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ERRATUM

The review 'Antiviral treatment for chronic hepatitis B virus infection – immune modulation or viral suppression?' by E.H.C.J. Buster and H.L.A. Janssen, published in Neth J Med 2006;64(6):175-85, unfortunately contain the following incorrect items:

- HbeAG should read HBeAG
- HbsAG should read HBsAG
- On page 178, section 'Pegylated interferon-a', IFN-a should read INF-α

Our apologies go out to the authors of this article and to our readers. A correct version of the article can be found on www.njm-online.nl.

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EDITORIAL

The Dutch guidelines for treatment with infliximab for Crohn's disease

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In the current issue of the Netherlands Journal of Medicine, inflammatory bowel disease (IBD) specialists from the Dutch University Medical Centres publish their guidelines for the use of infliximab in patients with Crohn's disease.[1] Infliximab is a monoclonal antibody to tumour necrosis factor-alpha (TNF-α) and is the first biological therapy registered and approved for the treatment of this chronic inflammatory gastrointestinal disorder.
In these guidelines, the authors give a detailed and very practical overview of all aspects related to the use of infliximab. The indications and the effectiveness of the drug in each of these indications are given, based on the results from the controlled studies, together with practical guidelines on dosing, interval and monitoring of patients. The second part of the guidelines deals with safety aspects of infliximab. The authors need to be congratulated for this large and detailed piece of work. This manuscript provides very useful and practical flowcharts that will undoubtedly help the (Dutch) gastroenterologist in treating patients with Crohn's disease.

There are a few issues which merit additional attention and maybe some updating since inevitably, new results and additional safety information are gathered on an almost weekly basis.
The first relates to safety and in particular the risk of developing malignancies or infections. TNF plays an important role in host defence and in tumour growth control. Anti-TNF therapies may therefore potentially increase the risk of infections and malignancies. Although I agree with the authors that the safety data from the controlled trials, as well as from post-marketing surveillance and prospective safety registries (TREAT registry in particular), do not support an increased risk for malignancies with use of infliximab, we need to bear in mind that the follow-up for this kind of side effect is still modest and that longer and closer follow-up remains necessary.[2] In this respect, a recent meta-analysis of patients treated with infliximab for rheumatoid arthritis has shown an increased risk for malignancy with a pooled odds ratio (OR) of 3.3 (95% CI 1.2 to 9.1) as well as for serious infections with a pooled OR of 2.0 (95% CI 1.3 to 3.1).[3] The risk of malignancies was dose-dependent and only increased in patients receiving higher (≥6 mg/kg) doses of anti-TNF (OR 4.3; 95% CI 1.6 to 11.8) as compared with patients treated with lower doses (≤3 mg/kg) (OR 1.4; 95% CI 0.3 to 5.7). Although no data in this meta-analysis are given on concomitant therapies or illnesses, nor on subgroups of patients at risk, these figures warrant careful follow-up, also in patients with Crohn's disease treated with infliximab, especially since the dose used (5 mg/kg) is close to the high-dose group described in rheumatoid arthritis. To date, the large prospective TREAT registry has not shown an increased risk for malignancies or serious infections related to the use of infliximab in patients with Crohn's disease.[2] The results of a European registry are expected soon.
The safety concern has also been brought back to attention recently following the report of six cases of hepatosplenic γδ T cell lymphomas (HSTCL) in patients treated with infliximab. HSTCL is a rare and often fatal form of non-Hodgkin's lymphoma that preferentially occurs in children and adolescents receiving azathioprine or 6-mercaptopurin. The six reported cases all occurred in adolescents or young adults (five males, one female, aged 12 to 31 years) who had received between two doses and three years of infliximab in combination with azathioprine. Therefore, safety of anti-TNF remains an important question, especially when the drug is used in combination with immunosuppressive agents such as azathioprine.

Combination therapy with immunomodulators has been demonstrated to reduce immunogenicity to infliximab and this is the main reason to recommend combination therapy at the onset of treatment.[4] Our group recently completed a randomised trial where 80 patients, who had been on combination therapy with infliximab and azathioprine or methotrexate (MTX) for at least six months and were in

stable clinical remission, were randomised to continuation (n=40) or discontinuation (n=40) of the immunosuppression (Infliximab Maintenance Immunosuppression Discontinuation (IMID) study).[5] After two years of follow-up, continuation of immunosuppressive drugs was not superior in preventing the need for change in dosing interval or loss of response or intolerance to infliximab. Also no differences in infliximab trough levels were observed after discontinuation of the immunomodulators, suggesting that immunomodulators may safely be stopped after a period of six months, provided of course that infliximab is continued every eight weeks to maintain remission.
The recommended dose of infliximab in patients with Crohn's disease is 5 mg/kg. It is still unclear what the ideal induction regimen is. The original evidence to support a 0-2-6 induction regimen is not very convincing and some authors have suggested an induction of two infusions at week 0 and 4.[6] The three doses were launched for commercial rather than scientific reasons. It is our current clinical practice to apply the 0-2-6 induction regimen in patients treated for fistulising disease (following a baseline MRI and examination under anaesthesia, and followed by a repeat MRI at week 10), and for luminal disease to give a single infusion of infliximab at week 0 and reassess the patient after four weeks, with a second infusion at that time if symptoms persist.

A question that is even more difficult to answer at this moment is how long to continue treatment with infliximab? Controlled studies are indeed lacking and at the moment one depends on expert opinion or data from small retrospective, uncontrolled studies. The authors do not provide clear guidelines on this issue either. Instead, they suggest that after induction treatment one should wait to see if relapse occurs. This is probably only true for patients in whom immunomodulation has recently been started, changed or optimised. In patients on a background therapy that is already optimal and has not been changed, starting maintenance therapy immediately after induction should be considered, since relapse is highly predictable.
In a patient in stable clinical remission with infliximab, it is our current practice to treat for at least one year.[7] If, after this period, the patient is in sustained clinical remission without corticosteroids or has complete external healing of fistulas, we may try to discontinue infliximab and continue immunosuppression. This decision may be supported by a repeat MRI (for fistulising disease) to confirm absence of fistula tracks or by repeat colonoscopy or small bowel imaging to confirm mucosal healing. For fistulising disease, Van Assche *et al*. showed that healing on MRI is associated with a better outcome.[8] Therefore, incomplete healing on MRI is an indication for continued treatment, whereas full healing may be a reason to discontinue.
However, the presence of mucosal healing has not yet been shown to guarantee successful discontinuation of infliximab. In any case, if the disease relapses, maintenance infliximab needs to be resumed in the long term. The situation for extraintestinal manifestations of Crohn's disease is slightly different since in the case of skin or eye manifestations, infliximab is given until the signs have disappeared and will then be stopped. Patients with Crohn's disease experiencing arthralgias and/or arthritis need to be treated long term with infliximab every eight weeks.
Prospective inflximab discontinuation studies have just been started and will give us more answers to this question.

In conclusion, the Dutch guidelines provide a good working document for all gastroenterologists, internists and surgeons treating Crohn's patients with infliximab. This drug has lead to a revolution in the treatment of this chronic and often complicated disease by the rapid and sustained induction of remission and mucosal healing. Infliximab is also the first drug that has been shown to heal and close perianal fistulas in a rapid and profound way. However, as always, the benefit of this treatment needs to be balanced against the potential risks of increased infections and possible malignancies. Until then, the use of this drug as first-line therapy in all patients is most likely not justified. Instead, the search for predictive molecular markers of complicated disease is of great importance in selecting the ideal candidates for this therapy and to enable earlier treatment in these high-risk patients.

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REVIEW

Infliximab use in patients with Crohn's disease: quality of life, costs and resource use

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KEYWORDS

Crohn's disease, infliximab

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract which primarily affects young adults, with the highest incidence rates reported from Northern Europe, the United Kingdom and North America ranging from 6.9 to 15.6 cases per 100.000 person-years.[1] The course of disease is characterised by episodes of remission and flare-up. The impact on the physical, social, as well as the emotional well-being of patients is substantial and the disease profoundly decreases the quality of life (QoL).[2-4]

The treatment of CD is unsatisfactory, since none of the existing treatments such as aminosalicylates, corticosteroids, immunosuppressive drugs or surgery are curative. Although these treatments have a positive effect on most patients, there is a high incidence of relapse and particularly morbidity from side effects.

Infliximab (Remicade®), a monoclonal antibody directed against tumour necrosis factor-alpha (TNF-α), was introduced in 1998 and has revolutionised treatment. It is indicated for fistulising CD and the treatment of moderately to severely active luminal CD resistant to conventional therapy.[5,6] The initial response rates for these indications are 61 to 69% and 58 to 65%, respectively.[7-10] An infusion of 5 mg/kg infliximab can be given as induction treatment at week 0, 2 and 6, or as maintenance treatment every eight weeks after induction treatment. Maintenance therapy sustains fistula closure, clinical remission and clinical response significantly more than induction treatment only. This implicates an additional therapeutic option for patients previously thought to be refractory to therapy.[7-10]

Although the efficacy of infliximab treatment in CD patients is proven, prescription of infliximab is hampered in daily practice due to its costs (the Netherlands: up to € 14,000 yearly) and the funding system (such as in the Netherlands, Belgium, Canada and the USA).

In general, CD has an expensive course of disease, since diagnosis is at an early age and life expectancy is normal. Annual direct medical costs for CD patients are predominantly caused by surgery and other inpatient services, such as hospitalisation, resulting in 81% of the costs, whereas medications only account for 10% of the costs. All other costs are produced by initial diagnostic workups, outpatient services and long-term complications.[11,12] Annual costs for CD patients per year are difficult to obtain since costs depend on the natural course of the disease. Feagan *et al*. estimated annual costs varying from US $ 6,277 to $ 37,135 by employing different disease severity groups.[13] Remarkable is the fact that only 2% of the CD patients generate 28.9% of the total costs.[11]

Unemployment, disability compensation, compromising of professional career, lost time from work and early retirement are indirect non-medical costs of CD which are difficult to assess but account for high costs to society.[14] An average of 25% of patients with moderate to severe luminal CD in the USA, Europe, Canada and Israel received disability compensation, 39% were unemployed with only 14% of them feeling well enough to work.[15] A Swedish study, which used Swedish national registry data to calculate costs for both ulcerative colitis and CD, reported annual indirect costs at US $ 58.4 million for 40,000 patients, which is a twofold higher than the direct costs.[16] Remission of CD increases employment and is associated with a reduced number of hospitalisations and operations, as well as a normalised QoL.[17] Because infliximab can induce and sustain remission in most patients with refractory and fistulising CD, the most severe type of the disease, this strategy could be cost-effective despite its costs.

The aim of this review is to critically appraise the cost-utility and cost-effectiveness of infliximab in patients with CD by summarising all available evidence with respect to the effect of infliximab on QoL, medical costs and use of resources.

MATERIALS AND METHODS

Literature search

A search in MEDLINE and EMBASE was performed combining different synonyms for CD and QoL, as well as for costs and resource use (*table 1*). No limits were added and the search was run until July 2005. Additionally, we searched the Cochrane Library 2005 and reference lists of relevant systematic reviews and randomised controlled trials (RCT) for articles missed by the computer-based search strategy.

Study selection criteria

Studies in which the efficacy of infliximab was evaluated in adult patients with moderate to severe active luminal or fistulising CD were selected for this review. The outcome of the QoL had to be described with the Inflammatory Bowel Disease Questionnaire (IBDQ) or the SF-36 Health Survey (SF-36). The IBDQ is a disease-specific questionnaire which contains 32 items concerning bowel, systemic, emotional and social subjects and correlates significantly with the Crohn's Disease Activity Index.[18,19] Total IBDQ scores range from 32 to 224, corresponding to a very poor and perfect QoL, respectively. The SF-36 is a generic QoL evaluation which contains two summary measures, the physical component summary (PCS) and the mental component summary (MCS), with a mean summary score of 50 and a standard deviation of 10 for the general population.[20] Previous studies have shown no[21] or only a weak correlation[22] between the SF-36 and the IBDQ.

Costs had to be described by direct financial medical costs or resource use. Another, indirect, tool for assessing costs is a Markov model. Markov models are theoretical models and estimate long-term costs by combining time spent in different distinct health states (e.g. remission, mild, drug dependence, surgery) and the calculated costs. The proportion of time spent in each health state and its costs has to be determined retrospectively.[23] The validity of a such model is critically dependent on the underlying assumptions and on the reliability of the data input. Because infliximab has only been available since 1998 and long-term effects have not been published, cost estimates are commonly based upon unpublished industry models. We question the validity of these estimations and do not consider clinical conclusions drawn from Markov models based on these data to be feasible.

Validity assessment and data abstraction

Only RCTs and clinical cohort studies were included. Three independent reviewers selected potentially relevant references based on title, abstract and keywords. From articles for which disagreement existed, full texts were obtained and in all of these cases consensus was reached at a later stage.

The number of patients, similarity of treated groups, blinding of patients and physicians, randomisation, setting, follow-up, dropout rate and intention-to-treat analysis were used as criteria to evaluate the selected studies.[24] Quality of evidence was assigned based on regulations formulated elsewhere, in decreasing value from meta-analysis, RCTs, nonrandomised (observational) comparative cohort study, nonrandomised historical cohort studies to case series.[25] If publications were based on the same study population the most relevant reference was selected. Furthermore, we corresponded with some authors to obtain missing data.[21,22,26,27]

Table 1. *Search strategy MEDLINE and EMBASE*

#	Terms	Number of articles	
		MEDLINE	EMBASE
1	Search ("Crohn Disease"[MeSH] OR "Inflammatory Bowel Diseases"[MeSH]) OR Crohn's disease[Title/Abstract] OR crohn disease[Title/Abstract] OR Inflammatory bowel disease[Title/Abstract]	39,071	96,822
2	Search "infliximab"[Substance Name] OR infliximab[Title/Abstract] OR remicade[Title/Abstract]	1976	4822
3	Search "Value of Life"[MeSH Terms] OR "Quality of Life"[MeSH Terms] OR "Quality Adjusted Life Years"[MeSH] [Terms] OR "Health status indicators"[MeSH Terms]) OR "Value of Life"[Title/Abstract] OR "quality of life"[Title/Abstract] OR "quality adjusted life years"[Title/Abstract] OR "health status indicators"[Title/Abstract]))	141,171	229,820
4	Search #1 AND #2 AND #3	88	125[§]
5	Search "Costs and Cost Analysis"[MeSH]) OR cost of illness[Title/Abstract] OR surger*[Title/Abstract]) OR hospitalizations[Title/Abstract] OR "Resource use"[Title/Abstract] OR "Patient Care"[MeSH]	839,489	409,638[†]
6	Search #1 AND #2 AND #5	86	143

[§]**Limits: English, human;** [†]**without surger*** **and hospitalizations.**

RESULTS

Characteristics of selected studies

Quality of life

In total 88 and 125 potential relevant references were found in MEDLINE and EMBASE, respectively (*table 1*). Of all these references only five RCT and two cohort studies met the inclusion criteria and were considered suitable for evaluation in this review.[10,21,22,28-31] These articles all turned up in both search strategies. The publication by Targan *et al*. was added after searching the Cochrane Library.[10] Two of the RCTs, those by Rutgeerts *et al*. and Feagan *et al*., were based on the same patient population (ACCENT I).[22,29] Since Feagan *et al*. specifically described the QoL while Rutgeerts *et al*. focussed on the effects on resource use, these trials were selected for their different endpoints. Three other RCTs, from Targan *et al*., Lichtenstein *et al*. and Rutgeerts *et al*., were based on the same cohort of patients as well.[10,28,31] The publication by Lichtenstein *et al*. described the QoL most specifically and was selected for this purpose. Thus, four studies were eventually used for our assessment (*table 2*).

The publications by Lichtenstein *et al*. and Feagan *et al*. are both double-blinded, multicentre RCTs supported by the manufacturer of infliximab. With a follow-up time of 54 weeks, Feagan *et al*. compared infliximab maintenance treatment with placebo in patients responding to a single 5 mg/kg infliximab infusion at week 0. Participants in the study had had moderately to severely active CD for at least three months. After 14 weeks patients were able to cross over to a 5 mg/kg infliximab regimen and their results were carried forward. QoL was assessed, with the IBDQ and SF-36. Results from the responders were used for the assessment of the QoL.

Lichtenstein *et al*. compared placebo and single doses of infliximab (5, 10 and 20 mg/kg) in patients with a diagnosis of luminal CD. The initial follow-up time was four weeks. For the present analysis only the results of the 5 mg/kg subgroup were used since this dosage is commonly administered in daily practice.

Cadahia *et al*. and Van Balkom *et al*. both published cohort studies in which parameters of QoL following infliximab use were compared with baseline data (*table 2*). Cadahia *et al*. included only patients with fistulising CD, while Van Balkom *et al*. included patients with fistulising as well as luminal disease. The study by Van Balkom *et al*. was supported by the manufacturer.

The quality of evidence of the RCT by Feagan *et al*. was considered to be the highest followed by the RCT by Lichtenstein *et al*. and the cohort studies by Van Balkom *et al*. and Cadahia *et al*.[32]

Table 2. *Overview of papers assessing the clinical outcome and the quality of life*

	Quality of evidence	Country (centres)	Indication (number of patients)	Inclusion criteria	Exclusion criteria	Intervention (n)	Comparator (n)	Primary outcome	Secondary outcome	Period of follow-up
Feagan (2003)	RCT (ACCENT I)	North America, Europe, Israel (55)	Luminal CD (335)	CDAI 220-400 responding to infliximab	+	Infliximab week 0, 2, 6 and every 8 weeks: 5 mg/kg (113) 10 mg/kg (112)	Infliximab week 0 (110)	Clinical remission Time to loss of response	HRQOL: SF-36 and IBDQ	54 weeks
Lichtenstein (2002)	RCT	North America, Europe (18)	Luminal CD (108)	CDAI 220-400	+	Infliximab week 0: 5 mg/kg (27) 10 mg/kg (28) 20 mg/kg (28)	Placebo week 0 (25)	Clinical response	HRQOL: IBDQ	4 weeks
Van Balkom (2002)	Cohort study	The Netherlands (5)	Fistulising and luminal CD (56)	CDAI ≥200	+	Infliximab week 0 for luminal CD and week 0, 2, 6 for fistulising CD	Baseline week 0	HRQOL: IBDQ	-	8-10 weeks
Cadahia (2004)	Cohort study	Spain (1)	Fistulising CD (25)	Single or multiple draining abdominal or perianal fistulas	+	Infliximab week 0, 2, 6	Baseline week 0	Clinical response	HRQOL: SF-36 and IBDQ	10 weeks

+ = Well described; - = not described; RCT = randomised controlled trial; CD = Crohn's disease; CDAI = Crohn's Disease Activity Index; HRQOL = health-related quality of life; IBDQ = Inflammatory Bowel Disease Questionnaire; SF-36 = SF-36 Health Survey.

Costs and resource use

Of the 86 and 143 references found by MEDLINE and EMBASE, only three studies met the inclusion criteria and were selected for evaluation of costs and resource use in this particular category of patients treated with infliximab (*table 1*).[26,27,29] The Cochrane Library search did not yield additional studies. Two RCTs, the ACCENT I by Rutgeerts *et al.* and the ACCENT II by Lichtenstein *et al.*, presented data on the effect of infliximab on the number of hospitalisations, hospitalised days and operations with a high quality of evidence. Both are large RCTs in which patients with luminal and fistulising disease, respectively, were enrolled. In these trials, maintenance use of infliximab was compared with placebo, after infliximab induction of remission with a follow-up time of 54 weeks. Both studies were supported by the manufacturer. Rubenstein *et al.* retrospectively compared resource use before and after infliximab administration in the same cohort of patients. Since most patients had severely active CD in the period preceding infliximab use, data were averaged over a timeframe of three years, allowing for a more accurate estimation of healthcare resource use. No studies were found comparing the direct medical costs or indirect costs in CD patients treated with infliximab *vs* conventional drugs (*table 3*).

All studies selected for this review were carried out in university hospitals. Patients were at least 18 years of age and had no severe infections. Infliximab was administered in a dose of 5 mg/kg.

