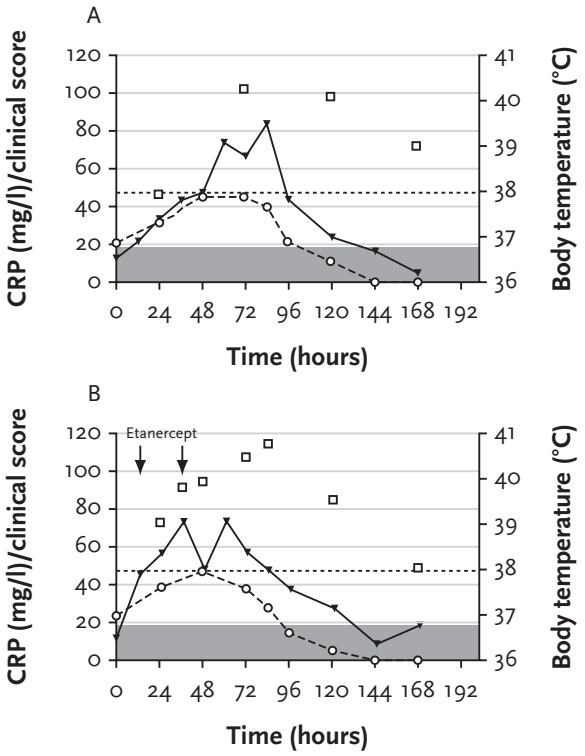
In this 26-year-old woman the diagnosis of HIDS was made three years ago, after she had experienced fever episodes since two months after birth. Childhood vaccinations consistently precipitated febrile attacks. The diagnosis was confirmed by mutation analysis, and an immeasurably low mevalonate kinase enzyme activity (*table 1*).
The attack during which she was monitored appeared to be uncommonly severe with respect to the accompanying symptoms. She did not receive treatment during this attack which lasted seven days; she experienced massive cervical and iliac lymphadenopathy and developed oral and vaginal aphthous ulcers. CRP concentration rose to a maximum of 315 mg/l after 72 hours, while her body temperature was maximally 38.4°C (*figure 2A*).
A subsequent fever episode is depicted in *figure 2B*. The patient only reached our hospital some 48 hours after onset of symptoms, and immediately after admission etanercept

Table 1 *Patient characteristics*

Case	Age (years)	Age at onset (months)	Mevalonate kinase genotype	Mevalonate kinase enzyme activity*	Serum IgD (U/ml†)
1	35	3	V377I/G202R	ND	2330
2	26	2	V377I/417insC	<0.1%	745
3	38	1	V377I/I268T	10.7%	616

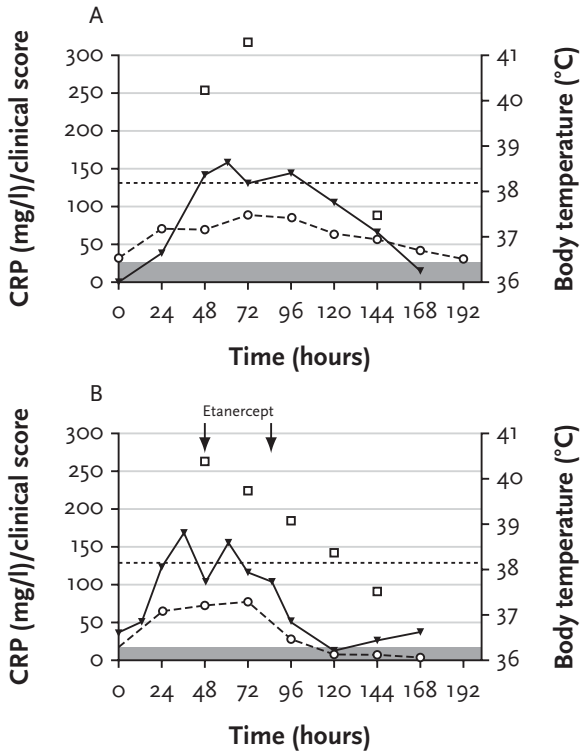
*Expressed as % of normal; †reference value IgD <100 U/ml; ND = not determined.

Figure 1 *Two inflammatory attacks in patient 1*



□ = Serum CRP concentration (mg/l) on left Y-axis; ▼ = body temperature (°C) on right Y-axis; ○ = total clinical score on left Y-axis; grey area depicts area of clinical score below 20 points. a) untreated inflammatory episode; b) inflammatory episode treated with etanercept at 12 and 36 hours after onset of symptoms (denoted by arrows).

Figure 2 *Two inflammatory attacks in patient 2*



□ = Serum CRP concentration (mg/l) on left Y-axis; ▼ = body temperature (°C) on right Y-axis; ○ = total clinical score on left Y-axis; grey area depicts area of clinical score below 20 points. One untreated inflammatory episode (a), and one inflammatory episode treated with etanercept (b, denoted by arrows).

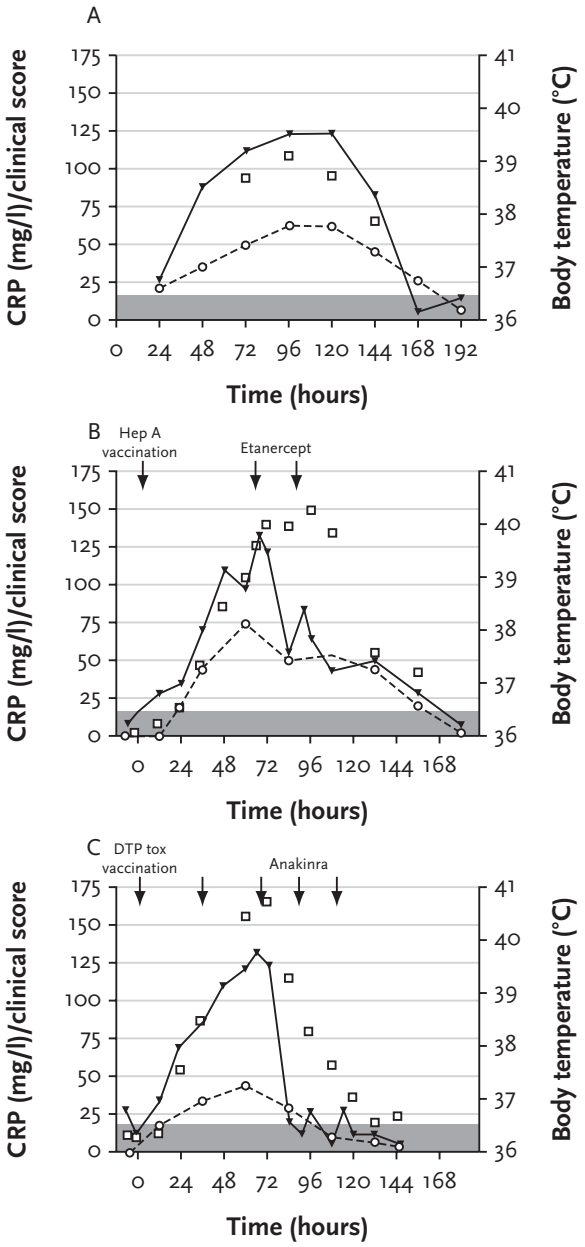
treatment was initiated. At that time, serum CRP concentration was similar to that at 48 hours into the first attack (263 *vs* 252 mg/l), as was her clinical score (74 *vs* 71 points) (*figure 2*). After institution of treatment, the CRP concentration declined steadily, and within three days her symptoms had disappeared (*figure 2B*). Thus, etanercept seemed to shorten this attack in comparison with the previous one. The patient maintained that the second attack was milder and she was unconvinced of a beneficial effect.

Patient 3

Patient 3 is a 38-year-old woman with fever episodes since birth (*table 1*). She was admitted to the hospital for observation of an untreated fever episode. On admission, she complained of myalgia, arthralgia, abdominal pain and skin lesions. Her body temperature was 36.8°C, but rose to above 39°C within two days (*figure 3A*). The symptomatic attack persisted for six days, with a concomitant rise and fall of CRP concentration.

Because of a planned trip to China she required several vaccinations, and given her earlier childhood experiences of fever attacks after vaccinations, we decided to admit her for administration of the injections. We gave her one vaccination for hepatitis A (a primo vaccination in her case) and one (repeat) DTP toxin vaccination, separated by one month. Some 24 to 48 hours after administration of each vaccine she developed a characteristic HIDS attack with fever, abdominal pain, myalgia and fatigue. In both instances this was accompanied by an intense acute-phase response (maximum CRP 166 mg/l; *figures 3B* and *3C*). We used this model of vaccination-precipitated HIDS attack to test the efficacy of early institution of treatment to abort the fever episode. In the first attack, two doses of etanercept (25 mg, subcutaneously) were administered 72 and 86 hours after vaccination (48 and 72 hours after onset of the first symptoms). The symptomatic attack lasted for 5.5 days in total, with maximum CRP concentration reaching 36 hours after the first dose of etanercept (*figure 3B*). At the next vaccination one month later, we administered anakinra, a recombinant selective IL-1 receptor antagonist (100 mg per dose, subcutaneously), at three time points from 72 hours after vaccination, with an interval of 24 hours. Body temperature normalised and symptoms disappeared within 17 hours after the first injection, peak CRP concen-

Figure 3 *Inflammatory attacks in patient 3*



□ = Serum CRP concentration (mg/l) on left Y-axis; ▼ = body temperature (°C) on right Y-axis; ○ = total clinical score on left Y-axis; grey area depicts area of clinical score below 20 points. a) untreated inflammatory episode; b) inflammatory episode provoked by hepatitis A vaccination, and treated with etanercept (denoted by arrows); c) inflammatory episode provoked by DTP vaccination, and treated with anakinra (denoted by arrows).

tration was reached at 12 hours after the first injection and CRP gradually returned to baseline values (*figure 3C*). Thus, anakinra aborted the attack after some three days of symptoms, in contrast to the 5.5 and 6 days of the other witnessed attacks.

Antibody titres were found to be adequate after both vaccinations (data not shown), thus demonstrating that the administration of cytokine antagonists 72 hours after vaccination did not have an influence on the effectiveness of the vaccinations.

DISCUSSION

In this pilot study monitoring fever attacks in HIDS, we closely followed seven fever episodes in three patients, with and without intervention.