Quality of life

All studies showed a significant improvement in the QoL of CD patients following administration of infliximab (*table 4*). A significant short-term improvement in the IBDQ was shown by Lichtenstein *et al.*, Van Balkom *et al.* and Cadahia *et al.* In addition, Feagan *et al.* concluded that maintenance treatment with infliximab induced a significantly longer improvement in the QoL. Measurement of the QoL using the SF-36 resulted in a significant improvement as well. Cadahia *et al.* reported an overall improvement in the PCS after four and ten weeks ($p<0.05$), as did Feagan *et al.* after ten and 54 weeks. The MCS did not significantly change in the trial by Cahadia *et al.* Feagan *et al.* only showed a significant increase in the MCS after 54 weeks in the 10 mg/kg infliximab group, $p<0.05$. Overall, the individual

Table 3. *Overview of papers assessing resource use*

	Quality of evidence	Country (centres)	Use of infliximab	Indication (number of patients)	Inclusion criteria	Exclusion criteria	Intervention (n)	Comparator (n)	Primary outcome	Secondary outcome	Period of follow-up
Lichtenstein (2005)	RCT (ACCENT II)	North America, Europe, Israel (45)	Clinical trial	Fistulising CD (282)	Single or multiple perianal/ entero-cutaneous draining fistulas	+	Infliximab week 0, 2, 6 and every 8 weeks (143)	Infliximab week 0, 2, 6 (139)	Time to loss of response	Hospitalisations Hospitalised days Operations Procedures Safety	54 weeks
Rutgeerts (2004)	RCT (ACCENT I)	North America, Europe, Israel (55)	Clinical trial	Luminal CD (573)	CDAI: 220-400 >3 months	+	Infliximab week 0, 2, 6 and every 8 weeks: 5 mg/kg (192) 10 mg/kg (193)	Infliximab week 0 (188)	Study treatment Efficacy	HRQOL: IBDQ Steroid sparing Mucosal healing Hospitalisations Operations Safety	54 weeks
Rubenstein (2002)	Cohort study with a pre and post test	USA (1)	Daily care	Luminal and fistulising CD (79)	>1 year data available before and after infliximab use	-	Infliximab week 0 for luminal CD and week 0, 2, 6 for fistulising CD	Treatment before infliximab use	Hospitalisations Hospitalised days Operations Procedures	Endoscopies Emergency room visits Radiology Parenteral nutrition	1-3 years retrospective + 1 year prospective

+ = Well described; - = not described; RCT = randomised controlled trial; CD = Crohn's disease; CDAI = Crohn's Disease Activity Index; HRQL = health-related quality of life; IBDQ = Inflammatory Bowel Disease Questionnaire; SF-36 = SF-36 Health Survey.

scales of the SF-36 demonstrated greater improvement in scales relevant to the physical aspects of health as opposed to psychological measures.

Cost and resource use

The results are given in *table 5*. The ACCENT I and II studies described the differences in resource use in patients treated with infliximab for induction and for maintenance use only as a secondary endpoint.[26,29] In both studies a significant reduction in the number of hospitalisations and operations in patients in the maintenance treatment arm was found. In the ACCENT II study, maintenance treatment was shown to result in fewer days in hospital in patients responding to infliximab. They also reported a decrease of 18.9 to 8.6% in patients hospitalised in the maintenance group (follow-up period 54 weeks, p<0.05).[26] Moreover, a significant reduction in major operations, such as fistula excision and anal fistulotomy was shown (13 *vs* 2 per 100 patients, p<0.05). The cohort study by Rubenstein *et al*. showed a significant decrease of 59% in patients with fistulising CD in the number of hospital admissions (p<0.05), a decrease in all surgery of 38% (p<0.01) and a trend towards a reduction in hospitalised days. Additionally, a significant reduction in the number of endoscopies, emergency room visits, radiology exams and gastroenterology outpatient visits was noted (43, 66, 12 and 20% in all patients, respectively).[27]

DISCUSSION

In this review we appraised all the available evidence about the QoL and costs of infliximab use in CD patients. We performed a systematic search and evaluated all relevant articles. However, the number of appropriate, high-quality articles is limited. Four and three publications with respect to the QoL and costs, respectively, were considered suitable for evaluation. Of all studies, only the RCT by Lichtenstein *et al*. compared the use of infliximab *vs* placebo.[31] The other studies compared the effect of infliximab within one study population or maintenance *vs* induction treatment.

Table 4. *Results of articles assessing the quality of life, presented in descending quality of evidence*

	Crohn' s Disease Activity Index		Inflammatory Bowel Disease Questionnaire	
Feagan (2003)	**Week 0**	**Week 54**	**Week 0**	**Week 54**
	Single dose: 298 ± 50	254 ± 132	Single dose: 129 ± 27	138 ± 41
	Maintenance: 309 ± 52	201 ± 122*	Maintenance: 130 ± 25	152 ± 43§
Lichtenstein# (2002)	**Week 0**	**Week 4**	**Week 0**	**Week 4**
	Placebo: 288 ± 54	271 ± 82	Placebo: 128 ± 29	133 ± 28
	Infliximab: 312 ± 56	166 ± 76§	Infliximab: 122 ± 29	168 ± 36§
Van Balkom (2002)	**Week 0**	**Week 4 and 10**	**Week 0**	**Week 4 and 10**
	Active CD: 311 ± 83.4	133.32 ± 110.6	Active CD: 117.5 ± 17.7	168.7 ± 31.8§
	Fistulising CD: 203 ± 131.0	131.0 ± 120.3	Fistulising CD: 151.8 ± 33.9	179.3 ± 25.5†
Cadahia## (2004)	**Week 0**	**Week 10**	**Week 0**	**Week 10**
	Active CD 220.5 ± 79.0	110.9 ± 61.2**	Active CD 174.6 ± 45.7	209.5 ± 35.6

#Results are described from the 5 mg/kg subgroup, since infliximab is now prescribed in this dosage; ##Cadahia used a Spanish version of IBDQ validated from the 36-item version of Love *et al*.[63] *p<0.05; †p< 0.01; §p<0.001, **p<0.0001.

Table 5. *Results of the articles assessing resource use, presented in descending quality of evidence*

	Hospitalisations[a]		Hospitalised days[b]		Surgeries[a]	
	Control	**Intervention**	**Control**	**Intervention**	**Control**	**Intervention**
					All surgeries and procedures	
Lichtenstein (2005)#	All patients: 31	14*	2.4	0.8°	118	60†
	Responders: 31	11*	2.5	0.5*	126	65*
					CD-related intra-abdominal surgeries	
Rutgeerts (2004)	38	23*	-	-	7.5	2.6*
					Gastrointestinal surgeries	
	All: -	-	2.35	2.14$	0.28	0.18*
Rubenstein (2002)[c##]	Fistula : 0.46	0.19*	2.13	1.16$	0.39	0.14†
	Luminal: -	-	-	-	-	-

The results are shown as comparator versus intervention group for RCT and as difference for cohort studies. [a]The mean number per 100 patients; [b]the mean number of days hospitalised per patient; [c]the means per patient year. #Lichtenstein et al. divide their results in all patients and the responders of infliximab; ##Rubenstein *et al*. distinguish between fistulising and luminal Crohn's disease; *p<0.05; †p<0.01; ‡p<0.001; °not significant; $p=0.06.

Notably, only two studies were not financially supported by the pharmaceutical industry.[21,27] The results of both studies were in line with the studies supported by the manufacturer.[22,26,29-31]

A clear conclusion can be made with respect to QoL. All studies described a significant improvement in short-term and one study in long-term analyses. Another clear conclusion can be made concerning the use of resources: all studies showed a decrease in the number of operations and hospitalisations. Although it might be appealing to combine these two conclusions, the statement that infliximab use is cost-effective is premature. The available studies do not provide empirical evidence for this assumption: costs were not calculated and cost-effectiveness ratios were not constructed.

We chose to disregard Markov models, because reliable data to feed these models are currently not available. This is underscored by the wide range of costs per quality adjusted life years for infliximab *vs* alternative treatments in CD, calculated using Markov models in recent literature.[33-35]

Different reports and guidelines on the use and the cost-effectiveness of infliximab were recently published, highlighting the importance of this subject and the need for consensus. Notwithstanding the presumed high incremental costs, infliximab is considered a valuable therapeutic alternative in patients with refractory CD. For example, the National Institute for Clinical Excellence (NICE) in the UK recommended treatment with infliximab in patients with severe active luminal CD, refractory to other treatment or inappropriate for surgery, and for fistulising CD meeting aforementioned criteria.[36] NICE suggested that infliximab would be cost-effective when only used in patients with severe active CD, refractory to conventional treatment and inappropriate for surgery. The Canadian Coordinating Office for Health Technology Assessment did not support infliximab therapy for CD on the grounds of cost-effectiveness, but emphasised that indirect cost savings by attenuating productivity losses and the lack of therapeutic alternatives in a specific group of patients with severe, refractory CD justifies the use of infliximab.[37]

Until now, cost effectiveness has not been studied prospectively as a primary endpoint. We feel that this should be done and that the study design should include parameters of indirect costs. Since the majority of CD patients are younger than 65 years of age, the indirect costs are high because of unemployment allowances, sickness relief allowances and handicap-related income allowances of the CD.[11,12] We expect infliximab therapy to result in a considerable decrease in these costs, possibly rendering the drug cost effective in the long run. In conclusion, treatment with infliximab has been shown to be effective in terms of disease activity and QoL. Since cost-effectiveness has not been specifically studied, no definitive conclusions can be drawn regarding this subject. Although evidence regarding decreases in the number of operations and hospitalisations following infliximab therapy is accumulating, a straightforward, well-designed and prospective cost-effectiveness analysis is needed.

NOTE

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REVIEW

Guidelines for treatment with infliximab for Crohn's disease

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ABSTRACT

Infliximab is an accepted induction and maintenance treatment for patients with Crohn's disease. The effectiveness of infliximab has been demonstrated for both active luminal disease and for enterocutaneous fistulisation. In addition, infliximab can be administered for extraintestinal symptoms of Crohn's disease, such as pyoderma gangrenosum, uveitis and arthropathy. Maintenance treatment with infliximab is effective and is regarded as safe as long as the necessary safety measures are heeded. Infusion reactions occur in 3 to 17% of the patients and are associated with the formation of antibodies to infliximab. A reduction in infusion reactions is possible by the concurrent administration of steroids and the use of immunosuppressants (azathioprine, 6-mercaptopurine, methotrexate). Furthermore, immunosuppressants increase the duration of the response to infliximab. For these reasons, the concomitant use of immunosuppressants with infliximab is recommended. Infections and most specifically tuberculosis need to be ruled out before infliximab is administered. Up to now, there are no indications for a connection between an increased risk for malignancies and treatment with infliximab.

KEYWORDS

Crohn's disease, guideline, infliximab

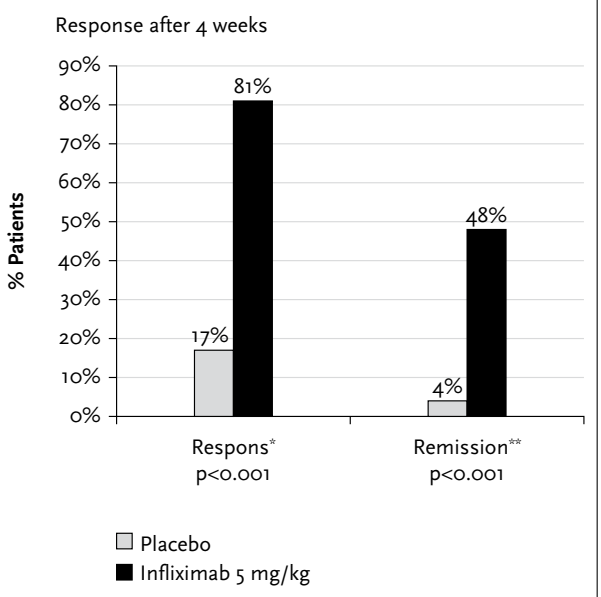
INTRODUCTION

Infliximab (Remicade®) is a chimeric monoclonal antibody (75% human, 25% mouse) against tumour necrosis factor-alpha (TNF-α), a cytokine which plays an important role in the development of inflammatory reactions. Increased TNF-α concentrations are found, for instance, in patients with Crohn's disease and rheumatoid arthritis and are associated with increased disease activity.[1] In Crohn's disease, neutralising TNF-α-induced effects with infliximab often result in a rapid reduction in intestinal inflammation with a good clinical response as outcome. Infliximab has been registered in the Netherlands since 1999 for the treatment of serious, active Crohn's disease in patients who do not respond to an adequate course of treatment with corticosteroids, or to an immunosuppressive therapy (azathioprine, 6-mercaptopurine, methotrexate), or in those with an intolerance to or a contraindication for these therapies. In addition, infliximab is registered for the treatment of draining enterocutaneous fistulas in Crohn's patients not responding to an adequate conventional therapy.

Since the registration of infliximab for these indications, and the publication of a consensus text about the use of infliximab for Crohn's patients,[2] numerous studies have been carried out on the effects of infliximab on Crohn's disease. Now that greater insight has been gained into the effectiveness and long-term safety of infliximab for Crohn's disease, an updated consensus text is desirable. There are, after all, great differences around the country in treatment regimens with infliximab, and greater unequivocalness is needed.

The guideline for induction and maintenance treatment with infliximab for Crohn's patients is described in this article. The objective of this guideline is to provide more clarity about the indications and safety of infliximab and to present a practical treatment plan. Based on this guideline, local treatment protocols can be developed. The guideline was drawn up by specialists in the field of inflammatory intestinal diseases from all Dutch University Medical Centres. Data from the literature as well as the specific experience of a large group of Dutch Crohn's patients over the past six years form the basis for this guideline. In this article, the following subjects will be discussed in succession: induction treatment of luminal Crohn's disease, induction treatment of fistula disease associated with Crohn's, maintenance treatment with infliximab, remaining treatment indications, the safety of infliximab, and practical treatment instructions.

Figure 1. *Remission induction for luminal Crohn's disease after a single administration of the study medication*[3]



*Reduction in Crohn's Disease Activity Index (CDAI) by ≥70 points; **CDAI <150.

INDUCTION TREATMENT OF ACTIVE LUMINAL CROHN'S DISEASE

Indications

1. Active luminal disease in patients who do not respond or inadequately respond to an adequate dose of corticosteroids, alone or in combination with an immunosuppressive drug. This includes both steroid-resistant and steroid-dependent patients. A patient is regarded as being steroid-resistant when the disease does not respond to intravenous steroids (1 mg/kg, maximum 60 mg). A patient is called steroid-dependent when an exacerbation recurs during the reduction of the steroids (5-10 mg/week to 20 mg thereafter 2.5 to 5 mg/week) or within one month of stopping the steroids.
2. Active luminal disease in patients where corticosteroids or immunosuppressants are contraindicated as a result of a previous history of clinically relevant side effects.

Objective

The ultimate goal is to induce a complete clinical and endoscopic remission. When this objective is achieved, the patients are largely free of symptoms, the corticosteroids can be reduced, and surgical intervention may be avoided (see under Maintenance treatment).

Effectiveness

Clinical efficacy of a single induction treatment with infliximab has been demonstrated in a double-blind, placebo-controlled study with 108 therapy-resistant patients with Crohn's disease.[3] Out of this group, 27 patients were treated with the recommended dose of 5 mg/kg. The higher doses of 10 and 20 mg/kg delivered less positive results. A response was observed within two weeks, with a maximum response after four weeks (*figure 1*).[3] After 12 weeks, a response could still be observed in nearly half of the patients. In another placebo-controlled study, it appeared that after four weeks a single infliximab treatment did not only result in a positive clinical response, but also in a significant endoscopic improvement.[4] Histological examination confirmed that a complete reduction in the inflammation infiltrate could only be seen in the patients treated with infliximab.

Dosage

In dose-response studies, a better result was not found at doses higher than 5 mg/kg.[3,4] A slightly better clinical response was observed after ten weeks at the 5% significance level with a treatment regimen of infusions at 0-2-6 weeks (65%) compared with a regimen which was started with one single infusion (52%).[5] However, this is unlikely to be clinically significant. In most of the patients, infliximab could still be detected in the serum for at least eight weeks after a once-only administration of the recommended dose of 5 mg/kg.[6] For this reason an induction regimen of two or three infusions every eight weeks could also be selected. Arguments which speak in favour of the 0-2-6 week induction regimen are that this regimen appears to result in less infusion reactions, delayed allergic reactions, and decreased formation of antibodies.[5] For this reason, the recommended intravenous dosage regimen for induction treatment is 5 mg/kg at 0-2-6 weeks.

TREATMENT OF ACTIVE FISTULA DISEASE ASSOCIATED WITH CROHN'S DISEASE

Indications

Symptomatic and draining enterocutaneous or perianal fistulas which do not respond or which respond inadequately to an adequate antibiotic course of treatment, alone or in combination with an immunosuppressive.

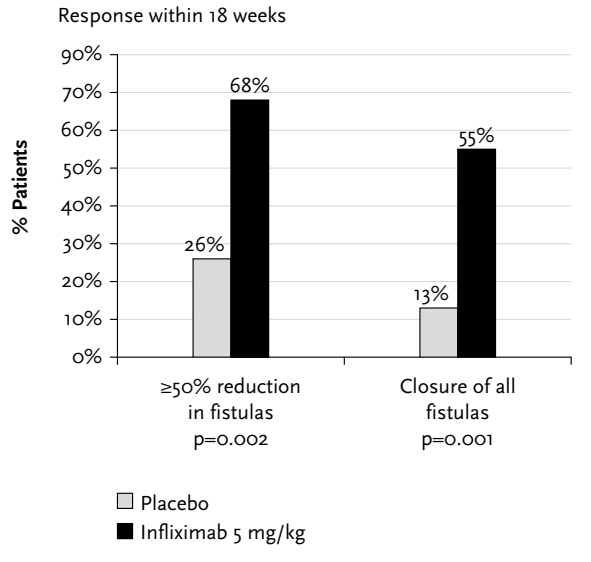
Objective

The aim is to reduce the number of active draining fistulas and complete or partial closure of the fistulous ducts. Complete radiological healing of fistulous ducts is usually not achievable, but patients in whom healing on MRI is achieved usually do better.[7-9]

Effectiveness

A rapid effect of infliximab on fistula formation associated with Crohn's disease has been demonstrated in a double-blind, placebo-controlled study of 94 patients who had one or more fistulas in a period of at least three months.[10] These patients received three infusions (with placebo or infliximab) at 0-2-6 weeks. Infliximab in a dose of 5 mg/kg (*figure 2*)[10] appeared to be more effective than the higher dosage of 10 mg/kg. A response was seen after an average of two weeks. The median duration of the response came to 12 weeks. Closure of all fistulas was achieved in 55% of the patients treated with infliximab 5 mg/kg and in 13% of patients treated with placebo.

Figure 2. *Remission induction for fistula disease associated with Crohn's after a treatment regimen of three doses of study medication at 0, 2 and 6 weeks*[10]



Infliximab does not seem to be indicated for enteroenteral fistulas or enterovesical fistulas. These types of complicated intra-abdominal fistulas usually require surgical intervention. The use of infliximab for rectal-vaginal fistulas seems disappointing.[11] However, in an infliximab maintenance study, ACCENT II, rectovaginal fistulas healed reasonably well.

Dosage

For the treatment of active fistula disease associated with Crohn's disease, a similar dosage (5 mg/kg infliximab) and treatment regimen can be selected as for luminal disease (see previous comments). Here too, consideration has to be given to the selection of an intensive remission-induction regimen of 0-2-6 weeks, or a series of two to three infusions at an interval of eight weeks.

MAINTENANCE TREATMENT

Indication

Patients with luminal Crohn's disease and fistulising Crohn's disease who, after a successful induction treatment with infliximab and despite an adequate treatment with immunosuppressants, have a rapid exacerbation, an increased risk for this, or whenever the dosage of corticosteroids cannot be reduced or phased out.