To quantify the accompanying symptoms of the inflammatory attack, we developed a clinical scoring system consisting of the total scores on a visual analogue scale on a range of 12 symptoms and signs. At the moment the patients felt the onset of a fever attack, they documented a total score of between 20 and 25. Since concentration of CRP and some of the symptoms, most notably tiredness, may persist for a longer period, the end of a HIDS attack may be difficult to define. We found that the proposed clinical score may help with this as well: we suggest defining the end of the attack as the moment at which the clinical score decreases to 20 or less.

The results of monitoring fever episodes in patients 1 and 2 demonstrate the variability of this disorder. It was our aim to start with intervention at the earliest possible moment and we therefore instructed patients to contact us as soon as possible after the start of symptoms. However, even in our small pilot study there was a difference of 36 hours between the time of first administration of etanercept in the two patients. The wide variation seriously impedes the comparability of results in a larger trial set-up. Part of the delay was caused by the fact that we admitted patients to our clinic, which might also induce a selection bias towards more severe attacks. However, treatment at home would make it more difficult to objectively observe symptoms, body temperature and CRP concentrations.

A HIDS patient who needed vaccinations allowed us to observe the power of these vaccinations to provoke a fever episode. In both instances, vaccination resulted in a fever episode within 12 to 24 hours. These attacks were comparable with attacks not precipitated by vaccinations. There are some advantages to using this provocation model: it is simple, easy to use and offers an opportunity to closely monitor the onset of the attack from the very beginning, and thus to standardise the time to starting treatment. Since patients are hesitant to receive (necessary) vaccinations because of the risk of a febrile attack, a closely monitored setting will be helpful to ensure that they do receive them.

With respect to the observations of effect of treatment-on-demand in these patients: these first results appear to be mixed, and this pilot study does not allow us to draw firm conclusions on that point. Anakinra seems to be more

successful than etanercept; this warrants further examination. HIDS is part of a group of hereditary autoinflammatory syndromes,[8] whose common pathogenic background seems to involve IL-1 signalling. Recently several groups have reported treating hereditary autoinflammatory syndromes successfully with the recombinant form of IL-1ra, anakinra.[9-13]

It remains difficult to get solid evidence on treatment efficacy in orphan diseases, especially when periodic and variable in phenotype, such as HIDS. Most published reports concern clinical observations in one or two patients. Drug trials set up on established lines such as randomised controlled trials will often remain underpowered because of too few patients and too few episodes of illness.[3,4] Also, because the frequency of fever attacks in adult HIDS patients will usually diminish to between 6 and 12 per year, patients will be more interested in an on-demand treatment which shortens an attack than in continuous treatment (and continuous life-long risk of side effects), unless this continuous treatment will abolish all further symptoms. We suggest that the vaccination provocation model in combination with the clinical score described in this pilot study will offer an opportunity for more rigorous and standardised study of on-demand treatment in HIDS.

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ORIGINAL ARTICLE

Diagnostic work-up for faecal incontinence in daily clinical practice in the Netherlands

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ABSTRACT

Background: To study variation in Dutch hospitals in applying diagnostic and treatment options for faecal incontinence.
Methods: Surgeons, gastroenterologists, internists and gynaecologists were contacted by phone or mail and requested to complete a questionnaire. The questionnaire asked for general information about patients with faecal incontinence, the use and availability of diagnostic techniques, the use of incontinence scores and therapeutic options.
Results: In total 306 specialists were contacted and data were collected from 203 specialists from 86 hospitals (response rate 66%). The most frequently applied diagnostics were sigmoidoscopy (64%), endoanal sonography (58%), evacuation proctography (56%) and/or anorectal manometry (51%). The choice seemed to be related to the availability of the techniques. Sigmoidoscopies were performed significantly more often in local hospitals ($p<0.001$), while in academic medical centres significantly more endoanal MRI examinations were conducted ($p<0.05$). The most stated treatment option was physiotherapy (90%), followed by dietary measures (83%), medication (71%) and surgery (68%). However, in general, combinations of treatment options were used.
Conclusions: A substantial variety exists in the diagnostic work-up of faecal incontinence. In general, at least one anorectal functional test and an imaging technique are the diagnostic techniques of choice. Pelvic floor physiotherapy is the first choice in conservative treatment.

KEYWORDS

Anorectal functional test, diagnostic work-up, endoanal sonography, faecal incontinence, manometry, MRI, therapy, survey

INTRODUCTION

Faecal incontinence is defined as 'the involuntary loss of flatus, liquid or solid stool that is a social or hygienic problem.'[1] The incidence and prevalence of faecal incontinence in the Netherlands are not exactly known.[2] The estimated prevalence is about 100,000 subjects in the Netherlands. The actual prevalence may be even higher due to underreporting as a consequence of the social stigma of this disorder.[3] The main causes of faecal incontinence are obstetric trauma (anal sphincter and/or pudendal nerve damage) and anorectal surgery (anal sphincter trauma).[4-6] Apart from medical history and physical examination, there are several diagnostic techniques that can be performed: anorectal functional testing, endoscopy and imaging.[7-9] Anorectal functional tests comprise anorectal manometry (measurement of sphincter pressure in rest, during squeeze and straining), measurement of pudendal nerve terminal motor latency (PNTML) (to establish pudendal nerve injury), electromyography (EMG) (conventional EMG to identify the quality of sphincter tissue as well as to determine whether the muscle contracts or relaxes; single-fibre EMG to identify denervation-reinnervation potentials indicative of nerve injury), rectal capacity measurement (to detect the threshold of the first detectable sensation, sensation of urgency and maximum tolerable volume) and sensory testing (to determine the sensitivity of the anal canal and rectum).[10,11]

A sigmoidoscopy can be performed to exclude organic disease, such as a benign or malignant obstructing lesion or inflammation.[10] With imaging techniques, such as endoanal sonography and magnetic resonance imaging (MRI), both internal and external anal sphincter abnormalities will be assessed.[7] Evacuation proctography (defaecography) involves imaging of the rectum and observation of the process, rate, and completeness of rectal evacuation.[10]

At present there is no consensus on the best diagnostic techniques for patients with faecal incontinence in the Netherlands. As a consequence of the increase in number and availability of diagnostic modalities, variation in diagnostics exists and an unambiguous strategy is lacking.[10] To assess if and to what extent variation exists in diagnostic work-up and treatment of patients with faecal incontinence in daily clinical practice in the Netherlands, we developed a survey. We restricted ourselves to an inventory of diagnostic modalities and treatment in secondary and tertiary centres.

MATERIALS AND METHODS

From October 2002 to April 2004 surgeons, gastroenterologists, internists and gynaecologists from all Dutch hospitals were informed about the survey by phone. If approach by phone turned out to be impossible, information was sent out by (digital) mail. For every hospital a questionnaire was sent per discipline to the most experienced specialist in the field of faecal incontinence.

The questionnaire comprised five sections. In the first, physicians were asked for general information about patients with faecal incontinence, such as how often these patients were referred to the respondent, and the age, and gender of the referred patients. In the second section information was requested about the selection of diagnostic tests which were used as routine work-up in patients with faecal incontinence. Options were anorectal functional tests, endoscopy and imaging techniques (*table 1*). In addition to the options for routine diagnostic techniques, in the third part questions were asked about the availability of these techniques to gain insight into where the techniques were performed. The respondent had to indicate whether the diagnostic test in question could be performed in the respondent's own hospital or if referral was needed. The fourth section was about the use of an incontinence score to determine the severity of incontinence. Respondents could choose between the Parks, Vaizey, Wexner, Pescatori, or Millar scores, and/or the American Medical System score.[12] If an incontinence score was used, the respondent was asked whether the score influenced the choice of diagnostic and therapeutic options. The final section contained questions on the therapeutic options used (conservative therapy (dietary measures, medication, pelvic

Table 1 *Options for diagnostic techniques together with the availability of diagnostic equipment and referral of patients with faecal incontinence*

Diagnostic techniques	Options for diagnostics* n (%)	Availability of diagnostics n (%)	Referral n (%)
Endoscopy			
Sigmoidoscopy	120 (64)	166 (89)	2 (1)
Anorectal functional tests			
Anorectal manometry	96 (51)	71 (38)	72 (39)
Rectal capacity measurement	42 (22)	44 (24)	48 (26)
PNTML	37 (20)	47 (25)	43 (23)
Anal sensibility measurement	32 (17)	33 (18)	47 (25)
Rectal sensibility measurement	31 (17)	23 (12)	46 (25)
Conventional electromyography	26 (14)	57 (31)	26 (14)
Fine needle electromyography	6 (3)	24 (13)	22 (12)
Imaging techniques			
Endoanal sonography	108 (58)	86 (46)	60 (32)
Evacuation proctography	104 (56)	136 (73)	31 (17)
Endoanal MRI	25 (13)	28 (15)	31 (17)
Phased-array MRI	25 (13)	56 (30)	10 (5)
MR defaecography	3 (2)	11 (6)	13 (7)

*The chosen diagnostic is the routine diagnostic work-up in patients with faecal incontinence. The routine diagnostic work-up could be performed in the respondent's own hospital or in a referring centre. PNTML = pudendal nerve terminal motor latency; MRI = magnetic resonance imaging.

floor physiotherapy), surgery or another kind of therapy). All nonrespondents received one more reminder by phone and if necessary, a new questionnaire was sent out. If there was no response after three questionnaires had been sent out, a final nonresponse was determined. (Details of the questionnaire can be obtained from the corresponding author.)
Analyses were performed with descriptive statistics. Differences between groups were calculated with χ^2 test. The results were statistically analysed with SPSS 11.5. for Windows (SPSS Inc. Standard Version). We analysed the response per specialist instead of per hospital.

RESULTS

Response

In total 306 physicians were contacted (91 surgeons, 74 gastroenterologists, 24 internists and 117 gynaecologists) from the 100 Dutch hospitals (we did not take into account categorical hospitals such as cancer institutes and outpatient clinics). The response rate was 66% (n=203) from 86 hospitals and one private clinic. Sixteen percent (n=33 questionnaires) of the response rate originated from academic medical centres. There were differences in response rate per medical specialist: the response rate of surgeons and gastroenterologists was higher (76 and 72% respectively) than that of internists and gynaecologists (58 and 57% respectively). Seventeen (29%) responding gynaecologists referred their patients almost directly to another medical specialist or hospital. For the majority of physicians (75%) patients with faecal incontinence were sometimes referred, while only 12% indicated having these patients referred regularly and 3% often.
Sixteen questionnaires (8%) had to be excluded from analysis since the respondent reported no referral of patients with faecal incontinence or referred these patients immediately to another specialist. Consequently, there were 187 questionnaires remaining for analysis, from 80 different hospitals and of one private clinic.
The majority of physicians (92%) indicated that they treated their patients with faecal incontinence on an interdisciplinary and/or multidisciplinary basis.

Patients

Physicians indicated that on average 87% of the patients with faecal incontinence were female. On average almost half of these patients (47%) were more than 65 years of age. Age as well as gender was not significantly influenced by the numbers of patients referred to the physician.

Diagnostic techniques

The range of routine diagnostic techniques applied in patients with faecal incontinence varied from none to 11 examinations. On average 3.5 examinations were performed as the routine diagnostic work-up. In *table 1* the results of differences in options of diagnostic testing are shown. The majority of the respondents (64%) indicated the routine use of sigmoidoscopy. The most frequently applied imaging techniques were endoanal sonography (58%) and evacuation proctography (56%). Of all anorectal functional tests, anorectal manometry (51%) was most often used. The use of these techniques seems to be linked to the availability of the diagnostic techniques. The other diagnostic techniques were not performed on a regular basis.
Sigmoidoscopy and evacuation proctography were available for most of the respondents. The highest percentages of referral were for endoanal sonography and anorectal manometry (32 and 39% respectively). The most commonly used combinations of diagnostic techniques were endoanal sonography with anorectal manometry (41%), and sigmoidoscopy with evacuation proctography (41%). Twelve percent of all respondents reported that they did not perform any kind of additional testing; 38% mentioned not performing any anorectal functional tests and 3% reported that they did not make use of any kind of imaging technique.
When comparing the routinely performed diagnostics in academic medical centres with those performed in local hospitals, physicians in local hospitals reported significantly more use of sigmoidoscopy (p<0.001), while physicians in academic medical centres reported significantly more use of endoanal MRI examinations (p<0.05) (*table 2*).

Table 2 *Significant differences between academic medical centres and local hospitals*

	Sigmoidoscopy	Endoanal MRI	Incontinence score
University hospital	15%	29%	60%
	p<0.001	p<0.05	p=0.001
Local hospital	73%	11%	28%

Incontinence score

Thirty-one percent of the respondents used an incontinence score; 13.5% indicated that they always used a score and 17.5% sometimes. A score was significantly more in use in academic medical centres compared with local hospitals (p=0.001) (*table 2*). The most applied incontinence score was the Parks score (44%), followed by the more recently introduced Vaizey score (28%). The selection of diagnostic tests and therapeutic treatment options were influenced by an incontinence score in 6%.

Therapeutic treatment options

The most reported treatment option by the respondents was pelvic floor physiotherapy (90%), followed by dietary

measures (83%), medication (71%) and surgery (68%). A combination of treatment options was most frequently reported. Fifty-four percent of the respondents indicated that they applied dietary measures, medication, pelvic floor physiotherapy as well as surgery as treatment options. In 7% (academic medical centres) *vs* 26% (local hospitals) surgery was not considered a treatment option as patients only received conservative treatment. Other therapies, such as sacral neuromodulation and anorectal or oral water enemas, were part of potential treatment options in 7% of the respondents.

DISCUSSION

In the Netherlands the most performed diagnostics in patients with faecal incontinence are sigmoidoscopy, endoanal sonography, evacuation proctography and anorectal manometry. Since sigmoidoscopy is performed to exclude local pathology such as tumours, and evacuation proctography is not a diagnostic technique specifically for faecal incontinence,[7] it can be concluded that most applied diagnostic tests in patients with faecal incontinence in secondary and tertiary centres are anorectal manometry (anorectal functional test) and endoanal sonography (imaging technique).
Significantly more sigmoidoscopies were performed in local hospitals ($p<0.001$), while endoanal MRI examinations were significantly more frequent in academic medical centres ($p<0.05$). It is possible that availability does play a role, as well as the referral pattern. Almost every physician in a local hospital performs a sigmoidoscopy to exclude malignancy or proctitis, for example, while in general patients are referred to an academic medical centre if comprehensive anorectal functional testing and/or endoanal MRI is needed. There was a considerable variation in the use of the other diagnostic modalities.

Anorectal functional tests

Anorectal manometry appeared to be the most commonly applied anorectal functional test; it was relatively widely available and had the highest percentage of referral. PNTML, rectal capacity measurement, and anal and rectal sensory testing were part of routine diagnostic testing to a lesser extent. Nevertheless, approximately 25% of the respondents referred their patients for these tests. It seems that these functional tests are included in the work-up when more extensive diagnostic is mandatory.
Conventional electromyography was reported to be part of the available diagnostic techniques by 31% of all respondents, but only 14% performed it as a routine procedure. Fine needle electromyography was not regarded as routine. These tests are not considered to have any substantial value and to be outdated. The performance of EMG for the detection of an external anal sphincter defect has been replaced by the availability of other techniques, such as endosonography or MRI.[10,13] For establishing pudendal nerve injury, PNTML measurement will be performed when considered appropriate.[7] The technique has been suggested for distinguishing between muscle weakness caused by pudendal nerve injury and muscle weakness caused by muscle injury in patients with faecal incontinence, but has a poor correlation with clinical symptoms and histological findings. Therefore, the clinical usefulness is controversial.[10]

Imaging techniques

Endoanal and phased-array MRI are part of the routine diagnostic work-up for more than 10% of respondents. Endoanal sonography and endoanal MRI are comparable techniques for evaluating external anal sphincter abnormalities. For evaluation of the internal anal sphincter complex, there is no consensus about the most accurate technique.[8,14,15] However, the sensitivity and specificity for identifying external anal sphincter atrophy with MRI is higher than for endoanal sonography.[8]

MR defaecography is hardly available. Besides, the accuracy and reproducibility of conventional defaecography is not (yet) established and the technique is still in development.[14]

Incontinence score

Several incontinence scores have been developed.[7,8,12] Nevertheless, it appears that these scores are rarely used in daily practice. This is probably because the registration of scoring is often a complex matter and the consequences of use, other than for scientific research, have not been clearly pointed out. This study showed that scoring systems according to Parks and Vaizey are the most applied scores in the Netherlands for patients with faecal incontinence. Possible explanations are that the score according to Parks is the most uncomplicated one and the score according to Vaizey is the most complete scoring system.[12]

Treatment options

A combination of treatments was predominantly reported, comprising various conservative treatment options (pelvic floor physiotherapy, dietary measures, medication), if necessary complemented with surgery. Of all therapeutic options, pelvic floor physiotherapy was the most widely applied (90%). According to Kamm pelvic floor physiotherapy and surgery are the two most utilised treatment options if dietary measures and/or medication fail.[16] However, in this study the respondents reported that they more often used pelvic floor physiotherapy as initial therapy than other conservative measures. Nevertheless, we must consider that previous conservative treatment may have been prescribed elsewhere by others.

Limitations

Potential limitations of this study should be taken into account. One limitation was that the majority of the respondents reported a relatively infrequent referral of patients with faecal incontinence, which was defined as a range from 1 to 24 patients a year. Because of the wide range, it is possible that differences exist in selected diagnostic and therapeutic options between physicians with one to five referrals a year compared with those with 20 to 24 referrals on a yearly basis.
In some of the participating hospitals this questionnaire was completed by several medical specialities while in others it was completed by only one speciality. Since this questionnaire was completed for the greater part by different medical specialities divergently, we assume it is justified considering that all hospitals have the same weighting.

This study shows that substantial variety exists in the diagnostic work-up for faecal incontinence. In general, at least one anorectal functional test and an imaging technique are the diagnostic techniques of choice. Besides, there are differences in work-up between local hospitals and academic medical centres, partly related to the availability of equipment. In the literature, guidelines for the evaluation of faecal incontinence are described.[10,17-19] In summary they all recommend, next to a detailed clinical assessment, appropriate physiological and imaging tests of the anorectum. These three sources of information are complementary. The anorectal physiology testing of choise in the presented guidelines were anorectal manometry and endoanal sonography, conform the results of our study. Furthermore, between guidelines there was variation concerning the remaining diagnostic modalities.
To reduce variability we encourage developing guidelines for the diagnostic work-up of faecal incontinence in the Netherlands. We recommend that the scope of the guidelines is aimed at simplification of the diagnostic path in patients with faecal incontinence, based on scientific evidence. We want to emphasise the importance of evidence-based guidelines to reduce inadequate use as well as both overuse and underuse. As a consequence, an efficient diagnostic work-up in patients with faecal incontinence can be developed.

ACKNOWLEDGEMENTS

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CASE REPORT

ANCA seropositivity in HIV: a serological pitfall

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ABSTRACT

In systemic vasculitis, cytoplasmic staining in ethanol-fixed neutrophilic granulocytes, i.e. cytoplasmic antineutrophilic cytoplasmic antibody (c-ANCA), is generally considered a highly significant serological marker. When a patient presents with upper airway or renal symptomatology and seropositivity to c-ANCA, a Wegener's granulomatosis is usually easily diagnosed by performing a biopsy of the diseased organ. However, not every ANCA-positive patient with pulmonary inflammation is suffering from Wegener's disease. In some cases of upper airway or pulmonary symptomatology, the *a priori* chance of having Wegener's disease is low despite a positive ANCA. A coincidental positivity of ANCA may then lead to clinicians jumping to conclusions. We present a 40-year-old man who was falsely suspected of having Wegener's disease because of upper airway symptomatology and c-ANCA positivity. Specificity analysis revealed that he was negative to antibodies for proteinase-3, but positive to myeloperoxidase. The potential serological pitfall of the supposedly specific c-ANCA is discussed.