The choice to move forward with infliximab maintenance therapy is partially determined by the risk profile of the patient. Risk factors for a rapid exacerbation and a complicated course are earlier resections, a positive family history, smoking, and previous frequent exacerbations despite the use of corticosteroids with immunosuppressants.[12] In patients already on optimal concomitant immunosuppressive therapy, which remained unchanged at the onset of infliximab, maintenance therapy should be strongly considered immediately after induction of remission.

Objective

The aim is to prevent an exacerbation of luminal disease or reoccurring fistulas, the reduction of luminal disease or recurring fistulas, the reduction or obviation of corticosteroid treatment, the reduction or obviation of hospital admissions and surgical interventions.

Effectiveness

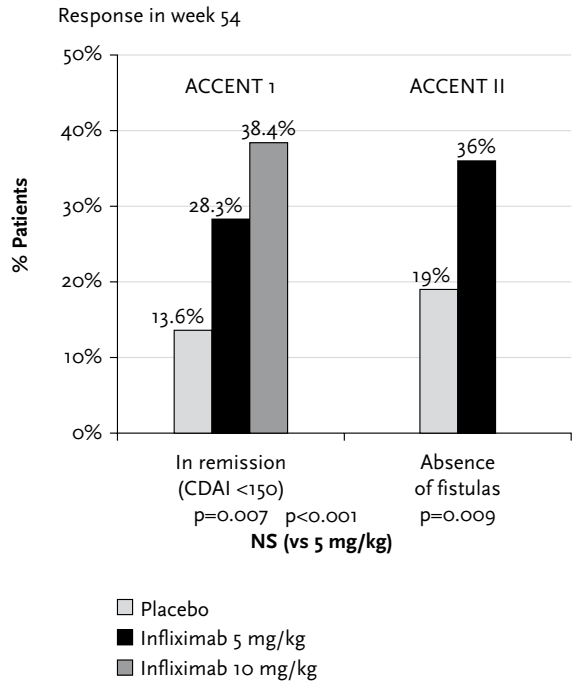
The efficacy of infliximab maintenance treatment for Crohn's disease has been demonstrated in three double-blind, placebo-controlled studies:

- After successful induction treatment with a single infusion of infliximab, patients (n=73) with luminal Crohn's disease received an infusion of infliximab (10 mg/kg) or placebo four times at eight-weekly intervals. In the infliximab

group, 53% remained in remission *vs* 20% in the placebo group. Most of the patients showed an exacerbation 8 to 12 weeks after the final infliximab infusion, which points to a duration of efficacy of about 8 to 12 weeks.[13]

- In the ACCENT I study, 573 luminal patients with Crohn's disease were treated with infliximab (5 mg/kg) according to the induction regimen of 0-2-6 weeks.[14] Those who showed a response (n=335, 59%) were randomised to maintenance treatment with a placebo or infliximab (5mg/kg or 10 mg/kg) every eight weeks over a period of 46 weeks. The results (*figure 3*)[14,15] indicate that retreatment with infliximab every eight weeks is effective in preventing of exacerbations after successful induction treatment.
- In the ACCENT II study, 195 (out of a group of 306) patients with fistula disease associated with Crohn's, had a clinical response after an induction treatment with infliximab (5 mg/kg at 0-2-6 weeks).[15] This group received maintenance treatment of placebo or infliximab (5 mg/kg) every eight weeks. The results (*figure 3*)[14,15] showed that maintenance treatment with infliximab over a long period (54 weeks) is effective in preventing new active draining fistulas.

Figure 3. *Maintenance of clinical remission after 54 weeks of maintenance treatment with infliximab for luminal Crohn's disease (ACCENT I study)[14] and fistula disease associated with Crohn's (ACCENT II study)[15]*



p-values: infliximab *vs* placebo. CDAI= Crohn's Disease Activity Index.

In two placebo-controlled studies of luminal Crohn's disease, the remission percentage was the largest in the group of patients who combined an infliximab maintenance treatment with an immunosuppressant (azathioprine, 6-mercaptopurine, methotrexate).[13,14] This possible synergistic effect of infliximab, combined with an immunosuppressant, has already been described for patients with rheumatoid arthritis.

In addition to the clinical effects noted above, it has been demonstrated in various studies that during a maintenance treatment with infliximab (5-10 mg/kg every eight weeks), the corticosteroids can be completely phased out in most patients,[14,16,17] the intestinal mucous improves considerably if not entirely cured,[14-18] the number of hospital admissions and surgical interventions is significantly reduced for luminal Crohn's disease[18] as well as for fistula disease,[19] and that the quality of life of these patients improves.[20,21]

Dosage

As maintenance treatment for luminal Crohn's disease as well as for fistulas associated with Crohn's disease, infliximab is administered on average every eight weeks in a dosage of 5 mg/kg. This dose may temporarily be increased to 10 mg/kg if loss of response occurs, to make patients responsive to the normal 5 mg/kg dose again.

Duration of maintenance therapy

The question when to discontinue maintenance therapy with infliximab is difficult to answer, because evidence is lacking. We believe that in azathioprine or methotrexate refractory patients, infliximab maintenance therapy should be continued after successful induction of remission, because on demand retreatment with infliximab with long intervals in between may increase the formation of anti-infliximab antibodies (ATI; antibodies to infliximab). In our experience, long-term remissions were observed in patients naive for immunosuppressants, after induction therapy with infliximab together with azathioprine or methotrexate maintenance therapy.

REMAINING INDICATIONS ASSOCIATED WITH CROHN'S DISEASE FOR INFLIXIMAB

Infliximab can be used for Crohn's disease localised in the mouth, oesophagus, stomach and duodenum, and for extraintestinal manifestations of Crohn's disease, such as pyoderma gangrenosum, uveitis and arthropathy including spondylitis. Infliximab is not indicated for primary sclerosing cholangitis.

Crohn's disease of the proximal digestive tract

In 5 to 15% of patients, disease is found in the proximal digestive tract. This is often coupled with distal luminal

disease. Usually, proximal lesions react in a similar way, i.e. well, to the therapy, which was originally initiated for distal disease. In some cases, proximal lesions of Crohn's disease persist, such as oral aphtosis or locations in the oesophagus, stomach or duodenum, although administration of infliximab for these localisations apparently has a beneficial effect.[22-24] Oral aphtosis is a painful condition.

Extraintestinal manifestations of Crohn's disease

Crohn's disease is often complicated by extraintestinal manifestations, such as inflammations of skin (erythema nodosum, pyoderma gangrenosum), eyes (uveitis), or of joints (arthropathy, enthesiopathy). Controlled studies of infliximab for these indications are still scarce. For pyoderma gangrenosum and psoriasis, a few studies have reported some therapeutic success.[25-30] A positive result for uveitis after infliximab treatment has also been published, but clinical response may vary greatly.[31] Joint manifestations can be distinguished as peripheral arthropathy, sacroiliitis, Bechterew's disease, and tendon pain or inflammation. Manifestations such as peripheral arthropathy are commonly associated with luminal activity and generally respond well to remission-induction therapy.[32,33] Infliximab also showed good results for sacroiliitis[31-34] and Bechterew's disease,[35-37] which run a more independent course. Psoriatric arthritis, Bechterew's disease and rheumatoid arthritis are now separate registered indications for infliximab. For primary sclerosing cholangitis (PSC), there is no indication at the moment. A controlled study on the effect of infliximab for PSC in ulcerative colitis patients was cancelled (no published data, Hommes *et al.*) For the above-mentioned proximal lesions and extraintestinal manifestations, a comparable treatment regimen is recommended as for luminal or fistula disease associated with Crohn's disease.

SAFETY OF INFLIXIMAB TREATMENT

There is a lively ongoing discussion about side effects of infliximab, which in rare cases can be very serious. A full understanding of the safety aspects is needed to use infliximab safely.

Infusion reaction

Acute infusion reaction

An acute infusion reaction generally occurs during infusion or within two hours after infusion. In Crohn's disease, this acute reaction occurs in 3 to 17% of patients treated with infliximab. In 0.1 to 1% there is an issue of a serious infusion reaction (*table 1*).[14,15,40,42,43] This is usually an anaphylactic allergic reaction, which is commonly IgE-independent.[38,39] The most common symptoms are headache, nausea, chest pain, dizziness, urticaria, other types of hives or itching, and shortness of breath. Symptoms such as acute urticaria, hypotension and bronchial spasms occur far less frequently, and are often based on an IgE-dependent, type I allergic reaction. Rare and serious side effects are anaphylactic shock, larynx oedema and stridor.[38,39]

Delayed infusion reaction

Delayed infusion reactions may occur as early as two days after infusion. For the most part, these late allergic reactions are observed 3 to 12 days after the infusion. The incidence of these 'serum disorder-like' reactions after a single infusion treatment, or during a maintenance treatment (an infusion every eight weeks) is 2 to 3%.[14,40] Typical symptoms of this reaction are artralgia or myalgia with fever, exanthema, facial, hand, or lip oedema, dysphagia, pruritus, headache, and sore throat.[38,40,41]

Table 1. *Incidence of side effects after infliximab treatment for Crohn's patients in various cohort studies*

	Amsterdam cohort[42]	ACCENT I[14]	ACCENT II[15]	Mayo cohort[40]	TREAT 2005[43]	
					Infliximab Crohn's patients	Non-infliximab Crohn's patients
Number of patients	71	573	306	500	3179	3111
Acute infusion reactions	17%	17%	9%	3.8%	4.6%	-
Serious infusion reactions	1.0%	1.0%	0.3%	0.4%	0.12%	-
Lupus-like syndrome	0	1	0	3		
Annual serious infections	0	4.0%	3.8%	2.1%	1.33%	0.70%*
Annual malignancies	0	1.0%	0%	0.4%	0.42%	0.51%
Annual mortality	0	0.7%	0%	1.3%	0.53%	0.43%

*p=0.012 (infliximab *vs* non-infliximab Crohn's patients).

Anti-infliximab antibodies

Infusion reactions are associated with the presence of ATI. High ATI values are associated with lowered serum concentrations of infliximab, and an attenuated and shorter response to infliximab.[44,45]

Prevention of an infusion reaction

Infusion reactions appear to occur more often after the second or third treatments,[38] especially if the last treatment occurred more than four months previously.[6] There are increasing indications that an induction treatment of three infusions at short intervals (at 0-2-6 weeks) diminishes the likelihood of the development of ATI and of an acute or delayed infusion reaction.[5] A maintenance treatment with an immunosuppressant, started prior to infliximab treatment, also reduces the formation of ATI, so the chance of an infusion reaction is reduced and the extent and duration of the response is increased.[5,6] The same applies for the administration of an infliximab infusion with hydrocortisone premedication.[45] For patients with a previous (delayed) infusion reaction, it is possible that in addition to a maintenance treatment with an immunosuppressive drug, premedication with a corticosteroid and/or an antihistamine can reduce the risk for a second infusion reaction (see under Practical treatment instructions).[5,6,38,41]

Treating an infusion reaction

If an acute infusion reaction occurs, slowing down or temporarily stopping the infusion is usually sufficient if the adverse reaction is mild.[38,41] After discontinuing the infliximab infusion, an antihistamine should be administered in combination with corticosteroids; in case of serious anaphylactic problems administration of adrenaline may be necessary.[38,41] Mild symptoms of a delayed infusion reaction often disappear spontaneously. For treatment of this type of delayed reaction, an oral antihistamine course of treatment can be given, in combination with acetaminophen.[38] For the more serious form of serum disease with myalgia and fever, a course of treatment with steroids can be considered. More details about the treatment of infusion reactions is provided under Practical treatment instructions.

Infection

The use of infliximab in patients with Crohn's is associated with an increased risk of infection. The average number of patients in clinical studies who develop an infection after infliximab treatment was 36%, *vs* 26% after placebo.[6] This primarily involved uncomplicated upper bronchial tube and urinary tract infections. From various large cohort studies, it appeared that the incidence of serious infections during (maintenance) treatment with infliximab only amounted to 1.3 to 4.0% per treatment year (*table 1*). Among other things, this involved fatal sepsis, pneumonia, gastroenteritis and abdominal abscesses. In the ACCENT I and II studies, the incidence of serious infections in the infliximab groups was not elevated in comparison with the placebo group.[14,15] The TREAT (Crohn's Therapy Resource, Evaluation and Assessment Tool) registration, in which data of more than 6000 Crohn's patients are collected, an increase in serious infections in connection with infliximab treatment was observed (*table 1*).[43] That is why it is of utmost importance to rule out abscesses or other ongoing infections before infliximab treatment.

An important observation is that treatment with infliximab increases the risk of reactivation of latent tuberculosis (TBC). As early as in 2001, 70 cases of TBC were reported among the 147,000 patients treated with infliximab (for rheumatoid arthritis or Crohn's disease),[46] while the annual incidence in the American population is normally approximately 6 per 100,000.[47] Most of the TBC cases manifested during the period of the first three infliximab infusions. Remarkably, 40 of these 70 cases involved extrapulmonary TBC.[46] In another study,[47] the incidence of TBC was investigated in a large population of patients with rheumatoid arthritis without and after treatment with infliximab. The TBC incidences amounted to 6.2 per 100,000 per year, *vs* 52.5 per 100,000 per treatment year, respectively, which is consistent with a clear increased risk of TBC during treatment with infliximab. Therefore, infliximab is contraindicated when there is a manifest presence of latent TBC. For this reason, it should be investigated whether a patient has a higher risk, for example because of previous contact with TBC or TBC treatment, or due to a long-term stay in a country or originating from a country where TBC is endemic (>50 per 100,000 inhabitants) prior to starting a treatment with infliximab. The presence of a latent or manifest TBC must be ruled out before the first infusion. A pretreatment chest X-ray is considered necessary, in addition to a Mantoux skin test.[48] For patients vaccinated for TBC earlier in their life, the Mantoux skin test is unreliable. Furthermore, there is a change of anergy among patients who are on corticosteroids and/or immunosuppressants. TBC and certainly the extraintestinal form, is difficult to rule out under these circumstances, so a thorough family history and clinical alertness remain important.

Autoimmunity

In various clinical studies, the percentages for antinuclear antibodies (ANA) and double-stranded DNA antibodies (anti-ds-DNA) after infliximab treatment amounted to 46 to 57% and 23 to 34%, respectively.[14,15,49] In the placebo groups, these percentages for ANA and anti-ds-DNA were significantly lower (18 to 35%, 6 to 23%, respectively).[14,15]

In rare cases, these elevated ANA values are paired with clinical symptoms which are consistent with the 'lupus-like' syndrome, such as polyarthralgia, myalgia, and butterfly-shaped hives on the face (*table 1*).[49]

Demyelinating diseases and neurological disorders
After treatment with infliximab or other anti-TNF-α therapies, new episodes of exacerbations have been described for demyelinating diseases and neurological disorders such as multiple sclerosis, myelitis, optic neuritis and Guillain-Barré syndrome, a worrying finding because of the elevated prevalence of demyelinating disease in the IBD population.[50,51] Although no causal relationship has been demonstrated, restraint is advised for patients with these disorders.

Malignancies
There is a concern about an increased risk for malignancy with the use of infliximab, because TNF-α may be protective against cancer development. However, up until now, no corroborating epidemiological data support this concern. In clinical studies, over a period of a maximum of 102 weeks, a malignancy developed in 18 of the 1678 patients treated with infliximab (including non-Hodgkin's lymphoma, breast cancer, skin cancer, rectal adenocarcinoma). On an annual basis, this incidence did not differ statistically from the expected incidence in a comparable population.[41] To make interpretation more complex, it is being debated whether patients with Crohn's disease have an increased risk for attracting lymphoproliferative disorders or bowel cancer. The use of infliximab in the various controlled studies did not show any increased incidence of any cancer (*table 1*). This is corroborated by the results of the TREAT registration,[43] where no statistical difference has been observed in malignancies between patients who were and those who were not treated with infliximab (*table 1*). However, longer follow-up of the safety data remains necessary.

Cardiac failure
Because of an increased mortality rate for patients who were treated with infliximab for severe cardiac insufficiency, infliximab is contraindicated in patients with chronic cardiac failure (New York Heart Association class III or IV).[52]

Liver disease
Infliximab has an immunosuppressive effect, which theoretically can result in hepatic, viral inflammation becoming reactivated after the use of infliximab.[53] Furthermore, there are indications that TNF-α plays a causal, pathogenetic role in viral hepatitis.[54] In clinical practice, cases have been described where infliximab was safe and effective for Crohn's disease patients with chronic viral hepatitis.[54-57] On the other hand, there are reports of patients with Crohn's disease getting a serious exacerbation of viral hepatitis after treatment with infliximab.[53,58,59]

Pregnancy
The first publications suggest that the possible risks of infliximab treatment during pregnancy outweigh the risks of active Crohn's disease during pregnancy.[59-61] In the TREAT registration, data of 98 pregnant patients with Crohn's disease were recorded (59 treated with infliximab and 39 not treated with infliximab); no children with deformations were registered and the percentages of miscarriages and neonatal complications were equal in both groups.[43] Formal recommendations, however, are premature, and a restrictive approach is advised.

PRACTICAL TREATMENT INSTRUCTIONS

Based on the previously discussed insight into the effectiveness and safety of infliximab for Crohn's disease, practical treatment instructions are outlined below (see relevant chapters above for further comments).

Selection of patients
Infliximab is indicated for luminal Crohn's disease and fistula disease associated with Crohn's disease in patients who do not respond or who respond inadequately to an adequate therapy with a conventional immunosuppressive treatment.

Diagnostic examination
Symptoms of irritable bowel disease frequently occur in quiescent Crohn's disease. When in doubt about the nature of the symptoms, the disease activity needs to be assessed by endoscopy for localisation in the colon or terminal ileum, or with imaging of the small intestine and duodenoscopy for more proximal localisations. Determination of C-reactive protein levels is advised.

Precautions
Liver function needs to be checked before treatment and infliximab must be stopped in patients who develop jaundice or liver function problems (>3 times the normal values) after treatment with infliximab.
Patients who smoke react significantly less favourably to infliximab.[12,62] Patients with Crohn's disease are strongly advised not to smoke.

Contraindications
An anaphylactic shock, the occurrence of stridor, or serious hypotension (or reduction in blood pressure of more than

40 mmHg) with a previous infusion of infliximab are a contraindication for repeating infliximab infusions.
The presence of serious infections such as sepsis, abscesses and TBC needs to be ruled out before starting treatment with infliximab. For patients with fistulas associated with Crohn's disease, abscesses must be ruled out, preferably by means of local ultrasound or MRI of the perianal region or with an ultrasound/CT for enterocutaneous localisation.

Before the start of infliximab treatment, latent or manifest TBC must be ruled out with at least:
- Extensive history to detect possible prior TBC contacts
- Mantoux test
- X-ray of the thorax

For a proper interpretation of the Mantoux test, it is important that the previous history of the patient is well documented: here attention needs to be paid to factors which could influence the outcome of the test, such as previous contact with TBC, a long-term stay in (or origin from) a country where TBC is endemic (>50 per 100,000 residents), use of immunosuppressive drugs and risk factors such as diabetes mellitus, kidney insufficiency, and haematological disorders.[5] During infliximab treatment, it is important to be alert for TBC when the patient complains of fever (noctural transpiration), coughing, unexplained stomach complaints and weight loss.

Dosage
In general, the following dosage is used for the various indications:
- induction regimen: 5 mg/kg at 0-2-6 weeks, usually followed by:
- maintenance treatment: 5 mg/kg every eight weeks, when response is inadequate increase to 10 mg/kg on strict verified indication.

A good clinical assessment as to whether the remission-induction regimen is effective is essential, preferably within four weeks. With the initiating regimen of 0-2-6 weeks, it may prove difficult to decide within four weeks whether the therapeutic effect is adequate, and waiting until four weeks after the second infusion is an option. If there is no clear and clinically significant response at that time, there is no indication for continuing further infusions or elevating the dosage to 10 mg/kg.

Optimising infliximab treatment
The use of immunosuppressants (azathioprine, 6-mercaptopurine of methotrexate):
- prevents the formation of ATI and thus the risk of an infusion reaction;
- increases the likelihood of an adequate response to infliximab;
- increases the duration of the response and with it, the infusion interval.

For an optimal effect, treatment with infliximab should be combined with the use of an immunosuppressive.

The optimal effect of an induction treatment with infliximab (using the 0-2-6 week regimen), combined with a concurrently started treatment with an immunosuppressant, is only noticeable about eight weeks after the third infusion. Because the effective duration of infliximab is an average of 12 weeks, while immunosuppressants such as azathioprine and methotrexate only become effective after about three months,[63] the eventual synergistic effect of this combination can only be evaluated after this period of three months.
For the protocol for administration of infliximab see *figure 4* and for the protocol for treatment of acute infusion reaction see *figure 5*.

CONCLUSION

Without doubt, infliximab is the most spectacular medicine in recent years for patients with Crohn's disease. It is obvious that this drug has a significant place in current treatment, where the roles of mesalazine and corticosteroids are increasingly being discussed. Even quite recently, it has become clear that Crohn's disease may be treated without corticosteroids, as the combination of azathioprine with infliximab has proved effective and safe as primary therapy for recently diagnosed patients.[64]
However, infliximab can also be a dangerous drug if the correct measures are not taken to prevent side effects and (serious) complications. This guideline attempts to produce clarity about these precautionary measures for the purpose of optimising the result of a treatment with infliximab.
Pharmacoeconomic considerations have shown a reduction in both direct and indirect costs in various Western countries, some of which can be compared with the Netherlands.[65-67] It is therefore remarkable that reimbursement for this expensive drug is still not sufficiently taken care of by the government and health insurance companies in the Netherlands.

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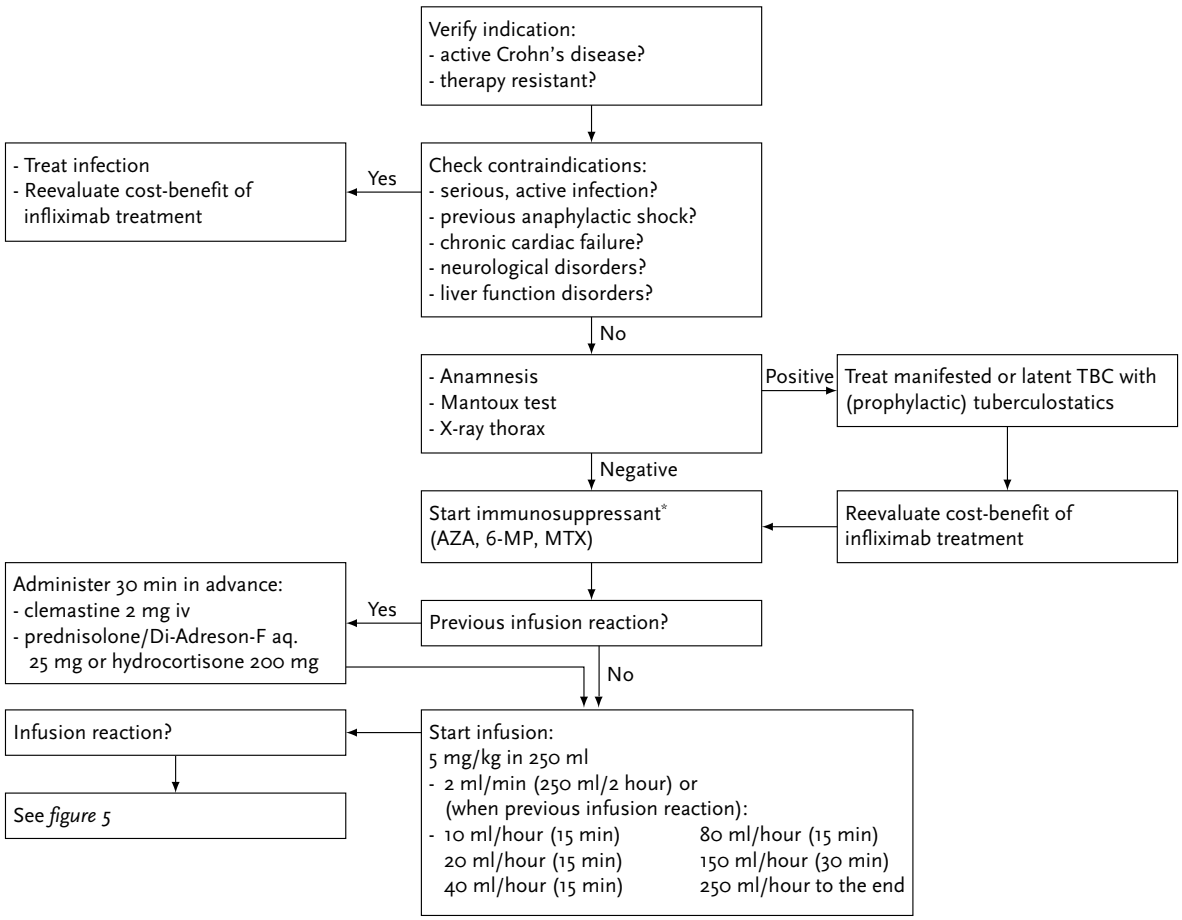
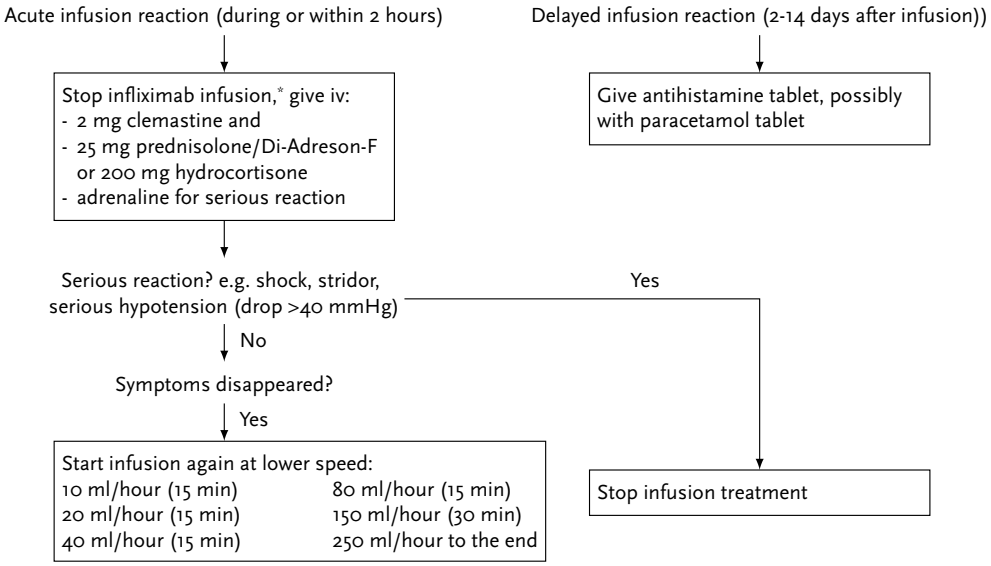
Figure 4. *Algorithm for the administration of infliximab*



*For patients who are not yet using an immunosuppressant. 6-MP = 6-mercaptopurine; AZA = azathioprine; MTX = methotrexate.

Figure 5. *Algorithm for the treatment of an acute or delayed infusion reaction*



*With mild complaints, one can also consider switching over to a reduced infusion speed.

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ORIGINAL ARTICLE

Establishment of reference values for endocrine tests – part V: acromegaly

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ABSTRACT

Background: Plasma insulin-like growth factor 1 (IGF-1) and the response of growth hormone (GH) to oral glucose are frequently used in the evaluation of patients suspected of acromegaly. Because of the implementation of new assay methodology for GH and IGF-1, we have established the reference values for these tests, as well as for urinary GH excretion.

Methods: From the general population, 50 subjects were recruited, equally distributed according to sex and age between 20 and 70 years. Two consecutive 24-hour urine samples were collected to determine urinary GH. Plasma IGF-1 was measured as well as the GH response during an oral glucose tolerance test (OGTT) with 100 g glucose. Basal plasma IGF-1 was also measured in 250 subjects recruited likewise from the general population who had participated in previous studies on reference values.

Results: The following reference ranges were established: urinary GH <5-46 μU/24 h; nadir GH after OGTT ≤1.5 mU/l for males and ≤2.0 mU/l for females. IGF-1 was divided into age groups: 20-30 years 8-61 nmol/l; 31-40 years 8-41 nmol/l; 41-50 years 7-36 nmol/l; 51-60 years 5-37 nmol/l; and 61-70 years 7-27 nmol/l.

Conclusion: We have established reference values with state-of-the-art assay methodology for the diagnostic tests frequently used in the evaluation of patients suspected of acromegaly.

KEYWORDS

Acromegaly, endocrine tests, reference values

INTRODUCTION

Acromegaly, a rare disease with a prevalence of 40 to 70 cases per million,[1] is usually caused by overproduction of growth hormone (GH) by a pituitary adenoma. The delay between onset of GH overproduction and clear clinical manifestation can be more than ten years.[2]

A random blood sample for measuring GH is not a reliable test for diagnosing acromegaly because GH is secreted in a pulsatile manner; peaks of more than 40 mU/l are followed by episodes of immeasurable GH concentrations. A 24-hour plasma sampling every 15 to 30 minutes is used to determine 24-hour GH production, but this test is time-consuming and costly. GH is secreted in the urine, so determination of GH in a 24-hour urine sample can give a measure of 24-hour GH production as well.[3]

The degree of GH suppression during an oral glucose tolerance test (OGTT) is the traditional test[4] in the biochemical diagnosis of acromegaly, although the OGTT is not 100% sensitive.[5] The serum concentration of insulin-like growth factor I (IGF-1) may reflect overall GH secretion and therefore be a good marker for GH overproduction.[2] Thus, serum IGF-1 is frequently used as a screening test for acromegaly.

In recent years much has changed in immunoassay methodology, also for the determination of GH and IGF-1. Older polyclonal radioimmunoassays or immunoradiometric assays have been replaced by (automated, monoclonal) immunochemiluminometric assays. In addition, for GH, International Reference Preparations (IRP 66/217, IRP 80/505) have been replaced by International Standards (IS 88/624 and IS 98/574). The IRPs were of pituitary origin; the ISs are recombinant DNA-derived human GH. With these changes, the cut-off values reported for GH suppression after a OGTT have changed from 2.5 to 5 μg/l to 0.4 to 1 μg/l.[6]

The consensus[7] for baseline biochemical parameters states that a random GH level of <0.4 μg/l and an IGF-

1 within the age- and gender-matched reference range excludes acromegaly. If either of these levels is not achieved, an OGTT should be performed with subsequent measurements of glucose and GH every 30 minutes over two hours. GH concentration should fall to ≤1 μg/l to exclude acromegaly. In recent years there has been some debate about reducing the nadir GH after OGTT to 0.4 μg/l[8] or even to 0.21 μg/l.[9] Under ideal conditions any assay should be validated with a normal range for suppressed GH levels after an oral glucose load.[10]
In the Netherlands there were discussions on which unit should be used to express GH concentration, i.e. μg/l or mU/l. Although the IS is expressed as μg/l, clinical endocrinologists and the board of the Endocrinology Section of the Dutch Foundation for Quality Assessment in Clinical Laboratories (SKML) have decided to use mU/l, because GH has a heterogeneous molecular composition. To reduce the inter-laboratory variation (especially for the diagnosis of GH deficiency) caused by, among other things, different specificities of the anti-GH antibodies for the different GH isoforms, GH assays have been harmonised in the Netherlands. A harmonisation sample from native serum with an assigned consensus value is used for this purpose.

In the light of these changes, we decided to obtain reference values for GH concentrations after OGTT, urinary GH excretion and IGF-1 in 50 subjects recruited from the general population, not suspected of having acromegaly. Because of the small number of subjects for establishing age- and gender-matched IGF-1 concentrations, we included 250 subjects from previous projects, equally distributed according to sex and age between 20 and 70 years. Reference values for other endocrine tests have previously been published in this journal.[11-14]

SUBJECTS AND METHODS

Subjects

Volunteers were recruited through an advertisement in a local newspaper with a free house-to-house distribution in the Amsterdam region: 104 subjects responded. The respondents were asked by telephone whether they met the inclusion criteria. The inclusion criteria were age between 20 and 69 years and self-reported good health. Exclusion criteria were diabetes mellitus or intravenous drug abuse, recent surgical treatment, hospital admission in the past month, having given birth in the past six months and breastfeeding at the time of the study.
The first 50 volunteers who met the inclusion criteria were invited to our department, where they were interviewed about drinking and smoking habits, current use of medication and their racial background. Females were asked about their periods (first day of the last menstrual cycle). Informed consent was obtained from all subjects and the study was approved by the local hospital's ethics committee.
The 50 subjects were divided into five age groups, ranging from 20-70 years. Each age group consisted of ten subjects (five males and five females). One subject did not complete the tests because of a problem with inserting an indwelling venous catheter. The first volunteer on the backup list meeting the age and sex criteria, replaced this subject. Three subjects were excluded later on, because their test results showed that they had diabetes mellitus. They were not replaced.
The 250 added subjects for establishing age- and gender-specific IGF-1 reference values met the same inclusion and exclusion criteria.

Tests

The tests were performed in the following order:
- 24-hour urinary excretion of GH
- Basal plasma values of IGF-1
- GH response after oral glucose load

Twenty-four hour urinary excretion of GH

On two successive days prior to the OGTT, two 24-hour urine collections were taken in two separate containers. During the collection period the urine was kept cool. Heavy physical exercise was not allowed while the urine was being saved, because it is known that GH production is stimulated by exercise.
The total volume per 24 hours of the two separate urine samples was measured, as well as the concentration of GH, glucose and creatinine. Total creatinine excretion was measured to check whether collection was complete. Some 15 to 20% of the difference in intra-individual creatinine excretion may be due to variations in dietary intake. Since urine collection took place in an outpatient setting, an intra-individual difference of up to 30% was accepted. If total creatinine excretion in the sample with the highest creatinine excretion was more than 150% of the creatinine excretion in the other sample, both samples were excluded.[14]

Basal serum levels of IGF-I

The OGTT was performed the day after the second urine collection. We combined the IGF-I measurement with the OGTT. The subjects were in the postabsorptive state and were not allowed to smoke before or during the test. Body height and weight were obtained in advance. An indwelling venous catheter was inserted (t = -30 min) in an antecubital vein and blood samples were taken at t = -15 min and t = 0 min for measuring GH and glucose (see hereafter). IGF-1 was measured in the t = 0 min blood sample. In the extra 250 subjects, the IGF-1 was measured in a basal sample.

GH response after oral glucose load

After the blood sample at t = 0 min the volunteers drank a solution of 100 g glucose in 200 ml water. After 30, 60 and 90 minutes, respectively, blood samples were obtained for measuring GH and glucose. The subjects were in a sitting position during the test.

Analytical methods

Glucose and creatinine were measured according to standard clinical chemical techniques.

IGF-I was measured by a fully automated, two-site chemiluminescent immunoassay (Nichols Advantage®; Nichols Institute Diagnostics, San Clemente, CA, USA). In this system the antibody to the C-terminal 62-70 amino acid sequence is biotinylated for capture and the antibody to the amino sequences of 1-23 and 42-61 is labelled with acridinium ester for detection. Samples are acidified to separate IGF-1 from IGF binding proteins (BP). Then, excess IGF-II is added in the assay to block the IGFBP binding sites from recombining with the released IGF-1. The acidified samples are incubated simultaneously with the biotinylated capture antibody, excess IGF-II, and the acridinium ester labelled tag antibody. During the first incubation, IGF-1 form a sandwich complex with the capture antibody and the acridinium ester labelled antibody in the samples. After the initial incubation period, streptavidin-coated magnetic particles are added to the reaction mixture and a second incubation follows. The streptavidin-coated particles allow for a highly specific and efficient means of binding the sandwich complex to the solid phase via the high-affinity interaction between biotin and streptavidin. Free-labelled antibody is separated from the labelled antibody bound to the magnetic particles by aspiration of the reaction mixture and subsequent washing. The wells containing the washed magnetic particles are transported into the system's luminometer, which automatically injects an acid hydrogen peroxide solution and a sodium hydroxide solution to initiate the chemiluminescence reaction. The light generated by the reaction is directly proportional to the amount of bound-labelled antibody and to the concentration of IGF-1. The assay is calibrated against the World Health Organisation's (WHO) International Reference Reagent 87/518. The inter-assay coefficient of variation (CV) is 6% (at 7 nmol/l) to 4% (at 55 nmol/l); the detection limit is set in our laboratory at 5 nmol/l.

Serum GH was measured using a two-site chemiluminescent immunoassay (Nichols Advantage; Nichols Institute Diagnostics, San Clemente, CA, USA). It utilises one mouse monoclonal antibody and one goat polyclonal antibody to GH. The monoclonal antibody is coupled to biotin, while the goat polyclonal antibody is labelled with an acridinium ester for detection; GH is 'sandwiched' between these antibodies. Separation and quantification is the same as in the IGF-1 assay. The inter-assay CV is 7% (at 2 mU/l) and 6% (at 18 mU/l); detection limit is 0.3 mU/l. The assay is calibrated against the WHO's 2nd International Standard 98/574. By definition 1 µg of this standard corresponds with 3 mU.[15] To achieve the assigned consensus value of the harmonisation sample, an additional multiplication factor of 1.85 is used to convert µg/l to mU/l. This was done for all serum GH values.

Urinary GH was measured by desalinating 2.5 ml of urine using a Sephadex G25 column, after which GH in the eluate was bound to a capture antibody coated to a tube (DiaSorin, MP Products, Amersfoort, the Netherlands) by rotating end over end for 18 hours and after washing followed by an incubation of a radioactive labelled detecting antibody. Standards were prepared from IS 98/574, range 0 to 2000 µU/l. Detection limit is 5 µU/l and inter-assay CV is 8% at 45µU/l and 12% at 15 µU/l.

Statistical methods

Alcohol intake was defined as the intake of at least two units of alcohol a day and smoking as smoking on a daily basis. The postabsorptive serum glucose and serum GH values were calculated as the average of the t = -15 min and t = 0 min values. The lowest GH value from t = 30, t = 60 and t = 90 was used for expressing GH suppression during the OGTT. Results under the detection limit were considered to have the value of 50% of this detection limit. Sex differences and the effect of smoking and alcohol intake were tested using the Mann-Whitney test. The Kruskal-Wallis test was used to evaluate the effects of age. Correlation between variables was tested by linear regression. The computer programme Graphpad Prism 3.0 was used for statistical analysis and composition of the figures. In all tests p values <0.05 were considered as statistically significant. Reference ranges were calculated as mean ± 2 SD if the values were normally distributed. In the case of GH after OGTT the lower and upper limits are given.

RESULTS

Subject characteristics

The 50 subjects had a mean age of 44 ± 14 (SD, range 22 to 67) years, 19 were smokers (38%) and 20 used alcohol (40%). Body mass index (BMI) varied from 20.0 to 32.6 (median 25.1) in males and from 18.6 to 41.2 (median 23.9) in females. Eleven females were postmenopausal. After performing all tests three males (51, 55 and 66 years) with high serum glucose levels were taken out the study.

Urinary excretion of GH

One additional subject (female, age 29 years) was excluded on account of incomplete urine collection (the intra-individual difference in 24-hour creatinine excretion was more than 150%).

In subjects in whom urine collection was adequate, there was no significant difference in urinary excretion of GH between day 1 and 2, and for this reason the mean of the two days was used to determine the reference values. There were no significant effects of sex, smoking, alcohol, age or BMI on urinary excretion of GH in the included subjects. The mean was 23 μU/24 hour (range <5 to 46). There was no correlation between mean plasma GH concentration or nadir GH concentration and urinary GH excretion. The reference values are given in *table 1* and the distribution in *figure 1*.

Basal plasma levels of IGF-1

We found no sex differences in IGF-1 concentrations between males and females in our population (p=0.10); therefore, the males and females were analysed together in the age-groups. IGF-1 showed a significant correlation with age (p<0.001), with a decline with increasing age (*figure 2*). There was no correlation between mean basal plasma GH concentration, nadir GH concentration after oral glucose or urinary GH excretion and plasma IGF-1. The reference values are given in *table 1*.