KEYWORDS

ANCA, HIV, pitfall, screening

INTRODUCTION

Wegener's granulomatosis is a necrotising granulomatous vasculitis involving predominantly the upper and lower respiratory tract and kidneys. Other organs may be affected to a variable extent by this small-vessel vasculitis. Some authors have suggested that sensitivity and specificity of antineutrophilic cytoplasmic antibodies (ANCA) are adequate to merit inclusion of ANCA in classification systems of systemic vasculitis. Many clinicians therefore even use ANCA in a similar way to antinuclear antibodies (ANA) early in the diagnostic phase for screening purposes.[1] Positivity of these ANCA may be misleading if clinicians have requested the tests incorrectly in these screening diagnostic situations. They may even become a diagnostic pitfall.[2,3] Such patients enter specialist care via different disciplines, i.e. general internists, pulmonologists, nephrologists, ear, nose and throat (ENT) specialists or rheumatologists, depending on the predominant clinical sign or symptom. In addition to pulmonary and renal involvement, antineutrophilic cytoplasmatic antibodies with a typical cytoplasmic staining pattern on ethanol-fixed neutrophilic granulocytes (c-ANCA) with a proteinase-3 (PR3) specificity has proven to be a valuable serological hallmark in the differential diagnosis of Wegener's disease.[1] The majority of papers focus on ANCA being highly specific, whereas only a minority deal with ANCA positivity as coincidental, i.e. 'false-positive', for example in screening settings. In the presented case we demonstrate the potential of ANCA as a diagnostic pitfall in the screening setting of clinical practice.

CASE REPORT

A 40-year-old male was admitted to the general internal medicine department for analysis of malaise with fever. He had experienced progressive fatigue during the past six months. There was no weight loss or loss of appetite. He did mention having a paronychium with a tendency toward delayed healing for a three-month period. Surgical debridement with amoxicillin/clavulanic acid 500/125 mg four times a day was prescribed, but the problem with wound healing persisted. He also mentioned having a non-productive cough for about a year, with slight progression over the last two months. At rest he experienced slight dyspnoea, and he had nasal symptoms with haemorrhagic crustae. During admission to the ward he had a fever of up to 39°C and chills despite the aforementioned antibiotic regimen. Physical examination revealed a feverish man with red cheeks and normal tension without tachypnoea: respiration frequency was 12/min. On auscultation of the lungs, dry inspiratory crackles were heard in the basal fields. His left digit finger was slightly inflamed but appeared to be healing. Further examination was unremarkable. X-rays of the thorax revealed some interstitial abnormalities in the lower left and right pulmonary lobes, without further abnormalities (*figure 1*). Laboratory investigations were as follows: ESR >90 mm/h, haemoglobin 7.9 mM (normal >8.2 mM), leucocytes 2.2 x 10^9/l (normal 4.0-10.0 x 10^9/l), platelets 143 x 10^9/l (normal 150-300 x 10^9/l), with only 0.5 x 10^9/l lymphocytes. For further data see *table 1*.
In order to rule out the possibility of an autoimmune disorder, blood was drawn for ANA and ANCA. The latter was analysed via an indirect immunofluorescent (IIF) test with serum on activated, ethanol-fixed human granulocytes (prepared within our laboratory). The cytological appearance (*figure 2*) shows the staining pattern, originally interpreted as typical c-ANCA. To determine the specificity of the antibody, a dot blot (BMD) was incubated with the patient's serum. The expected PR3 specificity could not, however, be confirmed; instead the analysis resulted in an myeloperoxidase (MPO)-positive c-ANCA pattern. The discrepancy between the cytological pattern and specificity led us to offer the sample to two other laboratories for reanalysis. The results are given in *table 2*.

Figure 1 *X-ray of lungs: interstitial alveolitis in immunodeficient patient*

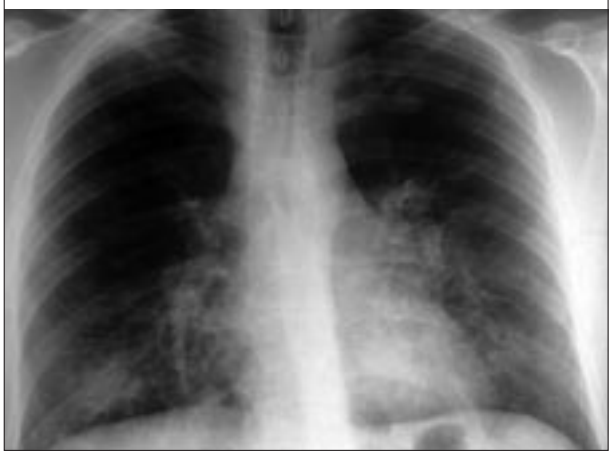

Figure 2 *Cytoplasmic ANCA*

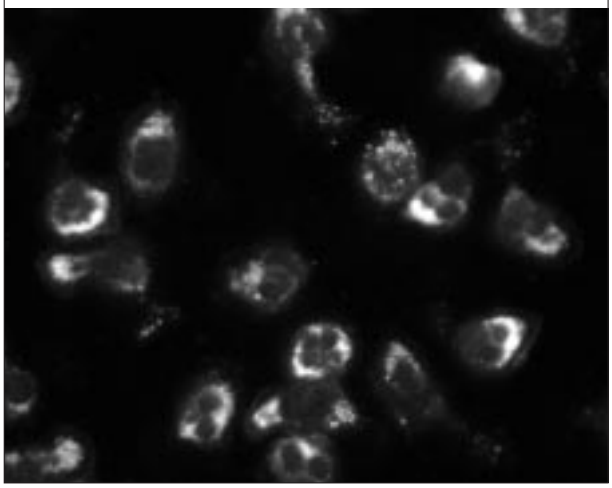

Table 1 *Biochemical findings in the presented patient*

Hb	7.9 (8.0-10.6) mmol/l	**Ht**	0,39 (0.40-0.52) l/l
ESR	>90 (<15) mm/h	**CRP**	10 (<10) mg/l
Leucocytes	2.2 (4.0-11.0) 10^9/l	**Neutrophils**	1.3 (2.0-7.5) 10^9/l
Lymphocytes	0.5 (0.8-3.2) 10^9/l	**Platelets**	143 (150-400) 10^9/l
Sodium	137 (136-146) mmol/l	**Potassium**	3.5 (3.5-5.0) mmol/l
Creatinine	71 (<105) µmol/l	**Urea**	2.5 (2.8-7.5) mmol/l
LDH	540 (<475) U/l	**ASAT**	30 (<45) U/l
ALAT	37 (<45) U/l	**AP**	90 (<120) U/l

Normal values are given between brackets. Hb = haemoglobin; ESR = erythrocyte sedimentation rate; LDH = lactate dehydrogenase; ALAT = alanine aminotranspeptidase; Ht = haematocrit; CRP = C-reactive protein; ASAT = aspartate aminotranspeptidase; AP = alkaline phosphatase.

Table 2 *Serological findings from our laboratory and two reference laboratories*

	Our lab	Lab 1	Lab 2
ELISA technique	Direct	Capture	Direct
ANCA pattern	c-ANCA	Atypical or c-ANCA-like	c-ANCA
Titre	1:80	1:320	1:80
Specificity	MPO	Non	MPO
Interpretation	MPO	Atypical	MPO

Lab 1 = serology laboratory university hospital 1; Lab 2 = serology laboratory university hospital 2.

Table 3 *Differential diagnosis of CD4 lymphocytopenia*[17]

Infection
Retrovirus: HIV-1, HIV-2, HTLV-1
Herpesvirus: varicella zoster virus, human herpes virus type 6, cytomegalovirus
Adenovirus/parvovirus/papillomavirus/rubeolavirus
Hepatitis B virus
Protozoa: leishmania
Ricketsia
Fungals: histoplasma, cryptococcus, coccidiomycosis
Bacterial: tuberculosis, brucella
Autoimmune disease
Lupus, primary Sjögren's syndrome,[18] etc.
Malnutrition
Medication
Corticosteroids
Lymphoreticular malignancy
Lymphoproliferation
Thymoma
Lymphangiectasia
Congenital disorders
Hypogammaglobulinaemia
Di George syndrome
Ageing
Pregnancy

Nasal biopsies were performed by the ENT specialist and the rheumatologist was consulted for confirmation of suspected Wegener's disease and for subsequent treatment options. On consultation no signs or symptoms were seen of arthritis, arthralgia, uveitis or neuropathy. The urine remained unremarkable. Additionally, history was taken focussed on the infectious diathesis and the patient confessed to having had various sexual contacts all over Europe without protection. There was a leucocytopenia, confirmed by a hand differentiation with specifically a CD4-positive lymphocytopenia: CD4-positive lymphocytes 18 x 10^6/l (normal 460-1450 x 10^6/l) and CD8-positive lymphocytes 123 x 10^6/l (normal 210-950 x 10^6/l) with a CD4/CD8 ratio of 0.15 (normal >1.0); see *table 3* for a differential diagnosis. A HIV infection was suspected and, after consent had been obtained from the patient, a rapid HIV test was requested. At the same time the following results came back: bacteriological analysis of the broncho-alveolar lavage revealed *Pneumocystic carinii* and the rapid HIV test was positive; further analysis revealed a high viral load. The patient was concluded to have AIDS category C3 with *Pneumocystic carinii* pneumonitis and a coincidental MPO-positive ANCA without clear signs of vasculitis. He was treated with cotrimoxazole-sulfamethoxazole and subsequently with highly active antiretroviral therapy. The positive ANCA gradually resolved.