Basal plasma levels of GH and GH response in OGTT

There was a significant difference between basal GH levels of males and females (p=0.002); the median for males was 2.1 (range <0.3 to 17 mU/l) and for females 6.8 (range 0.3 to 41 mU/l). The distribution of the GH values was non-Gaussian and could not be normalised after logarithmic transformation (*figure 3*).

During the OGTT 42 out of 47 subjects showed a decrease in GH. In four volunteers the GH concentration was undetectable at all time points and one subject showed a rise from 0.3 mU/l to 0.4 mU/l. Nadir GH was reached at 30 min in 24% of the subjects, at 60 min in 18% and after 90 min in 47%. In ten subjects there was a rise in GH concentration after the nadir. Males had a lower GH concentration after glucose (median <0.3; observed range <0.3 to 1.5 mU/l) than females (median 0.8; observed range <0.3 to 2 mU/l); p=0.01. No effects were seen in relation to age, BMI, alcohol intake or smoking. Also here no normalisation of the distribution could be achieved after logarithmic transformation. The results are depicted in *figure 4*.

DISCUSSION

In the biochemical diagnostic work-up for the diagnosis of acromegaly several tests are in use. The lack of widely available reference ranges for urinary GH, in addition to the difficulty in obtaining reliable 24-hour collection of urine samples, precludes this measurement in the routine diagnosis and follow-up of acromegaly.[2] Lack of information

Table 1. *Reference values for diagnostic tests in the evaluation of acromegaly*

	Mean	Reference interval*
Urinary GH	23 μU/24 hour	< 5 – 46 μU/24 hour
IGF-1 (age groups):		
• 20-30 years	34 nmol/l	8-61 nmol/l
• 31-40 years	25 nmol/l	8-41 nmol/l
• 41-50 years	22 nmol/l	7-36 nmol/l
• 51-60 years	21 nmol/l	5-37 nmol/l
• 61-70 years	17 nmol/l	7-27 nmol/l
GH after OGTT		
Male		≤1.5 mU/l (≤0.27 μg/l)
Female		≤2.0 mU/l (≤0.34 μg/l)

Reference interval: mean ± 2 SD, except for growth hormone (GH) after oral glucose tolerance test (OGTT).

Figure 1. *24-hour urinary GH concentrations in 46 healthy volunteers; mean of two 24-hour collections*

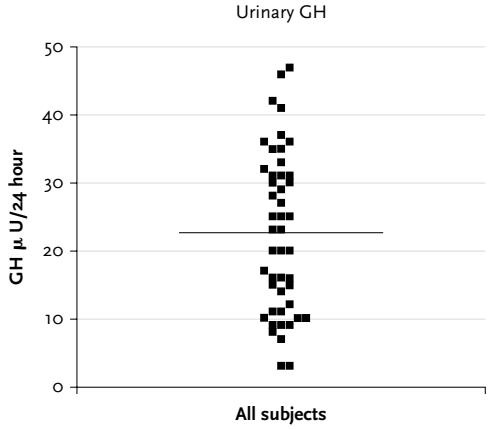


Figure 2. *Plasma IGF-1 concentrations in 297 healthy volunteers according to age groups*

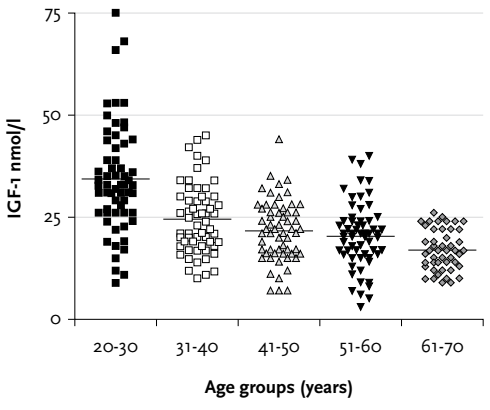
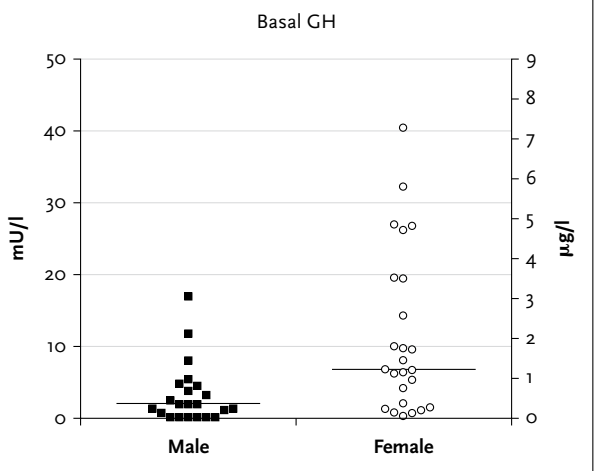
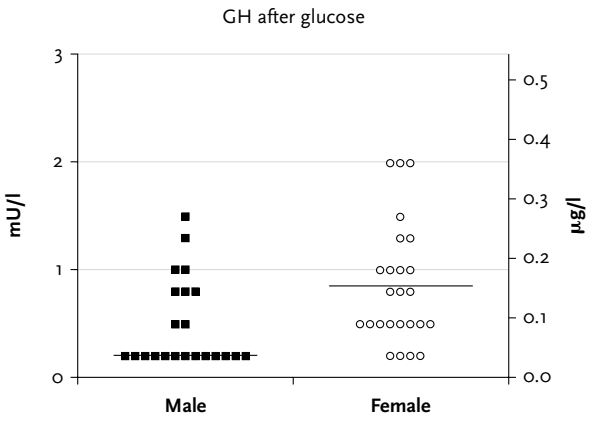


Figure 3. *Basal plasma GH concentrations in 22 male and 25 female healthy volunteers*



Conversion factor from µg/l to mU/l: 5.55.

Figure 4. *GH response on 100 g glucose in 22 male and 25 female healthy volunteers*



Conversion factor from µg/l to mU/l: 5.55.

on the standards used[16,17] and use of different standards have not been beneficial either for the establishment of uniform reference ranges. Our reference values are in agreement with a Danish study of 112 healthy adults, in which the upper reference limit was 49 µU/24 hours (using a conversion factor of 2.44 for ng to µU)[18] *vs* 46 µU/24 hour in our study. We did not find a sex difference in excretion of urinary growth hormone in contrast to other studies.[18,19] Whereas in some studies an age-related decline was observed in urinary GH excretion,[19,20] we did not found this relation. In acromegaly patients a (log) urinary GH and (log) serum GH correlation is found,[18,20] but not in healthy subjects; we did not find this correlation either. This discrepancy between patients and healthy subjects may reflect the large variation in urinary GH excretion in healthy controls as compared with the smaller variation in serum GH profiles.[18] This might be the explanation for us not finding a difference in urinary GH between males and females, while basal serum GH in females is higher than males. With our assays there was no relation between serum IGF-1 and urinary GH.

In recent years more robust assays have been developed to measure IGF-1. Since the world-wide calibration on WHO International Reference Reagent 87/518, published reference ranges are more in agreement with each other. The reference ranges established in this study are in a similar range to those of Brabant *et al.*,[21] who used the same methodology, although our upper limit of the reference range in all age groups is ± 5 nmol/l lower. In a recent study by Ranke *et al.*, comparing four immunoassays for IGF-1,[22] the measured reference range on the Advantage system is in agreement with our values. We did not find a gender difference in IGF-1 levels as other studies.[23] Before puberty, girls have significantly higher IGF-1 levels than boys, but after the age of 20 the values are more or less the same with a tendency of higher levels in males than in females.[21] Therefore, a reference range in age groups is more opportune than gender-related reference ranges.[23]

With the introduction of the WHO IS 98/574, based on recombinant DNA-derived GH, standardisation of GH assays has taken a tremendous step forward. Progress is, however, hindered by the discussion whether to express GH in mass units or arbitrary (activity) units. The supporters of mass units claim that the IS is derived by recombinant technology, so mass can be measured. The opponents say that GH in blood is not solely the 22 kd variant of GH and that different antibodies will not react in the same way with the other GH isoforms. This holds true in our case, where an additional multiplication factor of 1.85 (above the factor 3 for conversion from µg/l to mU/l) must be used. The endocrinologists in the Netherlands have chosen to use mU/l, so laboratories have agreed to harmonise their GH assays to an assigned consensus value of a native serum with a GH concentration of 17.5 mU/l. Also in a Belgian study such a harmonisation factor is proposed.[24]
In our study we saw an upper limit of nadir GH after OGTT of 1.5 mU/l in male and 2.0 mU/l in female volunteers. Of the male subjects, 56% reached undetectable GH concentrations *vs* 18% of the females. In the study by Freda *et al.* in 46 healthy volunteers (26 males, 20 females, age range 20-71 years) all had nadir GH after OGTT <0.14 µg/l;[25] in our situation this means <0.8 mU/l. They found no sex differences. In our study 7 male and 13 female

volunteers were above this level; we cannot explain this difference, other then perhaps the differences in antibodies used in the DSL kit *vs* the Advantage system. Chapman *et al*. studied only nine males and six females (age 21-34) and found a significantly higher nadir GH after OGTT in females than in males, with a mean of 0.25 µg/l *vs* 0.03 µg/l, and an upper limit of 0.72 µg/l *vs* 0.07 µg/l.[26] Other studies have set the cut-off level between disease and non-disease at 1 µg/l or 2 mU/l.[3,27,28] Looking at clinical studies, some report all acromegaly patients having GH after OGTT >2 mU/l,[28] or even >3 mU/l,[16] while Freda *et al*. found 5 out of 15 patients with a nadir GH between 0.4 and 1.0 µg/l.[5] Our levels of 1.5 mU/l (0.27 µg/l) for males and 2.0 mU/l (0.34 µg/l) for females are lower than that cut-off point, but still higher than in their healthy volunteers. On the other hand IGF-1 and perhaps urinary GH will add additional information, besides the clinical suspicion, for the diagnosis of acromegaly.

CONCLUSION

Urinary GH, IGF-1 and nadir GH after OGTT are used for the biochemical diagnosis of acromegaly. In our study we have established reference values for these tests.

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ORIGINAL ARTICLE

Trends in fungaemia and antifungal susceptibility in the Netherlands

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ABSTRACT

We retrospectively evaluated fungaemia over the period 1996 to 2001 in five university hospitals. Over 350,000 blood cultures were collected during more than 7 million days of hospitalisation. The average rate of fungaemia over the six-year period was 0.82 per 10,000 patient days (range 0.65 to 1.21 per 10,000 patient days). The proportion of bloodstream infections caused by *Candida albicans* remained stable throughout the study period with a mean of 53% (range 48 to 62%). This is a change from trends described in previous studies, including a survey performed in the Netherlands. This study shows a new, stable rate of fungaemia and no further signs of increasing rate of infections due to non-*albicans Candida* species. Susceptibility to all tested antifungal agents remained stable throughout the study period.

KEYWORDS

Candida, epidemiology, fungaemia

INTRODUCTION

Candida species are frequent causative agents of fungaemia. During the last decade, there has been a shift in the incidence of causative organisms of fungaemia for these species. Although in some studies *Candida albicans* was still the most frequently isolated species,[1-7] non-*albicans Candida* species have become increasingly prevalent.[8-10] Concomitantly, there may have been a change in the susceptibility for systemic antifungal drugs due to this changing distribution of *Candida* species.[11-15] Moreover, the increasing use of azoles in prophylaxis and treatment may have caused selection of azole-resistant yeasts or induced resistance. In 1996, Voss *et al*. determined the incidence of yeast infections in five Dutch university hospitals over the period 1987 to 1995.[8] That study showed an increase in the rate of fungaemia during this period. *C. albicans* was the most frequently isolated species, but overall non-*albicans Candida* species were increasing significantly. To assess whether this trend has continued in recent years, this study was repeated for the period 1996 to 2001. In addition, the susceptibility to antifungal drugs was determined according to the Clinical Laboratory Standards Institute (CLSI) protocol M27-A2.[16]

METHODS

Microbiology data from computer-generated lists of patients whose blood cultures had yielded yeasts during the period from 1 January 1996 to 31 December 2001 were analysed retrospectively. The data were provided by the five university hospital laboratories and an additional laboratory of a major hospital in Rotterdam. All patients admitted to the aforementioned hospitals were eligible. An episode of fungaemia was defined as at least one positive blood culture yielding yeasts during a single hospitalisation period. The number of blood culture sets were examined and the results were recorded. Automated blood culture systems were used in all participating hospitals. Data to determine patient days were retrieved from the hospital information systems.

Strain identification and susceptibility testing

Yeasts were cultured on Sabouraud-dextrose agar and incubated for four days at 37°C. Identification was performed with standard microbiological techniques. All the isolates were initially kept at -70°C in glycerol broth. The antifungal activity of amphotericin b (AMT, Bristol-Meyers-Squibb, Woerden, the Netherlands), 5-fluorocytosin (5-FC, Valeant, Zoetermeer, the Netherlands), fluconazole (FLU, Pfizer, Capelle aan de IJsel, the Netherlands), itraconazole (ITC, Janssen Pharmaceutica BV, Tilburg, the Netherlands), voriconazole (VOR, Pfizer, Capelle aan de IJsel, the Netherlands), posaconazole (PSZ, Schering-Plough, Maarsen, the Netherlands) and caspofungin (CAS, MSD, Haarlem, the Netherlands) was determined *in vitro* using a broth-microdilution method similar to the CLSI protocol M27-A2.[16] The concentration range for AMT, ITC, VOR, and PSZ was 0.016 to 16 mg/l and for 5-FC, FLU and CAS 0.062-64 mg/l.

For AMT and CAS the minimal inhibitory concentration (MIC) was defined as the lowest concentration that showed no visible growth. For the azoles and 5-FC the MIC was defined as the lowest concentration at which 50% growth inhibition was measured compared with that of the control. MIC was determined after 24 and 48 hours of incubation except for *Cryptocccus neoformans* isolates where the total incubation time was 72 hours. All susceptibility tests were performed in duplicate.

Statistics

ANOVA and Kruskal-Wallis tests were applied for comparing means and the χ^2 test for trends was used for comparing the contingency of causative organisms of fungaemia. MIC dilutions were transformed logarithmically before comparing means. Spearman's rank correlation was used for analysing trends for transformed MIC values over the observed period.

RESULTS

The participating hospitals delivered a total of 7,772,455 hospital days of care (mean 1,295,409 ± 215,042 per year) during the period 1996 to 2001 and the annual frequency did not change significantly during the study period (*table 1*). A total of 837,034 admissions were registered (mean 139,506 ± 21,465 per year) also with no significant changes over the study period. The number of hospital days per admission and the number of blood cultures per 10,000 admission days, 9.3 and 458 respectively, remained stable as well. A total of 355,708 cultures were collected throughout the study period, from which 56,270 (16%) yielded a positive culture. From these positive blood cultures 1688 (3%) yielded yeast divided over 626 episodes of fungaemia. The rate of fungaemia increased from 0.71 in 1996 to 1.21 episodes per 10,000 patient days in 2001; however, this increase did not reach a level of significance. The higher value of 1.21 per 10,000 admission days is an unexplained peak in 2001 whereas the rate over the period 1996 to 2000 only varied by 0.71 to 0.76 per 10,000 admission days (*figure 1*). *C. albicans* was still the most frequently isolated species and its proportion (mean 53%) remained stable during the study period; no significant shift from *C. albicans* to non-*albicans Candida* species was observed (*table 1* and *figure 2*). For susceptibility testing, 357 isolates were available: *C. albicans* (204), *C. glabrata* (70), *C. arapsilosis* (32), *C. tropicalis* (26), *C. krusei* (12), *C. lusitaniae* (6), C. *neoformans* (2), *C. dubliniensis* (1), *C. guilliermondii* (1), *C. inscopicua* (1), *C. kefyr* (1), and one isolate of an unspecified yeast. Of the 357 tested isolates, 53 (15%) were not susceptible to FLU (MIC >8 mg/l). These isolates were *C. krusei* (12), *C. glabrata* (39) and one isolate each of *C. albicans* and *C. tropicalis*. Generally, *C.albicans*

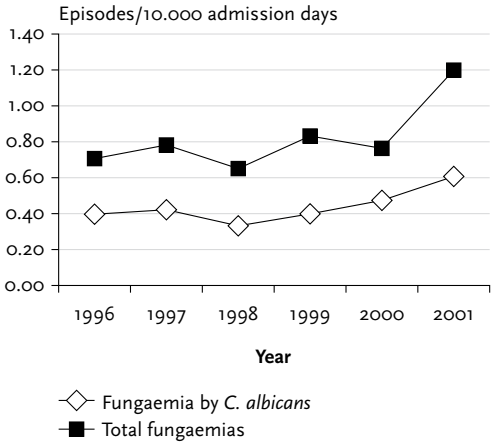
Table 1. *Selected epidemiological and microbiological characteristics over the observed period*

	1996	1997	1998	1999	2000	2001	Total
Admissions*	118,586	158,234	155,068	154,143	143,760	107,243	837,034
Admission days*	1,089,356	1,496,963	1,464,139	1,402,340	1,348,859	970,798	7,772,455
Days per admission	9.2	9.5	9.4	9.1	9.4	9.1	
Blood cultures*	52,282	62,223	61,960	66,012	66,435	46,796	355,708
Blood cultures per 10,000 admission days*	479.93	415.66	423.18	470.73	492.53	482.04	
Positive blood cultures	7324 (14%)	9557 (15%)	10001 (16%)	10660 (16%)	10364 (16%)	8364 (18%)	56270 (16%)
Positive blood cultures per 10,000 admission days*	67.23	63.84	68.31	76.02	76.84	86.16	
Blood cultures containing yeasts	263 (4%)	287 (3%)	332 (3%)	271 (3%)	296 (3%)	239 (3%)	1688 (3%)
Fungaemia*	77	117	95	117	103	117	626
Fungaemia with *C. albicans*	43 (56%)	63 (54%)	49 (52%)	56 (48%)	64 (62%)	59 (50%)	334 (53%)
Fungaemia/10,000 admission days*	0.71	0.78	0.65	0.83	0.76	1.21	
Fungaemia with *C. albicans* per 10,000 admission days*	0.39	0.42	0.33	0.40	0.47	0.61	
Fungaemias per 1000 admissions*	0.65	0.74	0.61	0.76	0.72	1.09	

*No significant changes occurred over the observed period.

isolates tended to be more susceptible to all tested antifungal agents, compared with non-*albicans Candida* isolates. This difference was more pronounced for the azoles. No significant changes in susceptibility of the tested yeast isolates to any of the tested antifungals were observed during the study period (*figures 3A-G*). The new azoles VOR and PSZ as well as the echinocandin CAS showed marked activity against all yeast isolates including the *C. krusei* isolates and non-*C. krusei* isolates with an FLU MIC >8 mg/l.

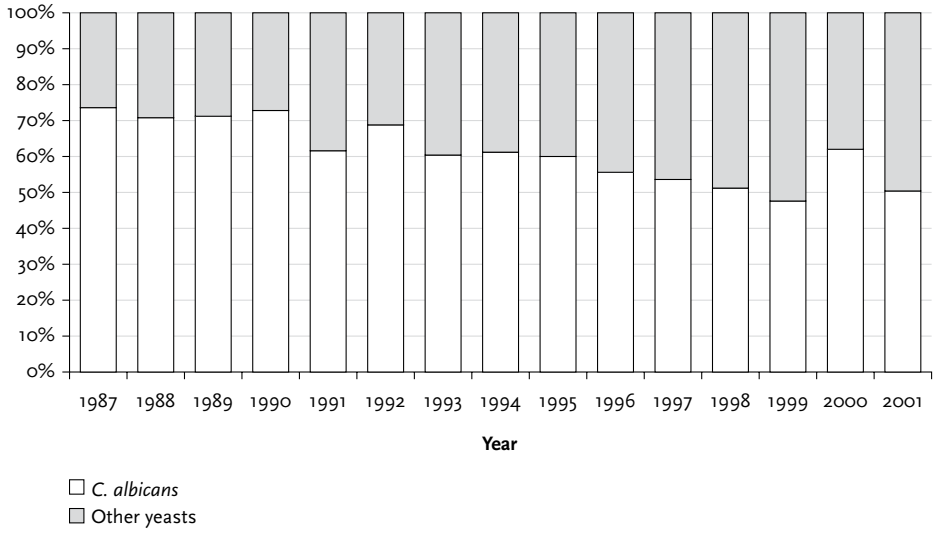
Figure 1. *Number of all episodes of fungaemia per 10,000 admission days and of those caused by C. Albicans per 10,000 admission days*



DISCUSSION

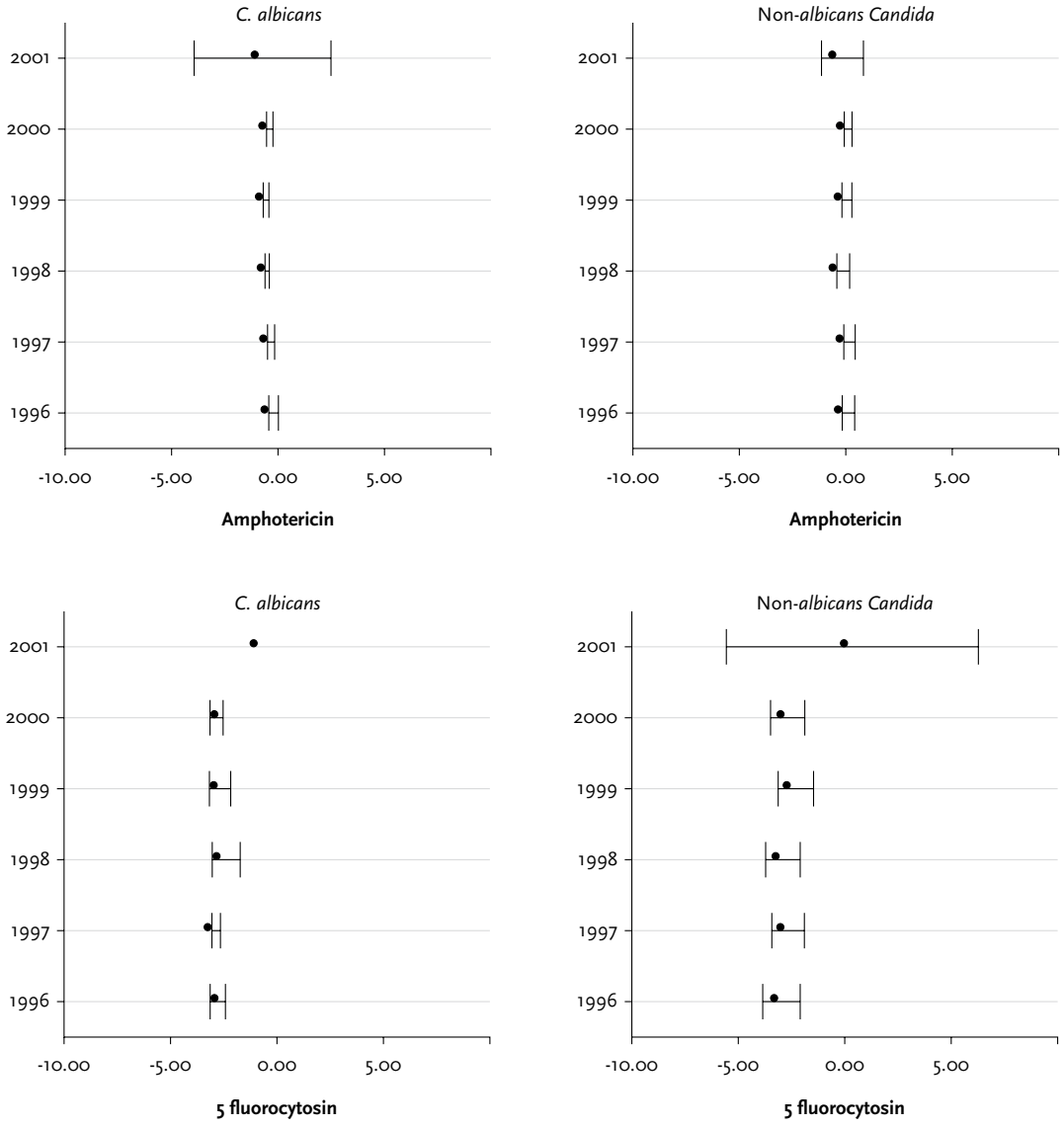
An increase in fungal infections was already reported in the 1980s.[17,18] The main causative agents were *Candida* species of which *C. albicans* was the principal representative. However, in the United States a gradual, relative, increase in non-*albicans Candida* species has been observed in intensive care units.[19] Other studies show similar shifts in fungaemia caused by non-*albicans Candida* species, mainly *C. glabrata*.[5,20] In leukaemia patients the proportion of fungaemia due to *C. krusei* and *C. glabrata* bloodstream infections increased, despite a significant decrease in the overall incidence of fungaemia.[21] This shift in frequency of non-*albicans Candida* infections has been attributed to increased use of FLU,[22] although in one study a causative link between these two parameters was not found.[23] Due to the increase of non-*albicans Candida* species, especially *C. glabrata*, susceptibility to the first-line azole FLU has decreased. More importantly, this decreased susceptibility appears to be accompanied by higher treatment failure rates.[24,25] However, in our study population, both the incidence of fungaemia as well as the proportion of non-*albicans Candida* species remained stable throughout the period 1996 to 2001. There was no significant change in the proportions of fungaemia caused by *C. albicans* and non-*albicans Candida* species. This suggests a break with the trend shown in the period 1987 to 1995, where a significant increase of fungaemia due to non-*albicans Candida* species was observed (*figure 2*).[8] The incidence of fungaemia varied from 0.71/10,000 patient days in 1996 to 0.76/10,000 patient days in 2000. The observed peak of 1.21 episodes

Figure 2. *Contribution of C. albicans candidaemia to total episodes of fungaemia*



Data from the episode 1987 to 1995 were extracted with permission from reference 8 and show a significant increase in the proportion of non-*albicans Candida* species. Over the period 1996 to 2001 this was not significant.

Figures 3A and B. *Mean ^{2}log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*

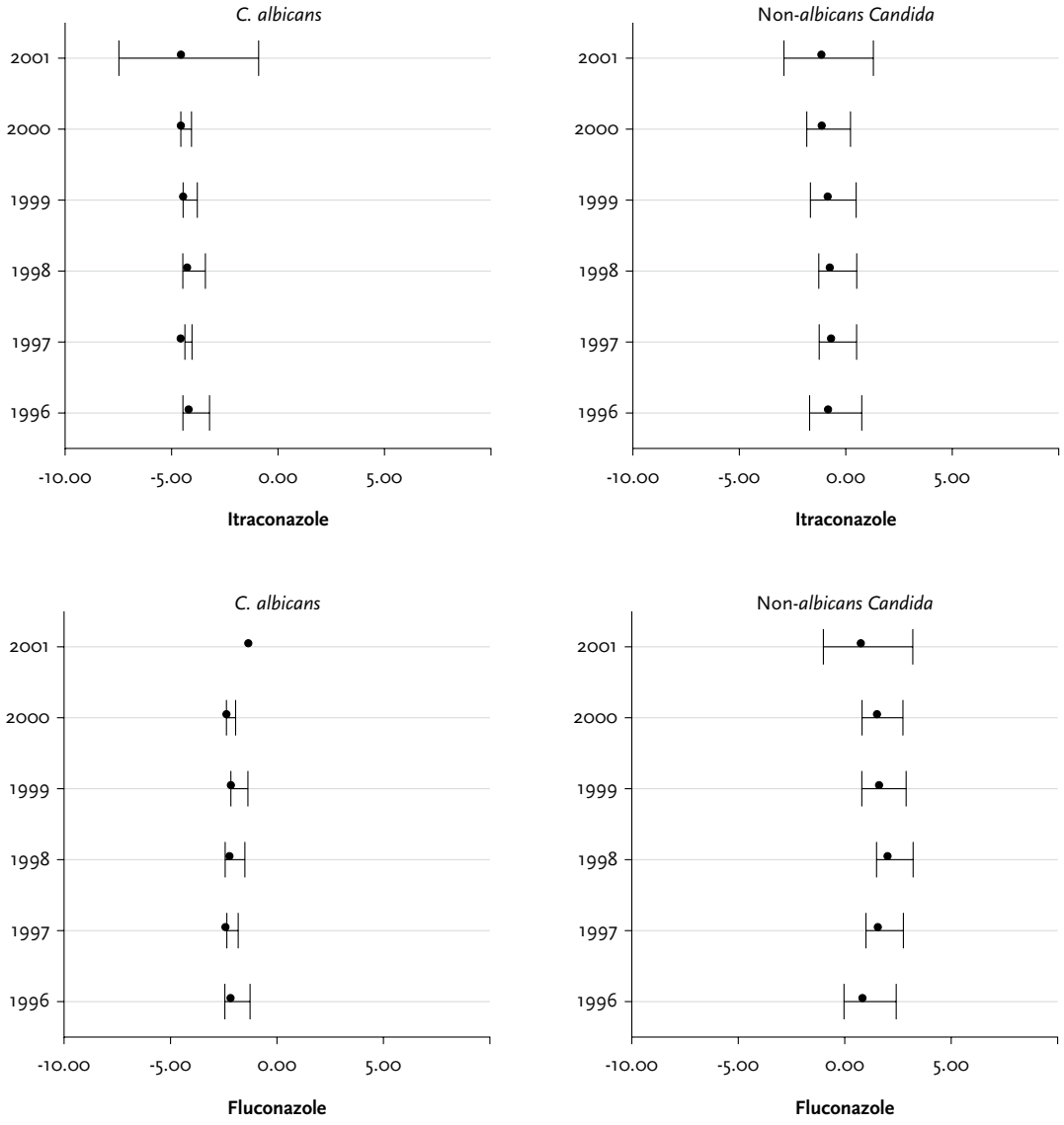


of fungaemia /10,000 patient days in 2001 was not significantly different to the rates in other years. However, additional observational studies are required for the years after 2001. The observed trends in the epidemiology of fungaemia are similar to those found in two Swiss studies, where no shift from *C. albicans* fungaemia to those caused by non-*albicans Candida* species was observed.[26,27] The incidence of fungaemia in Dutch hospitals is lower than the rates reported from hospitals outside Europe, even when the solitary peak of 1.21/10,000 patient days in 2001 is taken into account. In 2004, Hajjeh *et al*. reported incidence rates of 1.5/10,000 hospital days.[28] In a Canadian candidaemia study by Karlowsky *et al*., over the period 1976 to 1996, *Candida* species contributed to approximately 8% of the total bloodstream isolates;[29] this was more than twice the percentage we found (3%). In Iceland, the incidence of fungaemia had increased to 0.55/1000 admissions,[30] but remained lower than the incidence in our study.

C. albicans remains the most frequently isolated yeast in fungaemia; however, other species are on the rise. Over the period 1999 to 2003 Irish investigators observed an average annual incidence of 0.70 episodes /10,000 patient days in a tertiary care hospital. Here, the proportion of *C. albicans* decreased from about 80% in 1996 to 1999 to 58% in 2000 to 2003 in favour of the proportion of *C. glabrata* and *C. parapsilosis*.[20]

It is possible that the stable incidence of fungaemia in the Dutch hospitals in our study is due to FLU use as prophylaxis and vigilant infection control practices.

Figures 3C and D. *Mean 2log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*



However, the increased use of FLU might lead to selection of species such as *C. glabrata* and *C. krusei*, which are less susceptible to FLU, as described in a study from the Invasive Fungal Infections Cooperative Group (now Infectious Diseases Group) of the European Organisation for the Research and Treatment of Cancer.[31] We did not observe such a shift. In addition, no decrease in susceptibility was observed for any of the tested antifungal agents. Similar results were observed in a worldwide study on the susceptibility of *Candida* species to FLU over a ten-year period.[32] These observations show that FLU maintains its value for the treatment of systemic fungal infections. The antifungal agents showed good activity against the isolates, including isolates that were less susceptible to FLU.

In conclusion, the incidence of fungaemias in the Netherlands appears to have remained stable between 1996 and 2001, so the increase that we observed in the previous period (1987 to 1995) has levelled off. However, *C. albicans* was still the most frequently isolated species, being recovered from approximately 55% of the patients with fungal bloodstream infections.

ACKNOWLEDGEMENTS

We thank H.A.L van der Lee for his expert technical assistance and the Mycology Research Centre for the financial support.

Figures 3E and F. *Mean ^{2}log MIC-values after 48 hours of incubation (error bars show 95.0% CI of mean)*

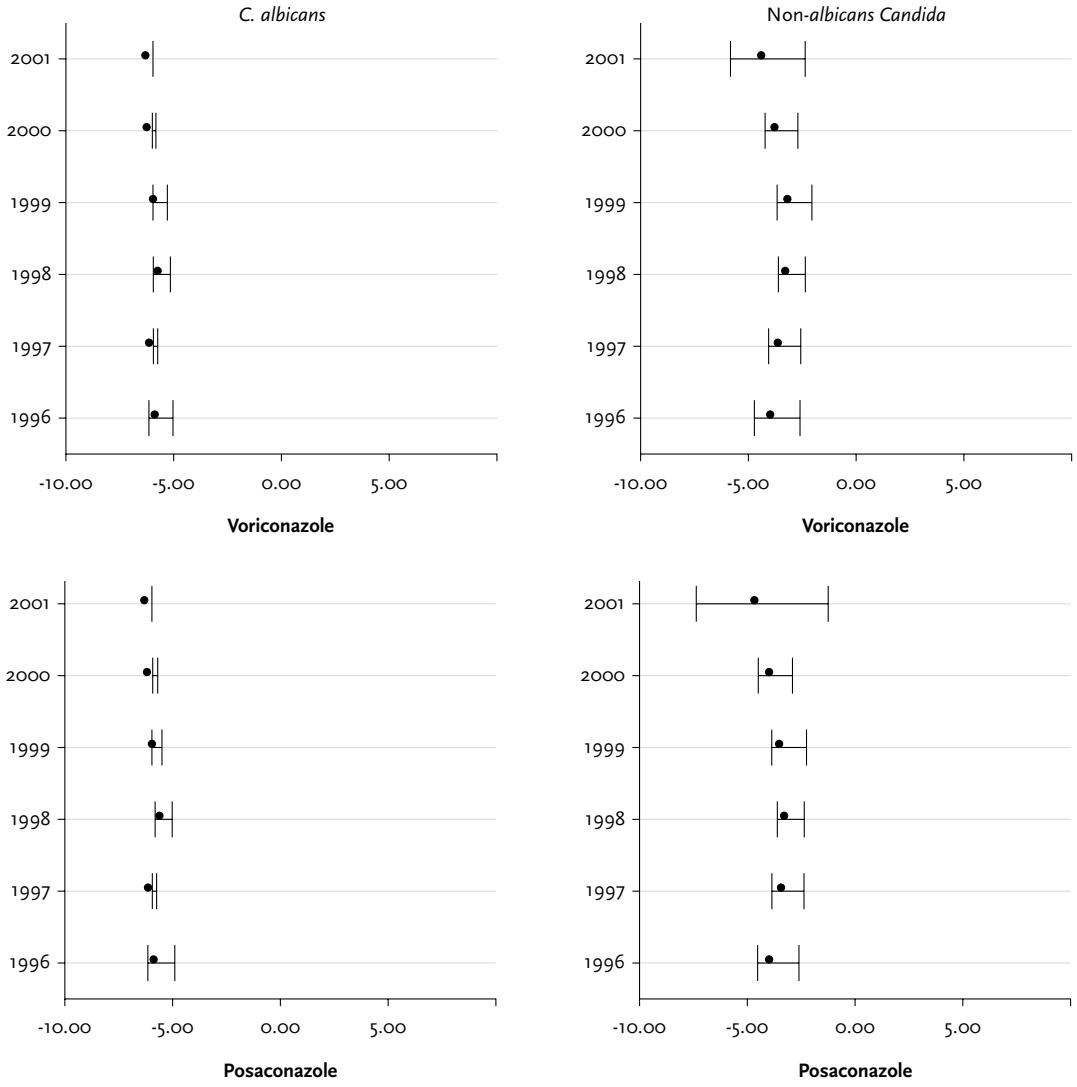
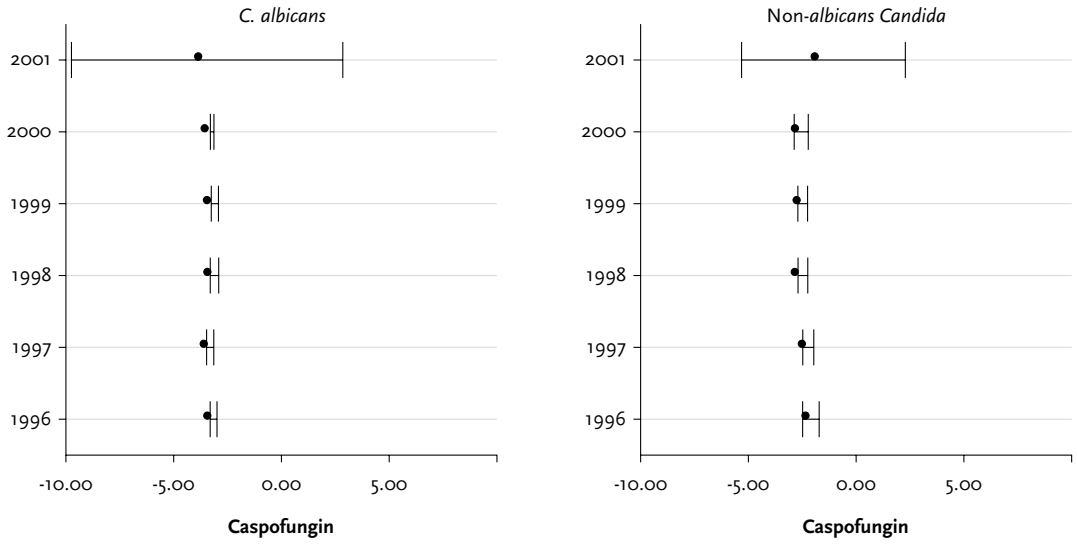


Figure 3G. *Mean ^{2}log MIC values after 48 hours of incubation (error bars show 95.0% CI of mean)*



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CASE REPORT

Paraneoplastic cerebellar degeneration preceding the diagnosis of Hodgkin's lymphoma

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ABSTRACT

Paraneoplastic cerebellar degeneration (PCD) can present as a severe and (sub)acute cerebellar syndrome. PCD can accompany different kinds of neoplasms including small cell lung cancer, adenocarcinoma of the breast and ovary, and Hodgkin's lymphoma. A 34-year-old patient is described with acute dysarthria, gait ataxia and diplopia. Despite extensive laboratory and radiological evaluations in this patient with rapidly deteriorating cerebellar syndrome, the diagnosis of a paraneoplastic syndrome was only made after several months, when an anti-Tr antibody was detected in his serum. The search for Hodgkin's disease as concomitant disorder was then started and resulted in stage IIB disease. The patient was successively treated with six courses of etoposide, bleomycin, vinblastine and dexamethasone and radiotherapy, which resulted in a complete remission of the Hodgkin's disease. After starting therapy the cerebellar degeneration stabilised.

The pathogenesis of neuronal damage in central nervous system paraneoplastic disorders such as the one we describe is not completely understood. Antitumour therapy is assumed to be the important cornerstone in stabilising the neurological condition. Improvement of the cerebellar syndrome in anti-Tr autoantibody paraneoplastic disease is a rare achievement. Early recognition of the concomitant disorders (anti-Tr autoantibody disease and Hodgkin's lymphoma) is of crucial importance.