DISCUSSION

Tests for circulating ANCA with specificity for MPO or PR3 are of considerable value in the diagnosis of the spectrum of vasculitides including Wegener's granulomatosis, microscopic polyangiitis, the Churg-Strauss syndrome, idiopathic necrotising and crescentic glomerulonephritis, and related overlapping forms of vasculitis.[1] Patients meeting two or more of the four American College of Rheumatology criteria can be classified as having Wegener's granulomatosis with a sensitivity of 88% and a specificity of 92%. The triggers that induce ANCA positivity, however, are largely unknown. Furthermore, in some cases ANCA positivity has been associated with medication: hydralazine,[4,5] minocycline[6,7] and propylthiouracil.[8,9] Cocaine abuse has sporadically been associated with ANCA.[3,10,11] Positivity of ANCA has also been found secondary to HIV infection. HIV infection may evoke ANCA in 20 to 83% of cases,[2,12-15] probably due to polyclonal activation of B cells,[12,15] but not associated with hypergammaglobulinaemia.[2] Savige *et al.* found 44 patients (42%) with ANCA on immunofluorescence testing out of 105 HIV-infected patients, including 26 with MPO specificity,[13] whereas Cornely *et al.* found 40 ANCA-positive patients (20%) out of 199 HIV-infected patients, 67 of whom revealed an atypical pattern and 33% a p-ANCA pattern.[14] Cornely *et al.* found MPO positivity in only one out of 199 HIV patients (0.5%),[14] whereas Koderich *et al.* found a faint c-ANCA positivity in 24 out of 29 HIV-infected patients (83%).[15] The last-mentioned 24 HIV-infected patients had homogeneous cytoplasmic ANCA probably representing nonspecific Fc-receptor binding of immunoglobulin G since this staining pattern was found particularly in 15 hyper-gammaglobulinaemic patients in this group. None of these HIV-infected patients showed p-ANCA, but five patients were repeatedly borderline positive in ANCA-enzyme-linked immunosorbent assays (ELISA) whereas three patients had positive MPO-ELISAs. Clinicians should be aware of the possibility of a false-positive ANCA, particularly in view of the current HIV

epidemiology. This warning may become more and more relevant for clinicians with the increasing application of protocol screening and new ANCA test systems in systemic vasculitis.[15]

Selective reading by young clinicians, as well as premature closure of the diagnostic process, and publication bias on the specificity of ANCA may lead to erroneous interpretation particularly with a confounding clinical copresentation of deformation of the nasal septum,[3] or pulmonary symptomatology as in the presented case. Our patient presented with nasal formation of crustae and an unproductive cough for a period of about a year. Physical examination revealed signs of inflammation with a subfever of up to 39.0°C with certain Wegener-compatible interstitial abnormalities on chest X-rays, which turned out to be due to *Pneumocystis carinii*. At first leucocytopenia and thrombocytopenia were both overlooked and the clinician's attention was drawn to abnormalities in serology, as our laboratory rapidly delivered a positive c-ANCA.

The diagnostic accuracy of ANCA was evaluated in a large multicentre European collaborative study including 169 newly diagnosed and 189 historic patients with idiopathic systemic vasculitis or rapid glomerulonephritis, and 184 disease controls and 740 healthy controls.[16] Both indirect immunofluorescence testing and anti-PR3 and anti-MPO ELISA were evaluated. The sensitivity of c-ANCA for Wegener's disease was 64% using the indirect immunofluorescence test, which could be improved to up to 73% by using a combination, either c-ANCA plus anti-PR3 or p-ANCA plus anti-MPO. In these validation populations no HIV-infected patients were included as far as we know. Even though clear symptoms of Wegener's disease are absent, there seems to be a real risk that clinicians may jump to conclusions in cases with a clinical suggestion of Wegener's granulomatosis. One may be asked for confirmation of the diagnosis and subsequent treatment. This may result in a potentially dangerous situation as cytotoxic therapy for Wegener's disease may well be fatal in AIDS. A similar situation has been described by Rowshani *et al.*, who reported a case with cocaine abuse and a subsequent erroneous interpretation of the clinical status with subsequent erroneous cytotoxic treatment.[3]

Specificity analysis of the antineutrophilic antibodies in the presented case, however, revealed MPO specificity. Others have reported on MPO-positive Wegener's disease; then staining specificity is, however, not cytoplasmic but perinuclear (as is expected with MPO specificity). Sera were sent for a second opinion to two university laboratories. The discrepancy between the results from these two laboratories was striking but this incongruence partly results from the different interpretation strategies and partly from a different laboratory technique: a direct ELISA has a different specificity than a capture ELISA. Capture ELISA is superior to direct ELISA with respect to specificity as it captures the MPO which therefore does not cross-react. The typical ANCA staining pattern shows accentuation of fluorescence intensity in the area within the nuclear lobes. Every positive IIF should be followed by an antigen-specific test for PR3 and MPO. PR3-ANCA is highly specific for idiopathic small-vessel vasculitis, whereas MPO-ANCA have been reported in various conditions not associated with vasculitis, i.e. specific drugs, infections and some connective tissue diseases.

The presented patient was sexually promiscuous and on presentation had a leucocytopenia with specifically a lymphocytopenia. Further analysis even revealed a specific CD4-positive lymphocytopenia. This leads to a differential diagnosis focussed on sexually transmittable diseases including HIV (*table 3*).[17,18] Indeed HIV serology was positive. The load of HIV replicates was determined and found to be sky-high. ANCA disappeared spontaneously during specific anti-HIV therapy. The patient did not develop other rheumatological problems i.e. no clinically apparent vasculitis, arthritis or arthralgia. Monteagudo *et al.* found a systemic necrotising vasculitis in only 1% of 106 cases with drug-induced AIDS.[19] In the presented c-ANCA positive, MPO-positive patient a vasculitis was not, however, found.

We conclude that in view of the increasing screening application of ANCA, one should be aware of false-positive results in all clinical presentations, in cases without specific *a priori* risk, and only vague upper airway/pulmonary symptomatology. History taking in ANCA-positive patients should include data on sexual promiscuity and other risk factors of HIV infection. A careful work-up is mandatory in patients in whom Wegener's granulomatosis is suspected, even when c-ANCA is suggested or even found positive. This report may serve again as a warning for the pitfall of positive ANCA in HIV-infected patients.

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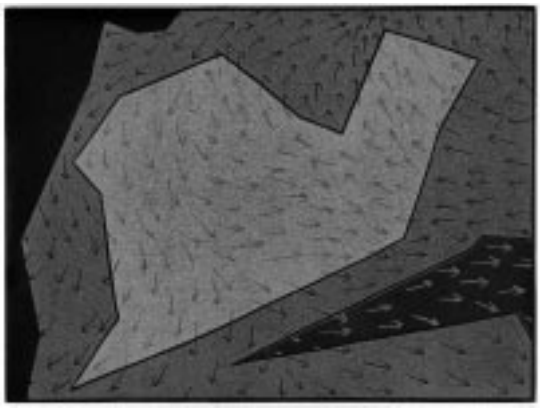
ABOUT THE COVER

'Biesbosch eilandenkaart VIII'

Miek Coppens



This month's cover is a linocut made by Miek Coppens entitled 'Biesbosch Eilandenkaart VIII'.

Miek Coppens (1944) attended the Academy of Arts in Tilburg. After graduation she studied in Hungary at the International Art Colony. From 1992 she exhibited her work in several group exhibitions in the Netherlands. In 1996 she made her breakthrough internationally and exposed her work from Japan to Russia and in many countries in between. Her work has been appreciated, which has resulted in her receiving many awards such as the Certificate of Merit, 3rd International Female Artists' Art Annual in Stockholm and the Certificate of Merit Mask II in Venice.

An original print of this linocut (limited edition of four) is available at a price of € 250 and can be ordered from Galerie Unita, Rijkkstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl or www.galerie-unita.com.

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CASE REPORT

Recurrent pancreatitis after trimethoprim-sulfamethoxazole rechallenge

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ABSTRACT

We report a female patient who repeatedly developed pancreatitis after trimethoprim-sulfamethoxazole (TMP/SMX) use. During childhood she had undergone an ureterosigmoidostomy after which she had been on TMP/SMX 480 mg daily as prophylaxis for pyelonephritis for many years. The patient presented with abdominal pain caused by acute pancreatitis. No other cause, except for TMP/SMX use, could be identified. A causal relationship was confirmed by relapse of the pancreatitis after rechallenge. Our case is unique in demonstrating that acute pancreatitis related to the use of TMP/SMX may occur even after long-term treatment. We advise that the medication is discontinued immediately if a causal relationship with pancreatitis is suspected.

KEYWORDS

Co-trimoxazole, drugs, pancreatitis, rechallange, trimethoprim-sulfamathoxazole

INTRODUCTION

Drug-related acute pancreatitis remains an uncommon clinical entity. Some cases of pancreatitis associated with the use of trimethoprim-sulfamethoxazole (TMP/SMX) have been reported previously (*table 1*).[1-6] In all of these, clinical symptoms occurred within days to weeks after the onset of treatment. We report a case that is unique in that acute pancreatitis occurred after many years of treatment with TMP/SMX. Furthermore, a causal relation was demonstrated by recurrent symptoms following drug rechallenge.

Table 1 *Documented case reports of TMP/SMX-induced acute pancreatitis*

Reference	Indication for TMP/SMX use	Daily TMP/SMX treatment (mg)	Onset (days) of pancreatitis after start of TMP/SMX	Onset of pancreatitis after rechallenge
1	Occipital abscess with *Nocardia asteroids*	960/4800	7	3
2	Urinary tract infection	320/1600	Only hepatitis after first exposure	'Several days' (hepatitis and pancreatitis)
3	Ear infection	320/1600	5	No rechallenge
4	Occipital abscess with *Nocardia*	1600/8000	42	No rechallenge
5	*Pneumocystis carinii* pneumonia (HIV pos.)	1280/6400	10	No rechallenge
6	*Pneumocystis carinii* pneumonia (HIV pos.)	900	6	No rechallenge

CASE REPORT

A 53-year-old woman presented with epigastric pain, radiating to the back. Her history revealed an ureterosigmoidostomy performed at the age of four years, according to the Coffey technique, because of extrophia vesicae. Next, she developed numerous episodes of pyelonephritis for which treatment with prophylactic antibiotics in the form of TMP/SMX (480 mg daily) had been initiated more than twenty years before. In addition, in later years hyperthyroidism and ulcerative colitis were diagnosed. Also, she developed a prolapsed uterus for which vaginal surgery was performed. At the time of presentation, she was not taking any drugs other than TMP/SMX.

The patient complained of continuous, left-sided epigastric pain, radiating to the back, which had been present for four days. The pain reminded her of an episode, one year earlier, during which she suffered from epigastric pain and nausea that increased after eating. At that time, the serum amylase had been normal.

During the current episode of epigastric pain she did not report nausea, vomiting or weight loss, and she had a good appetite. There was no alcohol or nicotine abuse. Physical examination was not remarkable, except for a mild tenderness in the epigastric region and some percussion pain in the left kidney region. There was no hepatosplenomegaly. Laboratory evaluation revealed an increase in the serum amylase, a metabolic acidosis and symptoms of inflammation (*table 2*). Other routine laboratory values were normal. Plain abdominal X-ray only showed a colon filled with faeces. Abdominal ultrasonography was not remarkable except for a uterus myomatosis. The pancreas could not be visualised due to air-filled intestines.