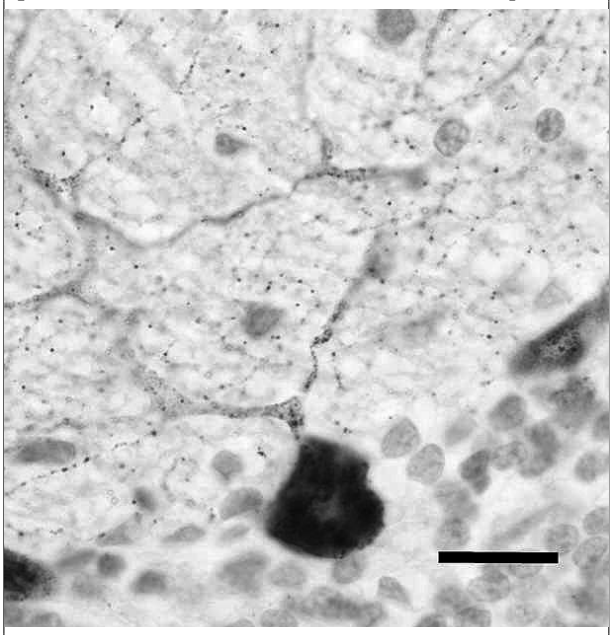
KEYWORDS

Anti-Tr, cerebellar degeneration, Hodgkin's lymphoma, paraneoplastic

INTRODUCTION

Paraneoplastic cerebellar degeneration (PCD) typically presents with (sub)acute, severe cerebellar ataxia.[1] PCD is most commonly associated with small cell lung cancer (SCLC), adenocarcinoma of the breast and ovary, followed by Hodgkin's lymphoma.[2] Sometimes the diagnosis of a malignant disease is made before the syndrome occurs. Usually, however, PCD precedes the underlying neoplastic disease, posing a diagnostic challenge. The detection of antineuronal autoantibodies directed against onconeural antigens helps diagnose the neurological syndrome as paraneoplastic and directs the search for an underlying tumour.[3-5] The autoantibodies associated with PCD include anti-Hu (SCLC), anti-Yo (breast and ovarian cancer) and anti-Tr (Hodgkin's disease). In 1976, Trotter *et al.* described an autoantibody in the serum of a patient with Hodgkin's lymphoma directed against cerebellar Purkinje cells and held the antibody responsible for the paraneoplastic symptoms.[6] Other reports followed, but it was not until 1997 that Graus *et al.* found an anti-Purkinje cell antibody in five Hodgkin's patients with PCD showing a characteristic immunoreactivity in the molecular layer of the cerebellum (*figure 1*). The antibody was named anti-Tr after the first two letters of Dr Trotter's name.[7-9] Recently, Bernal *et al.* analysed a series of 28 patients with PCD and anti-Tr antibodies. Of the 28 patients with anti-Tr immunoreactivity, 25 patients suffered from Hodgkin's disease while three had no demonstrable tumour.[1] Here we describe a patient presenting with an acute cerebellar syndrome leading to the diagnosis of Hodgkin's lymphoma several months later.

Figure 1. *Paraffin sections of rat cerebellum incubated with biotynilated immunoglobulin G from an anti-Tr positive serum, counterstained with haematoxylin*



The typical anti-Tr pattern with punctate reactivity of the molecular layer and staining of the cytoplasm and proximal dendrites of Purkinje cells is shown.

CASE REPORT

A 34-year-old male was admitted to the neurology ward because of acute dysarthria, gait ataxia and diplopia. He complained of headache accompanied by nausea and vertigo. There was no fever or any other systemic symptom at the time of presentation. Several months before admission, his family doctor had started him on paroxetine (30 mg daily) for depression. He smoked 20 cigarettes daily and had four to six alcoholic drinks during the weekend. Physical examination showed a slightly apathetic man without signs of meningeal irritation. He had normal blood pressure (135/85 mmHg), pulse (90 beats/min) and temperature of (37.5°C). His speech was dysarthric and there was a third-grade nystagmus to the left. No cranial nerve dysfunction or visual disturbances were noticed. Sensory examination and strength were normal. The finger-nose and heel-shin test were severely ataxic and the patient was incapable of walking without help, due to an unsteady and wide-based ataxic gate.

Laboratory tests showed no signs of infection, and the erythrocyte sedimentation rate was 13 mm (in the first hour). Renal function, liver enzymes, glucose, alcohol level, serological tests (Lues, HIV, Lyme's disease, herpes viruses, ANF), vitamin E level, angiotensin-converting enzyme and tumour marker tests did not reveal the cause of the symptoms. Cerebral CT scan (*figure 2A*) and MR scan (before and after administration of gadolineum contrast medium) were normal. Cerebrospinal fluid examination revealed pleiocytosis with lymphocyte counts of 462/3, glucose 3.47 mmol/l and total protein of 0.73 g/l. Furthermore a monoclonal IgG was present which was not found in the serum (IgG index 0.74). Microbiological examination of the cerebrospinal fluid (CSF) remained negative. Immunophenotyping of blood and CSF showed no monoclonal cell population. In the blood 16% of all mononuclear cells were B-cells and of all T cells, 43% showed CD4 expression, 26% CD8 expression. There was no increase in NK-cells. In the CSF 24% of all cells were B-lymphocytes and T-cell distribution CD4/CD8 cells equalled 3:1. CT scanning of the body did not show any malignancies, including lymphoma. A descriptive diagnosis of 'lymphocytic meningitis' was made but the cause of the severe cerebellar syndrome remained unclear. During the admission, the nystagmus disappeared spontaneously and the patient was discharged to a rehabilitation clinic. Some weeks later, the results of serum paraneoplastic antibody test, showed the presence of anti-Tr antibodies (titre 1:800), almost pathognomonic for Hodgkin's lymphoma. Physical examination showed a pathological lymph node in the right axilla. However, in the operating room, one week later, the lymph node had disappeared. Ultrasound examination also ruled out any axillary lymph node enlargement. FDG-PET scanning, again ten days later, showed increased FGD uptake in the right axilla and the neck (*figure 3*). Meanwhile, the patient was treated with plasmapheresis in an attempt to alleviate the cerebellar syndrome by decreasing the autoantibody titre. During the plasmapheresis his clinical condition stabilised. An axillary lymph node became palpable again and biopsy demonstrated a Hodgkin's lymphoma, nodular sclerosing type. Ann Arbor staging revealed stage IIB, weight loss occurred within several weeks as well as itching. At the start of his first chemotherapy cycle, i.e. etoposide, bleomycin, vinblastine and prednisone (EBVP), the antibody titre had risen to 1:3200. After two cycles of chemotherapy the titre had decreased to 1:800. The lymph nodes also decreased rapidly and were not palpable after two cycles. After the sixth cycle of EBVP the anti-Tr antibody was no longer detectable (*figure 4*). CT scan of the body showed complete remission after chemotherapy and involved field radiotherapy. The cerebellar syndrome had stabilised, but the patient still was incapable of walking without help and spoke in a dysarthric manner. No neurotoxic effects of vinblastine were observed. This was carefully examined, as it is a reported side effect of this cytostatic drug. CT scan of the brain six months after the first symptoms showed marked degeneration of gyri and widening of sulci of the cerebellum, indicating that supra- and infratentorial (cerebellar) tissue loss had occurred (*figure 2B*).

Figure 2. *CT scan of the cerebrum of the patient described at diagnosis (A) and showing widening of the supratentorial ventricular system and the sulci of the cerebellum as a sign of tissue loss six months later (B; arrows)*

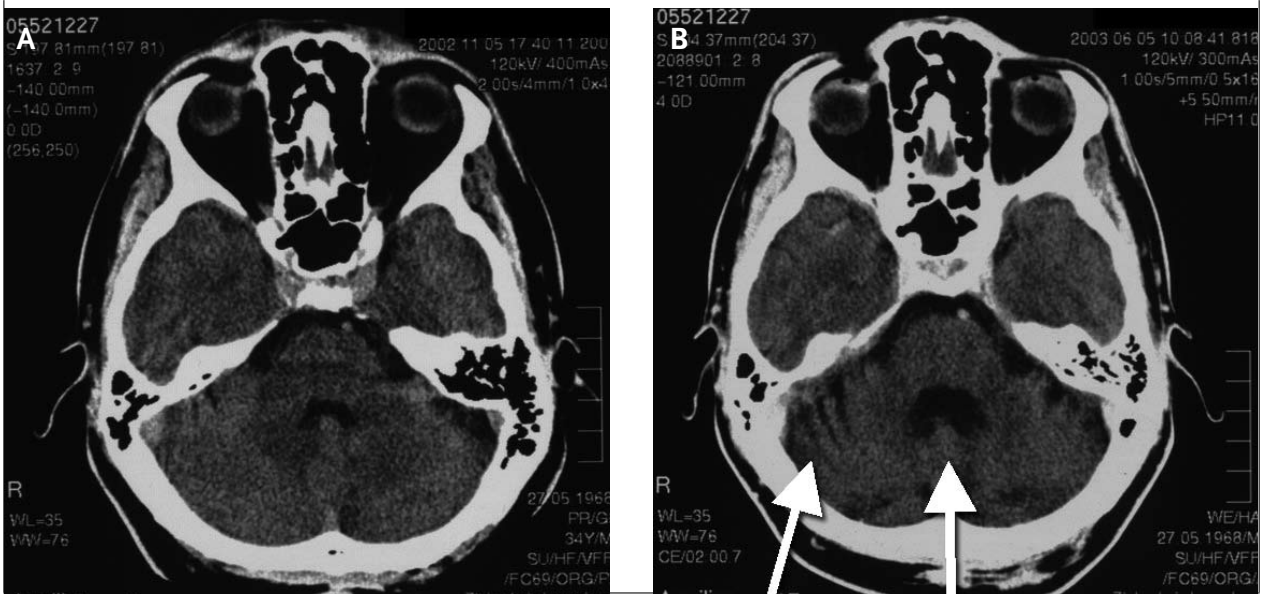
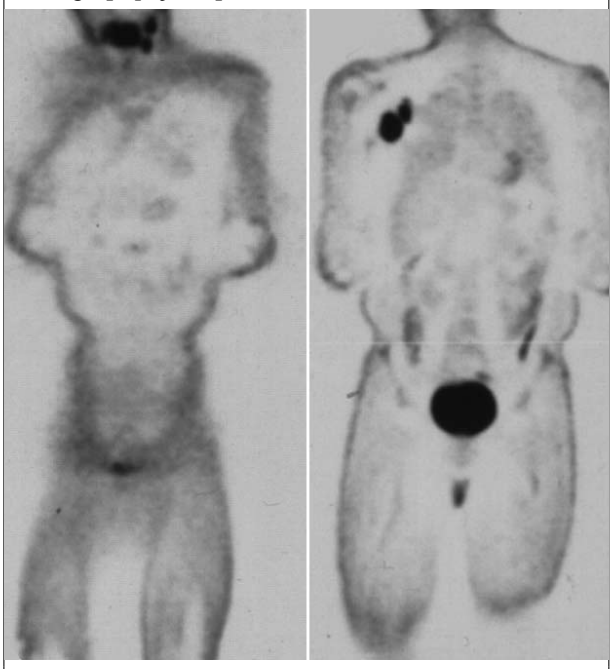
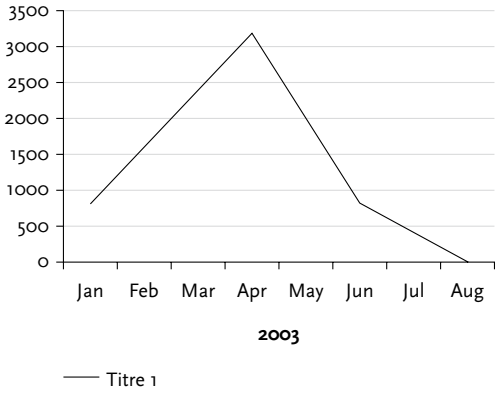


Figure 3. *^{18}Fluorodeoxyglucose positron emission tomography of the patient described*



FDG uptake is striking in the neck and right axilla as a sign of localisation of the malignant disease. Uptake of FDG in the urinary bladder is considered physiological.

Figure 4. *Concentration of anti-Tr antibody measured in serum of the patient described*



After the sixth cycle of chemotherapy the anti-Tr antibody titre had disappeared.

DISCUSSION

With small cell lung, breast and ovarian cancer, Hodgkin's lymphoma belongs to the malignancies most often associated with PCD.[2,3] The possible association between Hodgkin's disease and cerebellar degeneration was noted some years ago.[4,5] Although the pathogenesis of PCD is still not understood, the presence of high titre antibodies directed against antigens in cerebellar Purkinje cells, the intrathecal synthesis of these antibodies and the presence of inflammatory infiltrates in the cerebellum strongly point to an autoimmune process. Anti-Tr antibody was named after John L. Trotter, who described a 21-year-old female with stage I Hodgkin's disease and cerebellar ataxia.[6,7] Serum of the patient showed strong immunofluorescent staining of cerebellar Purkinje cells on sections of normal human cerebellar tissue.[6] Anti-Tr is identified by its immunohistochemical staining pattern in fixed frozen cerebellar sections. The antigen reacting with the antibody has not yet been identified. Anti-Tr is clearly associated with both PCD and Hodgkin's disease.[1,3,7-10] The antibody

can be detected in serum as well as in cerebrospinal fluid thus for screening purposes the testing of serum suffices. The CSF of our patient was not tested for paraneoplastic antibodies.

The largest series of patients with anti-Tr associated PCD was recently published.[1] In the majority of these 28 patients the diagnosis of PCD was made prior to the Hodgkin's lymphoma diagnosis, as was the case in our patient. Others, however, describe a reverse sequence of events in the majority of patients with Hodgkin's lymphoma associated PCD.[8] The majority of patients with PCD and Hodgkin's lymphoma are of the male sex, in both series up to 80%.[1,8] Although Hodgkin's disease is about twice as common in men than in women the reason for this discrepancy is not known.

The prognosis of PCD associated with Hodgkin's lymphoma seems to be very poor. Of 28 patients described by Bernal *et al*. 86% suffered irreversible damage to the cerebellum.[1] The patients who recovered from their symptoms were all relatively young, under 40 years (as is the case in our patient). No relation to type or stage of the Hodgkin's disease is known. There is also no evidence that a decrease of the antibody titre by means of treating the underlying Hodgkin's disease predicts better outcome. If treatment of the underlying neoplasm is successful, the antibody disappears in most cases.[1] In general, early recognition and intensive treatment of the underlying malignancy is advocated in most paraneoplastic neurological conditions. As in our patient, the neurological disorder develops rapidly and neurons are quickly disturbed. By the time the diagnosis was made and treatment undertaken, the neurons had probably been destroyed and no sign of regeneration was obvious. As the CT scan after six months showed, the cerebellar tissue had partially disappeared despite lowering of the antibody titre.

Treatment options other than treating the underlying malignancy to lower antibody titres in paraneoplastic neurological disease have been described mainly in anti-Hu and anti-Yo antibody related syndromes. Anti-Yo antibodies are found in women with ovarian or breast cancer. Anti-Hu antibodies coexist with SCLC. Therapeutic interventions consist of intravenous administration of immunoglobulins, plasmapheresis (also applied to our patient) and immunosuppressive medication (prednisone, cyclophosphamide).[11-17] One of four patients with anti-neuronal antibodies described by Blaes *et al*. suffered from cerebellar degeneration as a result of anti-Yo antibodies and showed improvement of clinical condition after plasmapheresis followed by high-dose immunoglobulines.[11] Graus *et al*. described several series of patients with anti-Hu and anti-Yo (paraneoplastic) antibodies and neurological syndromes.[12,13] The mean duration of the neurological syndrome was 3.3 months at the onset of plasmapheresis. Antibody titres in serum of all patients decreased to 10 to 20% of the initial levels with plasmapheresis alone or combined with prednisone or cyclophosphamide. No patients improved, some remained stable for at least six months if they also received treatment of the malignancy. In our patient the pre- and post-plasmapheresis antibody titres were not measured. Clinically he showed no improvement with two weeks of plasmapheresis, three times a week.

As noted before, the pathogenesis of neuronal damage in central nervous system paraneoplastic disorders is related to an autoimmune process. The hypothesis is that antigens normally expressed in the central nervous system are no longer restricted to this area, but are aberrantly (mutated or not) expressed in malignant tissue. This idea seems to hold true in case of, for example, PCD and anti-Hu or anti-Yo antibodies. The Hu- and Yo-antigens were identified as proteins normally expressed in neuronal cells and respectively SCLC and gynaecological- or breast tumour cells.[16] Tr-immunoreactivity however was discovered only in one out of 15 analysed samples of Hodgkin's lymphoma tissue of patients with PCD, anti-Tr antibodies and Hodgkin's lymphoma.[1] The biopsy specimen from the pathological lymphnode of our patient was not appropriately conserved to perform this test of Tr-immunoreactivity.

Bernal *et al*. also hypothesise that the character and origin of the immune process in PCD with Hodgkin's lymphoma may be different from paraneoplastic neurological syndromes in solid malignancies.[1] A polyclonal B-cell activation as described in patients with Hodgkin's lymphoma could be responsible for an autoimmune process in the cerebellum without a particular 'Tr antigen' being expressed by the malignant cells. It is also postulated that paraneoplastic disorders of the central nervous system can be the result of T-cell mediated cytotoxic response, antineuronal antibodies cause additional pathogenic effects.[17]

Whether lymphoma (progression) or the central nervous system condition will be the major prognostic factor in our patient is left for the future to decide.

CONCLUSION

PCD presents with (sub)acute, severe cerebellar ataxia and should lead to prompt extensive diagnostic work-up. Positron emission tomography can, as also shown in our patient, be helpful localising an underlying tumour when antineuronal antibodies are identified.[18] Treatment of the underlying disease, in this case Hodgkin's lymphoma, could then be initiated as soon as possible, before irreversible cerebellar damage has occurred. Improvement of paraneoplastic central nervous system disorders is a rare achievement., therefore the central nervous system condition can be a considerable prognostic factor in PCD.

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CASE REPORT

Sino-nasal bony and cartilaginous destruction associated with cocaine abuse, *S. aureus* and antineutrophil cytoplasmic antibodies

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ABSTRACT

Three male patients aged 29, 30 and 34 years and a 36-year-old female are reported with nasal septum perforation and a history of cocaine abuse. Two of the patients also had a perforation of the hard palate. In all four, antineutrophil cytoplasmic antibodies (ANCA) were found. One had a cytoplasmic immunofluorescence-staining pattern (c-ANCA), the other three showed a perinuclear staining pattern (p-ANCA). Furthermore, all patients were found to be nasal carriers of *S. aureus*. We hypothesise that tissue damage to the nasal and palatal area in patients using cocaine may partly be mediated by the presence of ANCA antibodies. Furthermore, we speculate that *S. aureus* facilitates the development of these ANCA antibodies.

KEYWORDS

ANCA, cocaine, *S. aureus*, sino-nasal destruction

INTRODUCTION

Cocaine, derived from the leaves of the coca plant (*Erythroxylon coca*), has central stimulatory as well as anaesthetic effects through the release of dopamine, norepinephrine and/or serotonin. It is bedrock knowledge that cocaine can cause tissue damage to the nasal area, lungs, cardiac conduction system, and the brain. Septum perforation of the nose alone or together with a hard palate defect are recognised as local complications of nasal cocaine abuse.[1-12]

Antineutrophil cytoplasmic antibodies (ANCA) were first described in 1982 by Davies *et al.*, who found these antibodies in patients with glomerulonephritis.[13] Later, ANCA was found to be associated with Wegener's granulomatosis. In addition to the classical cytoplasmic immunofluorescence-staining pattern (c-ANCA), a perinuclear pattern (p-ANCA) was recognised. In the majority of cases, the c-ANCA is directed against proteinase 3 (PR3), and the p-ANCA is directed against myeloperoxidase (MPO). In addition to Wegener's granulomatosis, the ANCAs are important in other systemic vasculitis syndromes as well, including Churg-Strauss syndrome, microscopic polyangiitis, idiopathic pauci-immune necrotising crescentic glomerulonephritis and in some cases Goodpasture's disease.[14,15] The diagnosis of Wegener's granulomatosis is based clinically on the presence of nose bleeds, nephritis (crescentic glomerulonephritis with necrosis) and pulmonary involvement. More than 70% of patients with Wegener's granulomatosis have PR3-ANCA, whereas in 10 to 30% of the cases MPO-ANCA is present. Although c-ANCA (PR3-ANCA) is predominately associated with Wegener's granulomatosis, and p-ANCA (MPO-ANCA) with microscopic polyangiitis, idiopathic pauci-immune necrotising crescentic glomerulonephritis and Churg-Strauss syndrome, there is not an absolute specificity.