Gastroduodenoscopy showed no abnormalities, especially not in the peripapillary region. Therefore, the diagnosis of acute pancreatitis was made. The patient was treated conservatively (nil by mouth) and received paracetamol 1 g/6 h, as a suppository, for pain relief. Bicarbonate was provided to correct the metabolic acidosis, which was ascribed to bicarbonate loss through the ureterosigmoidostomy. TMP/SMX was continued. The clinical condition improved within three days and the CRP and serum amylase level decreased to 124 mg/l and 151 U/l respectively. On the fourth day the patient was discharged. A magnetic resonance cholangiopancreatography (MRCP), which was performed after discharge, showed no abnormalities, especially no bile stones. The pain gradually decreased. Since pancreatitis can be caused by TMP/SMX, this antibiotic was replaced by ciprofloxacin. Eventually, the abdominal symptoms disappeared completely.

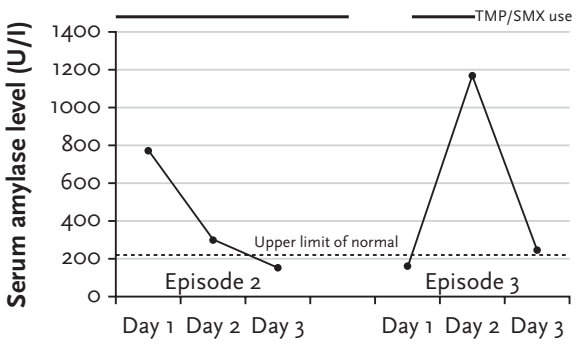
After some weeks of treatment with ciprofloxacin, the patient developed headaches, diarrhoea, fever and swollen axillary glands. Ciprofloxacin was stopped and TMP/SMX (480 mg twice a day) restarted. The serum amylase level was normal (156 U/l) on the day treatment was restarted (day 1, episode 3, *figure 1*). Within four days the epigastric pain recurred and the amylase level again increased to 1161 U/l (day 5, episode 3, *figure 1*). The diagnosis of a relapse of acute pancreatitis was made. TMP/SMX was now replaced by amoxicillin-clavulanic acid. After two days, the serum amylase decreased to 245 U/l and the epigastric pain disappeared. Because the patient developed abdominal discomfort while taking amoxicillin-clavulanic acid, trimethoprim was prescribed. In the following months she did not take any sulphonamides and did not have any further episodes of epigastric pain.

Table 2 *Laboratory values at admission (day one, episode two)*

CRP	141 mg/l
Amylase	782 (<220) U/l
pH	7.31
pO_2	11.6 kPa
pCO_2	5.1 kPa
Bicarbonate	18.9 mmol/l
Base excess	-6.8 mmol/l
Calcium	2.44 (2.20-2.60) mmol/l
Triglycerides	0.71 (0.8-2.0) mmol/l
Alkaline phosphatase	68 (<120) U/l
ASAT	25 (<40) U/l
ALAT	15 (<45) U/l
γGT	14 (<35) U/l

Normal values are given between brackets. CRP = C-reactive protein; ASAT = aspartate aminotranspeptidase; ALAT = alanine aminotranspeptidase; γGT = gamma glutamyl transpeptidase.

Figure 1 *Serum amylase (Y-axis) in time in the absence or presence of TMP/SMX exposure*



Since serum amylase was normal in the first episode of epigastric pain, this episode is not shown in the figure. Time between episode two and three is approximately two months. Day one of the second episode represents the visit to the emergency department because of epigastric pain. Day one of the third episode represents the day of restarting TMP/SMX.

DISCUSSION

Drugs are classified as having a definite relation with the development of pancreatitis if there is a temporal relationship between drug administration and the signs and symptoms of pancreatitis or a positive response to direct (re)challenge with the drug.[7] Previous case reports have demonstrated/suggested an association between pancreatitis and TMP/SMX (*table 1*).[1-6] Sulfamethoxazole is generally held responsible since sulphonamide derivates have been associated with pancreatitis while trimethoprim alone has not.[8,9] A definite causal relationship between sulfamethoxazole administration and pancreatitis, shown by rechallenge, has only been demonstrated in a few cases.[1,8] The pancreatitis-inducing mechanism remains unclear. An allergic reaction has been assumed.[8]

Here we report a patient who developed acute pancreatitis after having taken TMP/SMX for many years as prophylaxis for pyelonephritis. During her first episode of epigastric pain, the diagnosis of acute pancreatitis was considered, but based on her clinical condition and a normal serum amylase level, measured four days after the first symptoms, a diagnosis of gastric ulceration seemed more likely. Retrospectively, it seems probable that our patient had already suffered a bout of acute pancreatitis during that episode. During her second episode of epigastric pain, serum amylase levels were definitely elevated and acute pancreatitis was diagnosed. Known causes of acute pancreatitis include alcohol abuse, cholelithiasis, hyperlipidaemia, hypercalcaemia, pancreatic trauma, viral infections and drugs. Based on a negative history of alcohol abuse and pancreatic trauma, normal values of liver enzymes, triglycerides and calcium, and the absence of ulcers, bile stones and other liver or bile duct abnormalities on a plain abdominal X-ray, abdominal ultrasonography, gastroduodenoscopy and MRCP, all of the above-mentioned causes could be excluded with the exception of drugs. This is supported by the fact that discontinuation of TMP/SMX treatment resulted in her symptoms disappearing completely. However, a causal relationship was finally confirmed when we observed a relapse of acute pancreatitis within four days of restarting TMP/SMX because of adverse effects to prophylaxis with ciprofloxacin. Since trimethoprim alone has not been associated with acute pancreatitis, we eventually decided to use trimethoprim as a prophylactic drug for pyelonephritis.

The interval between starting the cotrimoxazole and the onset of pancreatitis was long. For various drugs it is well known that complications do not occur immediately after the start of the drug therapy, as for instance for azathioprine. In a recent review paper it was stated that 'Cutaneous reactions are hypersensitivity reactions and they usually manifest immediately after exposure or re-exposure to the drug, unlike other adverse reactions, which might only manifest at high doses or after prolonged therapy'.[10] The absence of a rash in the patient presented in this paper does not support the hypothesis of a hypersensitivity reaction as the cause of the pancreatitis.

Our case is unique in that it demonstrates a definite causal relationship between acute pancreatitis and TMP/SMX in a patient who had been taking this antibiotic continuously for more than 20 years. Based on this experience, we advise that this medication is immediately discontinued if a causal relationship with pancreatitis is highly suspected.

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PHOTO QUIZ

A patient with prolonged vague pain in the lower abdomen following a three-day period with diarrhoea and vomiting

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CASE REPORT

A 73-year-old woman presented to the emergency department with progression of pain in the lower abdomen. The pain developed two months earlier, following pneumonia treated with antibiotics. Initially, it was accompanied by a short episode of diarrhoea and vomiting, which spontaneously resolved. She had a documented history of rheumatoid arthritis, diabetes mellitus type 2, hypertension, hypercholesterolaemia, myocardial infarction and cholecystectomy.
On admission there were no other abdominal symptoms. She was only having one bowel motion a day with lactulose intake. Her daily oral food intake was normal and there was no history of weight loss. Her rheumatoid arthritis has been stable for years. She stopped smoking more than 20 years ago. Current medication consisted of insulin, prednisone, a depot methylprednisolone every ten weeks, nitrazepam, acetylsalicylic acid, diclofenac, omeprazol, bumetanide, metroprolol, folic acid and lactulose. Physical examination revealed a moderately ill patient with a blood pressure of 140/80 mmHg, a pulse rate of 85 beats/min and a body temperature of 37°C. On auscultation active bowel sounds were heard. On palpation a localised tenderness in the lower abdomen, somewhat more pronounced in the right lower quadrant, was found. Blood tests revealed 17.2 x 10^9 leucocytes (88% neutrophils, 8% monocytes, 4% monocytes), CRP 144 mg/l, creatinine 69 μmol/l, alkaline phosphate 146 U/l, ASAT 11 U/l, ALAT 22 U/l, γGT 25 U/l, amylase 63 U/l, lactate 1.8 mmol/l and glucose 15.1 mmol/l. The urine examination was normal, except for glucosuria. The gynaecological examination was normal. A contrast-enhanced CT scan of the abdomen is presented in *figures 1* and *2*.

WHAT IS YOUR DIAGNOSIS?

See page 285 for the answer to this photo quiz.

Figure 1 *A transverse contrast-enhanced abdominal CT scan of the patient*

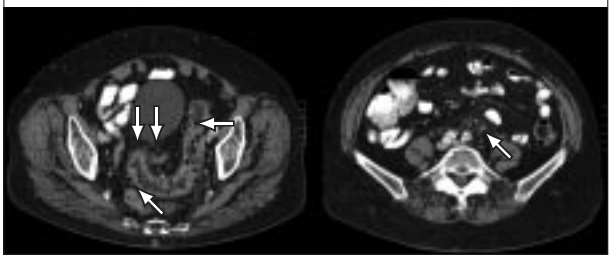
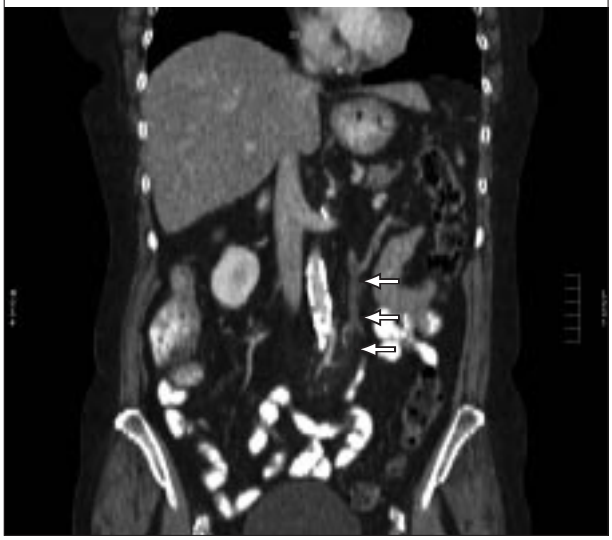


Figure 2 *Coronal reconstruction of the abdominal CT scan*



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SPECIAL REPORT

Global clinical performance rating, reliability and validity in an undergraduate clerkship

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ABSTRACT

Background: Global performance rating is frequently used in clinical training despite its known psychometric drawbacks. Inter-rater reliability is low in undergraduate training but better in residency training, possibly because residency offers more opportunities for supervision. The low or moderate predictive validity of global performance ratings in undergraduate and residency training may be due to low or unknown reliability of both global performance ratings and criterion measures. In an undergraduate clerkship, we investigated whether reliability improves when raters are more familiar with students' work and whether validity improves with increased reliability of the predictor and criterion instrument.

Methods: Inter-rater reliability was determined in a clerkship with more student-rater contacts than usual. The in-training assessment programme of the clerkship that immediately followed was used as the criterion measure to determine predictive validity.

Results: With four ratings, inter-rater reliability was 0.41 and predictive validity was 0.32. Reliability was lower and validity slightly higher than similar results published for residency training.

Conclusion: Even with increased student-rater interaction, the reliability and validity of global performance ratings were too low to warrant the usage of global performance ratings as individual assessment format. However, combined with other assessment measures, global performance ratings may lead to improved integral assessment.

KEYWORDS

Clerkship, disattenuation, global clinical performance rating, inter-rater reliability, predictive validity

INTRODUCTION

Evaluation of clinical performance typically takes the form of a global rating by a supervisor, halfway or at the end of a clinical rotation, covering learners' performance on a number of clinically relevant competencies over a certain period of time. Hereafter, we will refer to this type of rating as global performance rating (GPR). Despite the availability of new assessment methods, GPRs continue to be frequently used in both undergraduate and residency training, most probably due to the combined advantage of feasibility and face validity (the assessed performance represents the performance domain of interest). In undergraduate training, GPRs are often the primary determinant of the final grade a student receives at the end of a clerkship.[1,2] Moreover, despite measures to increase the reliability of GPRs, such as rater training, in practice most assessors are not trained. At best the items on a scale are anchored to descriptors of criterion behaviour. In the last two decades several studies have examined the reliability and validity of GPRs by untrained assessors in both undergraduate and residency training.

For inter-rater agreement among members of staff as a measure of the reliability of GPRs in undergraduate training the findings varied, with inter-rater agreement ranging from 0.29-0.42.[3,4] Studies performed in residency training have consistently demonstrated higher inter-rater agreement (0.79-0.87) than studies among undergraduate students.[5-8]

A possible explanation for this difference may be that clinical staff, who typically evaluate students' and residents' performance, generally have more opportunity to supervise the work of residents than that of students because residency rotations last longer than clerkship rotations.[9,10] Moreover, because residents treat patients, supervision of residents is necessary to ensure the provision of appropriate patient care. Staff members have a strong professional stake in an adequate performance by residents, because they may be held liable for adequate supervision.[11] Assuming that the reliability of assessment benefits from increased supervision, we designed a study in which we measured inter-rater agreement on GPRs in a setting where staff members supervised students' work more frequently than is customary in undergraduate clerkships. If our assumption is correct, we would expect inter-rater agreement in this setting to be moderate to quite high.
Studies have investigated both concurrent and predictive validity of GPRs by untrained assessors. Both concurrent and predictive validity indicate the extent to which GPRs predict scores on a selected criterion that is not directly measured by the assessment but that is assumed to be parallel. For concurrent validity the criterion measurement is performed at the same time, for predictive validity it is performed at some point in the future. *Concurrent validity* has been studied in both undergraduate and residency training by correlating GPRs with more objective performance measures for the same training period, such as written examinations, OSCEs or simulated patient exams.[6,12-14] Correlations ranged from 0.19 to 0.33 for undergraduate training and from 0.29 to 0.56 for residency training. Fewer studies have addressed the *predictive validity* of GPRs. In undergraduate training predictive validity has been determined by comparing GPRs of student performance in different rotations with GPRs of end-of-clerkship performance or of performance in residency training. Predictive validity ranged from 0.17 to 0.44.[12,15] Callahan examined the predictive validity of GPRs in clerkships for the results on United States Medical Licensing Examinations (USMLE) steps 2 and 3. The maximum predictive validity was 0.29 for USMLE step 2 and for USMLE step 3 it was 0.20.[15] In residency training the predictive validity of GPRs for performance at in-training and American Board of Internal Medicine certification exams was reported to be moderate for overall competence (0.19) and for specific competencies on a global performance rating scale (ranging from 0.11 to 0.41).[8,16] The validity coefficients reported in these studies suffered from attenuation, i.e. low or unknown reliabilities in predictor and criterion variables introduce inaccuracy into the calculation. When a measurement error is present in one or both of the variables that are being correlated, the correlation coefficient that is obtained will be attenuated. This implies that the observed correlation coefficient between less than perfectly reliable scores will tend to underestimate the true level of co-variation between the predictor and criterion variables.[17] Therefore, if the reliability of either the predictor or the criterion variables is low, validity coefficients will also be low. This effect might have been even stronger in studies in undergraduate training settings, where the reliability of global ratings was typically low and the reliability of the criterion variable mostly unknown. That is why we considered it worthwhile to examine the predictive validity of GPRs in undergraduate training using a criterion variable of known and acceptable reliability. If staff members have more opportunities to supervise students' performance the reliability of GPRs in undergraduate medical training might benefit.

We thus sought to answer the following research questions: what is the reliability of GPRs in an undergraduate clerkship with increased rater-student interactions? And, because of the inaccuracy in validity estimates of GPRs due to the low or unknown reliabilities of predictor and criterion variables (attenuation): what is the validity of GPRs when the reliabilities of both predictor and criterion variables are assumed to be perfect (disattenuation correction)?
We addressed the research questions by determining the inter-rater agreement for GPRs in an undergraduate clerkship with extensive interaction (detailed below) of staff members (raters) and students. These GPRs were then compared with a valid and reliable performance measure for the competencies demonstrated by the same students in the next clerkship that immediately followed, with less student-staff interaction. The performance measure used in the second rotation was the overall score on an in-training assessment programme (ITA) consisting of several assessments of clinical competence.

MATERIALS AND METHODS

Educational background

At the Vrije Universiteit Medical Centre (VU Medical Centre), Amsterdam, the Netherlands, four years of preclinical medical education are followed by two years of rotations in the major clinical disciplines. The clinical phase starts in year 5 with a three-week introductory clerkship in which the students are closely supervised by clinical staff. The students' main tasks are history taking and physical examination, medical record writing and practising skills in pathophysiological thinking and clinical reasoning in structured discussions both in groups supervised by a member of staff and in writing. Staff members are scheduled to supervise the group discussion, discuss the medical records and observe (parts of) history taking and physical examination. Every day a different staff member supervises the students in their daily structured group discussion, which lasts about an hour. The supervisor asks several

students to elaborate on their findings, interpret data, formulate differential diagnoses and propose additional investigations. Over the course of the clerkship, students are supervised twice by a member of staff while performing a (scheduled) complete patient interview and physical examination. Afterwards student and staff member discuss the student's performance. For morning and evening reports, radiology meetings, interdisciplinary meetings et cetera, students are not linked to residents but to members of staff, who are thus more focussed on students' contributions. In most cases, a student is supervised by six to seven members of staff during the three-week rotation. At the end of the three weeks, a staff member to whom this task has been assigned determines a GPR for the student's performance during the rotation. Next, the students move on to the ten-week internal medicine rotation in the VU Medical Centre or in one of the affiliated hospitals. This rotation is scheduled immediately after the introductory clerkship. In this rotation the students are usually supervised by residents instead of members of staff during student-patient interactions and for medical records. This rotation involves more participation by the students in day-to-day clinical practice, including multidisciplinary meetings and on call duties. In order to better monitor students' performance in the internal medicine clerkship, a programme of systematic observation and documentation of students' actual performance (detailed below) has been introduced in the rotation in the VU Medical Centre.

Global clinical performance rating

Eight supervisors of the introductory clinical rotation were approached during a staff meeting and asked to participate in the study. Participation entailed giving a global performance rating on a five-point Likert scale (1 = fail, 2 = borderline, 3 = pass, 4 = high pass, 5 = excellent) for every student doing the rotation in the study period. The raters received a brief description to be used in rating students' performance. The description mainly focused on the comparison of the student's performance with that of an average student in his/her first three weeks of clinical rotation. On a student's last day of this rotation, the members of staff received a form together with a scanned picture of the student concerned. The members of staff who had interacted with the student were asked to complete and return the form. The members of staff who had not supervised the student could return the form without filling it in. The entire procedure was computerised. We used a single-item rating (global performance) to preclude the use by raters of only one or two items (dimensions of performance) of a larger scale to judge global performance.[5,6,16,18,19] Each participating staff member was asked to complete one evaluation form for each student during the study period. In this way students could receive a maximum of eight GPRs from different examiners.

In-training assessment

All students proceed from the introductory rotation to the internal medicine rotation. In the internal medicine rotation in the VU Medical Centre a fully integrated ITA programme is used. ITA implies systematic observation and documentation of the learners' actual performance using several formats.[20] ITA in undergraduate clinical training has been described as a feasible assessment format that has reasonable reliability and good content validity.[20-22] The ITA programme used in this study consisted of observation and documentation of students' actual performance in five test formats.[22] A minimum frequency per student over the entire clerkship was specified for each test format, resulting in a required total of 19 assessments: three single-sample formats (student-patient encounter, critical appraisal session and case presentation) and two multiple-sample formats (12 case write-ups and four structured long cases). The student-patient encounter, critical appraisal session, case presentation and structured long cases were assessed by staff and the case write-ups by residents. All tests were rated on the five-point Likert scale and an overall score was obtained by calculating the mean of the scores and rounding it off to the nearest integer (1-5). The assessors of the ITA programme were not specifically informed about the current study.

Subjects: student participants

From April 2001 to October 2002, 91 students received global ratings of their performance in the introductory clerkship. We collected ITA scores for 48 of these 91 students. These 48 students did the subsequent ten-week internal medicine rotation in the VU Medical Centre, whereas the other students went to affiliated hospitals, where the ITA programme had not yet been implemented. A t-test on the means of the GPRs showed no differences between the GPRs of the students participating in the study and the students assigned to the affiliated hospitals.

Data analysis

First we counted the total number of GPRs per student. For further calculations we used the balanced dataset of the group of students for whom at least four GPRs were available.[23] For the analysis we used random samples of four GPRs per student.

We calculated means and standard deviations for the GPRs. Inter-rater reliability was estimated based on the generalisability theory. We used a one-facet design with raters (or GPRs) nested within persons (students) to estimate variance components. Subsequently, reliability coefficients, i.e. dependability coefficients, were calculated as a function of the number of examiners (or GPRs). In the clerkship studied, each member of staff supervised students in two to three group discussions and most probably several

times during reports and meetings. Only two members of staff witnessed complete student-patient contacts (interview and physical examination). As a result, these two staff members may have developed significantly different judgements than did other staff members. However, the fact that different selections of four members of staff did not yield significantly different ratings suggests that this was not the case.

We calculated means and standard deviations of the ITA scores. A similar generalisability design was used to estimate the reliability of the ITA programme, with observations across test formats nested within students.
The predictive validity of the GPRs was determined by correlating the mean GPR with the mean ITA score. We estimated the disattenuated correlation (the estimated correlation when both predictor and criterion measures have perfect reliability) using the reliability coefficient of the GPRs with four raters and the reliability of the ITA programme.

RESULTS

Of the 91 students whose performance was rated in the introductory clerkship, 87 received four or more GPRs (*table 1*). Four students were rated by fewer than four staff members. Each of the eight members of staff who participated in the study contributed to the GPRs throughout the duration of the study. Having had no interaction with the student, holidays and illness were the main reasons given by staff members for not having witnessed a student's performance and thus being unable to provide a GPR.
The mean GPR was 3.19 (SD 0.37). Inter-rater reliability with four GPRs per student was 0.41 (n=87). Twenty-five GPRs per student would be needed to reach sufficient reliability (0.8) (*table 2*).

Table 1 *The number of global performance ratings (GPRs) per student*

Number of GPRs	2	3	4	5	6	7	8
Number of students	1	3	13	30	16	23	5

Table 2 *Reliability coefficients as a function of the number of examiners or global performance ratings (GPRs)*

Number of GPRs	4	6	10	25
Reliability coefficient	0.41	0.51	0.63	0.81

Means and standard deviations of the different ITAs ranged from 3.61 (SD 0.65) for case write-ups to 4.35 (SD 0.65) for case presentations (*table 3*).
The reliability of the ITA programme was 0.71. The observed predictive validity of the GPRs for the ITA programme was 0.32 ($p<0.05$) and the disattenuated predictive validity was 0.59.

Table 3 *Mean scores (standard deviations; SD) for the different tests in the in-training assessment (ITA) programme*

ITA	Mean (SD)
Student-patient encounter	3.70 (.70)
Critical appraisal session	4.00 (.67)
Case presentation	4.35 (.65)
Write-up	3.61 (.65)-4.04 (.71)
Structured long case	4.00 (.67)-4.26 (.63)

DISCUSSION

Global ratings have some well-known disadvantages. They are often only given at the end of a rotation when assessors may have forgotten details of student's performance. In addition, they may be biased due to a halo effect, i.e. the phenomenon that an impression created by a student's good or poor performance in one area affects assessors' judgements of that student's performance in another area.[24]
In the introductory clerkship, we could easily have used structured assessment with rating forms, such as in-training assessment. However, we purposely used global ratings, because in this study we set out to investigate the possibility of improving the reliability and validity of such ratings, as they continue to be much used in undergraduate and graduate training. With improved reliability and validity, global ratings could make a truly positive contribution to assessment of clinical performance, the more so since they can cover more competencies than assessment formats focused on specific items.[25]
We investigated the reliability and the predictive validity of GPRs in undergraduate training. We studied the reliability of GPRs in an introductory clerkship where the members of staff who rated the students supervised students' performance more frequently than is customary in undergraduate clerkships. We expected that this would yield a better, i.e. moderate to high, reliability than is generally reported for GPRs in undergraduate clinical training. We observed an inter-rater reliability of 0.41, which is comparable with the literature on undergraduate inter-rater agreement. We speculate that the potentially positive influence of increased supervision of students' clinical work by staff may have been mitigated by the limited duration of the clerkship as compared with residency rotations.[26]

A relatively shorter period during which staff are in a position to supervise students may lead to a correspondingly diminished accuracy of the perceived levels of students' performance. Hence increased supervision did not result in improved reliability. Furthermore, reliability may have (slightly) suffered on account of staff not having participated in assessment training before this study was conducted.[27,28] The results showed that 25 GPRs from different examiners would be needed to achieve adequate reliability. Other studies have yielded estimates of between 7 and 14 ratings to attain a reliability of 0.80.[5,29,30] However, the assessment formats on which the GPRs in those studies were based included aspects that might potentially improve reliability, such as a long duration of the student-staff work relationship (up to one year), a highly detailed description of the behaviour associated with the low and high scale points on the rating scale and raters who were better acquainted with students' performance (e.g. resident ratings). We suspect that more ratings will be needed to reach acceptable reliability in undergraduate settings, where the working relationship of staff and students lasts only a short time and raters thus witness less of the students' work and have to judge performance without guidance from concrete descriptions of the behaviours corresponding to the different scale points.

The validity measure derived from the predictive validity of the GPRs for the scores on the ITA programme in the subsequent clerkship was 0.32. Although slightly higher than the predictive validity reported for overall competence in studies in both undergraduate and residency training, it is still quite low.[8,15] The GPRs in this study were based on staff members' evaluations of students at the end of a three-week rotation. Despite frequent student-staff interaction in these three weeks, details of the interactions can be lost quite quickly.[31,32] In contrast, the evaluations in the ITA programme were recorded immediately after the activity or behaviour that was evaluated and according to a checklist. Despite the more than usually intensive interaction between staff and students in the initial clerkship, the fact that the GPRs were based on less detailed information about students' clinical performance than the ITA scores may offer an explanation for the low predictive validity of the GPRs. Disattenuated predictive validity was 0.59, however, which is much higher. Our findings implicate that GPRs, despite being based on less detailed information, can still make a positive contribution to the evaluation of students' performance. In a recently published study, Kreiter and Ferguson found comparable disattenuated predictive validity when they compared global ratings of clinical clerkship performance with former measures of physical examination performance provided by simulated patients (SP) using ratings and checklists, and with SP ratings of rapport and communication.[33] They conclude that measures of skills by global ratings are correlated with other clinical performance measures and discuss that more studies of this topic are needed to conclude that global ratings make a positive contribution to students' evaluation and thus contributes to the conclusion that global rating can positively contribute to students' evaluation. The evidence in our study points in the same direction.

This study has one major drawback. We compared our findings to findings in the literature and not to those of a control group. The circumstances in which research in the presented literature was performed were certainly different from the circumstances of our study. However, it was practically not feasible to have a control group in the same clerkship at the same time due to staff shortage and the practical impossibility to have two educational programmes performed by the same members of staff during the same period of time.

Our results indicate that even when conditions in an undergraduate rotation are positively manipulated, reliability and validity of GPRs remain low. However, the reliability and validity we reached were not lower than those found for other assessment formats performed over a short testing time.[34,35] This means that GPRs can contribute to the assessment of undergraduate students' clinical competencies as long as they are sampled on many occasions and by many assessors. Nevertheless, sufficient reliability and validity are likely to be hard to achieve. In a recent review, Williams *et al.* concluded that GPRs by themselves were an insufficient measure of students' clinical competence, even though they might be an important source of information about it.[36] These authors recommended that GPRs should be supplemented with ratings of students' performance in standardised clinical encounters and assessment protocols. The results of our study point to a similar recommendation, i.e. to combine GPRs with more specific and reliable assessment formats, such as the ITA programme in this study, to arrive at an integrated assessment programme. Further studies will have to examine whether such an assessment programme can provide reliable and valid measures of students' competencies.

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ANSWER TO PHOTO QUIZ (ON PAGE 278)

A PATIENT WITH PROLONGED VAGUE PAIN IN THE LOWER ABDOMEN FOLLOWING A THREE-DAY PERIOD WITH DIARRHOEA AND VOMITING

DIAGNOSIS

The abdominal contrast-enhanced CT scan shows mild diverticulosis of the sigmoid (solid arrows, *figure 1*), with infiltration of the surrounding mesenteric fat (arrowheads, *figure 1*) extending along the inferior mesenteric vein (open arrow, *figure 1*). The wall of the inferior mesenteric vein is thickened and the lumen shows filling defects (arrowheads, *figure 2*) consistent with thrombosis. The splenic and portal veins are open, and there are no signs of appendicitis. These findings are very consistent with a thrombophlebitis of the inferior mesenteric vein complicating a mild sigmoid diverticulitis.

Thrombotic events of the mesenteric veins lack specific clinical symptoms and laboratory data. Mesenteric vein thrombosis is diagnosed in 5 to 15% of all mesenteric ischaemic events,[1] usually in the superior mesenteric vein. In about 75% of the patients it occurs secondary to abdominal inflammation, cancer, coagulation disorders, recent abdominal surgery, or cirrhosis in portal hypertension.[1,2]

Thrombophlebitis of the inferior mesenteric vein secondary to diverticulitis occurs infrequently,[3] and may be complicated by sepsis and intrahepatic abscesses. CT imaging helps to diagnose this complication at an early stage and can significantly improve the previously reported high mortality and morbidity rates associated with this condition. Conservative therapy with antibiotics, which target Gram-negative bacilli, anaerobes and enterococci, can lead to resolution of the thrombosis.[4] Although the effect remains controversial, in most cases anticoagulant therapy is started. Elective surgery may be performed to eradicate the primary inflammatory process when antibiotic therapy fails.

Immediately after admission, this patient was started on anticoagulant therapy. Because the abdominal pain persisted and the CRP concentration rose to 246 mg/ml, intravenous antibiotic therapy was started within 48 hours, resulting in a clinical improvement. Blood cultures obtained on the first and second day of admission were negative. After one week the antibiotics amoxicillin/clavulanic acid were continued orally and the patient was discharged. At follow-up the patient had made a full recovery, laboratory parameters normalised, and the CT scan no longer showed abnormalities.

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The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the editor are welcomed.

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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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