Positive ANCA titres have been described in drug-induced vasculitides as well, especially in association with propylthiouracil treatment for hypothyroidism.[16]

We report four patients suffering from sino-nasal destruction caused by cocaine abuse. Two of the patients also had destructive perforation of the hard palate. ANCAs were found in all four patients. Furthermore, all patients were *S. aureus* carriers. We speculate that production of ANCA, combined with the presence of *S. aureus*, contributes to this local toxic effect of cocaine.

CASE REPORT

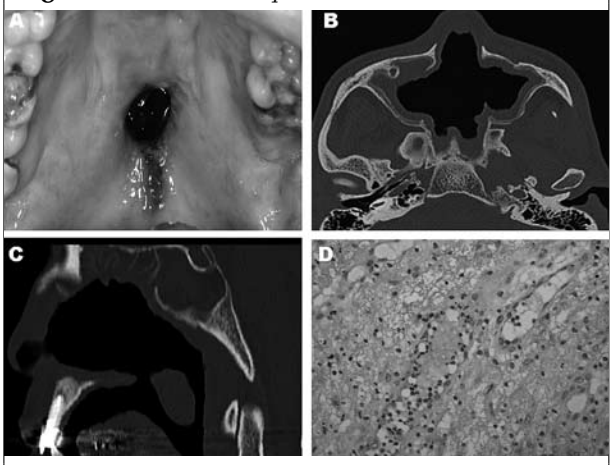
A 34-year-old male was seen at the ENT outpatient clinic because of persistent nose blockage for one year. His medical history was unremarkable. He was a semi-professional boxer and admitted to having used cocaine frequently. Examination showed a saddle nose deformity, crusts in the nasal cavity, and a subtotal septum perforation.

The initial diagnosis was septum perforation caused by cocaine abuse and repeated facial trauma. Local therapy (Sofradex® drops and nasal lavage) was started and he was strongly advised to stop using cocaine. Follow-up examination several months later revealed a perforation of the hard palate (*figure 1A*). Computer tomography showed destruction of the entire nasal septum and medial borders of the maxillary sinus and ethmoids (*figures 1B* and *C*). Microscopy of the nasal mucosa showed a chronic active, partly necrotising inflammation without a clearly granulomatous aspect (*figure 1D*). Microbiological culture of the nose revealed *S. aureus*. Because Wegener's granulomatosis was considered an alternative diagnosis, serological investigation was performed (*table 1*).

The serological findings led to the diagnosis of septum and hard palate perforation due to the combination of trauma, cocaine abuse and limited Wegener's granulomatosis (normal kidney function, normal urinalysis, and normal computer tomography of the lungs). Treatment initially consisted of sulphametoxazole and trimetroprim, followed by the addition of oral corticosteroids. The patient's symptoms improved dramatically. In six months' time, the c-ANCA titre had decreased significantly to 1:40. In addition, anti-PR3 antibodies were no longer detected. Local treatment was continued, and an obturator was placed in the palatal defect. Despite clinical and biochemical improvement, the patient still periodically requires local treatment, possibly due to persisting abuse of cocaine, confirmed by detecting cocaine in urine samples.

For the clinical, serological, microbiological and therapy data of the other three patients, see *table 1* and *figure 2*.

Figure 1. *The oral cavity*

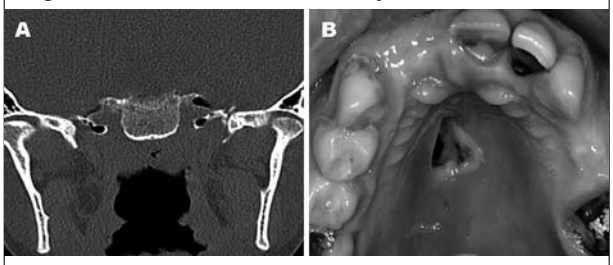


A. Defect of the palate. B. Sagital CT scan of the nasal cavity, demonstrating the absence of the nasal septum. C. Coronal CT scan of the nasal cavity, showing the absence of the nasal septum as well as the palate defect. D. A representative section of a nasal mucosa biopsy showing an aspecific inflammation, without evident signs of vasculitis.

DISCUSSION

In this paper we present four patients with local destructive changes in the nasal and palatal area, associated with cocaine abuse and the presence of ANCAs. This combination of findings caused difficulties in the diagnostic

Figure 2. *A transversal CT scan of the head*



A. Absence of the nasal septum on CT scan. B. Oral cavity with hard palate defect.

Table 1. *The patient characteristics*

Patient	Sex/age	Clinical manifestation	ANCA type (titre)	ANCA specificity	*S. aureus*	Treatment
A	M/34	Septum perforation Hard palate perforation	c-type (1 :320)	Anti-PR3	+	SMX/TMP Steroids
B	F/36	Septum perforation Saddle-nose deformity	p-type (1 :80)	Anti-PR3	+	SMX/TMP Steroids Mtx
C	M/29	Septum perforation Necrotising crescentic glomerulonephritis	p-type (1 :1280)	Anti-PR3	+	Steroids Cy
D	M/30	Septum perforation Hard palate fistula	p-type (1:320)	none	+	SMX/TMP

SMX/TMP = Sulphametoxazole and trimetroprim; Mtx = methotrexate; Cy = cyclophosphamide

process, because cocaine-induced toxicity may mimic the lesions resulting from (limited) Wegener's granulomatosis, especially in those cases when patients deny the use of cocaine.[1,8] With the recent report that ANCAs reacting with human neutrophil elastase (HNE) may be used as a diagnostic marker for cocaine-induced destructive lesions in the nasal and palatal area, this difficulty may be circumvented.[17] It may be easy to distinguish patients with cocaine-induced midline destructive lesions (CIMDL) from those with Wegener's granulomatosis. In their paper, Wiesner *et al*. characterised the reactivity of HNE-ANCA in 25 patients with CIMDL and compared this with a control group of 604 consecutive patients, including 64 patients with Wegener's granulomatosis, 14 patients with microscopic polyangiitis and 526 patients with other vasculitis together with 45 healthy volunteers.[17] Using three different assays (indirect immunofluorescence, capture ELISA and direct ELISA), HNE-ANCAs were detectable in 84% of the patients with CIMDL. No HNE-ANCAs were detected in patients with Wegener's granulomatosis or microscopic polyangiitis. Among the three assays, the capture ELISA was the most sensitive method, detecting HNE-ANCAs in 76% of the patients with CIMDL. In 13 of the 25 patients with CIMDL, PR3-ANCAs could be detected. Unfortunately, this paper appeared after we had seen and treated our patients. Therefore, we were not able to test our patients for HNE-ANCA positivity. Nevertheless, our study provides the literature with four patients with sino-nasal bony and cartilaginous destruction during/after use of cocaine and the association with *S. aureus* and ANCAs.

Positive ANCA has previously been reported in cases of nasal septum perforation attributed to cocaine abuse, but this usually involved lower titres than in the patients described in this study.[8,10,11] In only one of the four patients in our study was a c-ANCA pattern found with a higher titre than in the previously reported cases (*table 1*).

Thirteen cases have been reported before describing extensive palatal destruction attributed to cocaine abuse.[2-12] The c-ANCA serology was only reported in five of these cases.[2,8,10-12] This usually involved lower titres of c-ANCA, except in two cases.[2,12] This major lesion was found in two of our patients, one with c-ANCA (patient A) and one with p-ANCA pattern (patient D). Although in patient A specificity for the c-ANCA could be detected, in patient D the p-ANCA lacked any specificity. One possibility is that at the time of determination of the specificity in patient D the disease activity might have been low. Another possibility could be that other aetiological agents are more important in the destruction of the oronasal mucosa (*S. aureus*).

The exact mechanism behind the presence of p- or c-ANCA (in cocaine abusers) is not known. Several studies have demonstrated the pathogenetic role of ANCA. However, environmental factors, such as infection (particularly *S. aureus*) or drugs, are required for the production and/or activation of ANCAs.[15,18,19] It has been shown that (chronic) nasal carriage of *S. aureus* is associated with higher relapse rates in Wegener's granulomatosis.[20] It is remarkable that all our patients were carriers of *S. aureus* (*table 1*). A possible pathogenetic mechanism for the lesions in the oronasal region may be that due to the repetitive local vasoconstrictive effects of cocaine on the nasal mucosa when sniffing, ischaemia may occur. Subsequently, the damage to the mucosa causes the infiltration of *S. aureus*. This in turn is followed by the formation of ANCAs. Together with the direct toxicity of cocaine, the infection/infiltration/inflammation with *S. aureus* and the destructive effects of ANCAs, microscopic as well as macroscopic lesions occur. Thus ANCAs can develop upon exposure to 1) environmental factors, such as medication (hydralazine, propylthiouracil), toxic effects of silicon or cocaine; 2) infection, including *S. aureus* and 3) a certain susceptibility due to genetic factors, such as polymorphisms, the genes encoding Fcγ receptors, α1-antitrypsin, PR3 and MPO. These environmental and inherited factors together may break through the immunological barriers of self-tolerance, thus causing autoimmunity. We believe that ANCA should be measured in cocaine users presenting with nasal septum/palate perforation, particularly when signs of local inflammatory activity predominate. It has not yet been determined what the optimal therapy is for the cocaine-induced destructive lesions in the nasal and palatal area, but a short course of immunosuppressive therapy should be considered in patients with extensive disease or in patients who do not improve on cessation of cocaine use.

In conclusion, the association between cocaine (ab)use, nasal septum perforation, and positive ANCA serology is described. Cocaine use and the presence/production of ANCA may not be coincidental. We hypothesise that cocaine may trigger p- or c-ANCA production via *S. aureus* as an intermediate, which may partially explain the ischaemic necrosis seen in cocaine-associated lesions. Moreover, the presence of genetic factors (Fcγ receptor polymorphisms) may explain the susceptibility for the toxic effects of micro-organisms and drugs, leading to the formation of ANCAs.

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CASE REPORT

Mirizzi's syndrome

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ABSTRACT

A case is described emphasising rare complication of gallstone disease: the Mirizzi syndrome in which an impacted gallstone in the Hartmann's pouch or cystic duct causes common hepatic duct obstruction and by eroding a fistula. Diagnosis is made by endoscopic retrograde cholangio-pancreatography and treatment includes cholecystectomy.

KEYWORDS

Endoscopic retrograde cholangio-pancreatography, gallstones, Mirizzi's syndrome

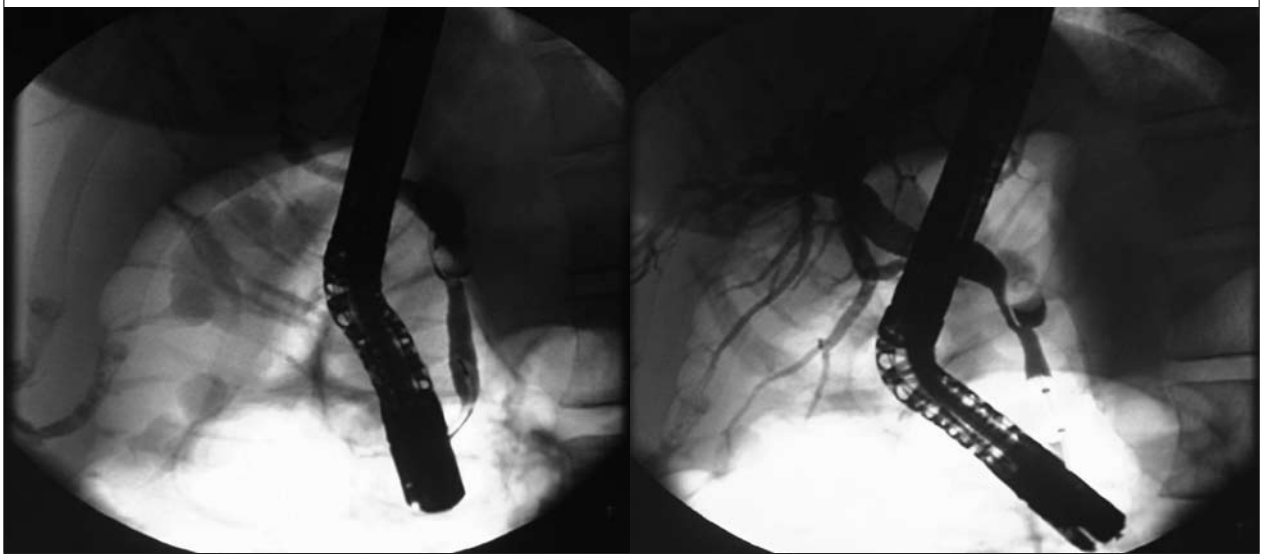
INTRODUCTION

In Western Europe, the prevalence of gallstones is high. The standard treatment of symptomatic gallstones is laparoscopic cholecystectomy and bile duct stones are treated endoscopically. Unfortunately, it is sometimes difficult to extract bile duct stones, for example in patients with Mirizzi's syndrome.[1,2] In this case report, we present a patient with this syndrome.

CASE REPORT

A 43-year-old woman visited our outpatient clinic in March 2004 with a six-month history of colic pain, nausea and vomiting. She had a past history of hyperthyroidism. Physical examination revealed a tender right upper abdomen and jaundice. The laboratory blood tests showed increased levels of bilirubin 67.9 μmol/l (normal 3-22 μmol/l), alkaline phosphatase 145 U/l (<140 U/l), γ-glutamyl-transferase 161 U/l (<40 U/l), alanine transaminase 1240 U/l (<40 U/l), aspartate transaminase 688 U/l (<35 U/l) and lactate dehydrogenase 888 U/l (<480 U/l), while the serum amylase was normal. Ultrasonography showed slightly dilated intrahepatic ducts, an extended gallbladder with multiple stones and a stone in the choledochal duct. Moreover, on endoscopic retrograde cholangio-pancreatography (ERCP) we found a stone located in the cystic duct distal to the orifice of the choledochal duct and with compression of the before common hepatic duct *figure 1*).

Figure 1. *Retrograde cholangiography*



DISCUSSION

Mirizzi's syndrome is named after Doctor Pablo Mirizzi, who described a peculiar bile duct obstruction due to gallstones in 1948.[1] However, this condition had been reported as early as in 1905. This syndrome refers to common hepatic duct obstruction caused by an extrinsic compression of an impacted stone in the Hartmann's pouch or cystic duct (type I). Predispositions are a low-lying cystic-choledochal duct juncture, contiguity of a large cystic duct with the common hepatic duct or a short cystic duct. Also inflammation contributes to the bile duct obstruction. If the obstruction persists the stone may erode into the common bile duct producing a cholecystocholedochal fistula (type II).[2,3]

Patients generally present with clinical and biochemical signs of biliary obstruction, sometimes in the setting of an acute cholecystitis, acute cholangitis or pancreatitis. There is usually a longstanding history of biliary symptoms. Approximately 50 to 77% of all patients reported are women. ERCP is the method of choice for diagnosing these patients. The radiological appearance of this condition may be misinterpreted as a tumour of the gallbladder or cystic duct, a cholangiocarcinoma, metastatic disease of the hilum or acute cholecystitis. These diseases should be excluded by a CT or an ultrasound.[4,5]

ERCP is not the therapy of choice because stone extraction fails due to inability to access or capture the impacted stone. The standard treatment of Mirizzi's syndrome is a cholecystectomy and stone retrieval followed by temporary T-tube insertion.[6,7] Normally, this should be performed by a laparoscopy, but the presence of acquired biliary abnormalities sometimes makes it difficult to interprete the anatomy, especially with extensive adhesions, and gives more risk for bile duct injury. If there is any suspicion of abnormalities, an open cholecystectomy should be performed, if necessary in combination with a peroperative cholangiography. In patients who are not fit for surgery, endoscopic treatment, endoscopic papillotomy, and/or stent placement with or without electrohydraulic lithotripsy should be preferred.

CONCLUSION

In our patient, the diagnosis was made on ERCP. In accordance with the guidelines, our patient underwent surgery, which confirmed the diagnosis: a stone eroding from the cystic duct into the choledochal duct (type II). Subsequently, a cholecystectomy was performed with extraction of the stone and a temporary T-drain was left *in situ*. The patient recovered completely without any complications within a few days.

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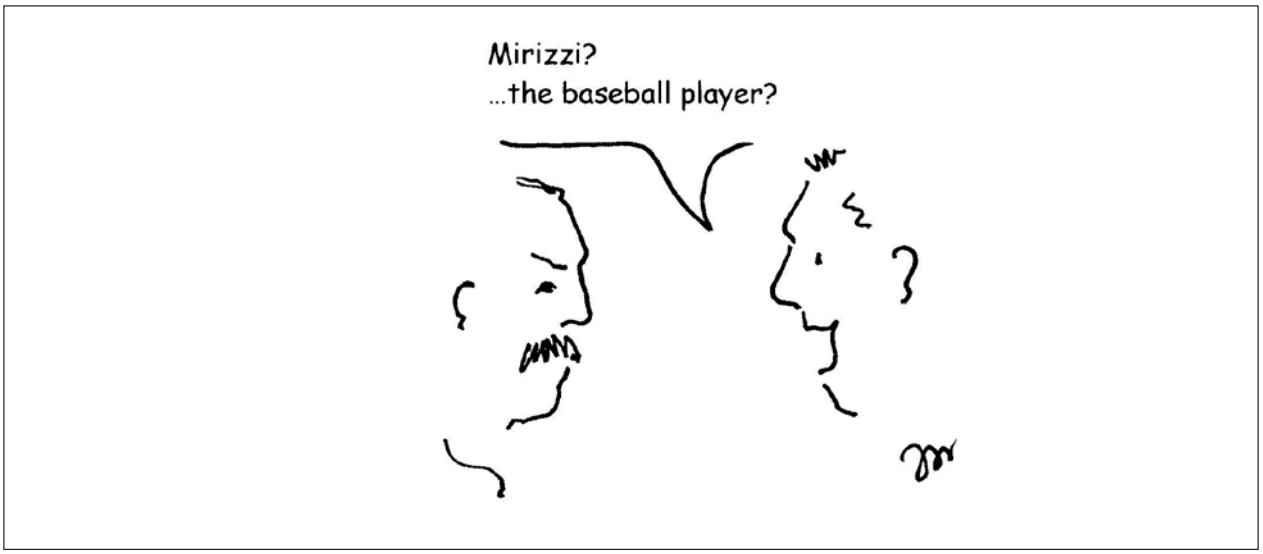

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Fatal *Clostridium septicum* infection in a young pregnant woman

CASE REPORT

A 27-year-old pregnant woman, with no previous medical history, presented to the hospital with a spontaneous abortion at nine weeks' gestation. Curettage was performed. Although there were no clinical abnormalities on physical examination, she complained of intense pain in both legs for one day. Swelling, a purple skin discolouration on the right thigh and signs of crepitus developed during the next night (figure 1). Because of respiratory distress and hypotension, the patient was admitted to the ICU and required mechanical ventilation, volume resuscitation and vasopressor support.

She was febrile (temperature 38.4°C), tachycardic (heart rate 132 beats/min), tachypnoeic (respiratory rate 40 breaths/min), hypotensive (blood pressure 82/50 mmHg), oliguric with dark-brown urine and she had cold extremities. Laboratory examination showed an ESR 13 mm/hour (0-20 mm/hour), Hb 9.5 mmol/l (7.5-10.0 mmol/l), Ht 0.44 l/l (0.36-0.46 l/l), MVC 88 (80-100), leucocytes 10.3/nl (4-11/nl) with left shift, platelets 226/nl (150-400/nl), D-dimer 25.61 µg/ml, ASAT 538 U/l (0-45 U/l), ALAT 140 U/l (0-45 U/l), CK 23953 U/l (0-170 U/l), LD 1349 U/l (0-450 U/l), CRP 343 mg/l (0-5 mg/l), and lactate 5.8 mmol/l (0.5-1.5 mmol/l). Arterial blood gas showed a pH 7.28 (7.35-7.45), $PaCO_2$ 3.1 kPA (4.5-6.0 kPA), PaO_2 40.6 kPA (9.5-13.0 kPA), HCO_3- 10.2 mmol/l (22-26 mmol/l), BE -14.6 mmol/l (-2.0-2.0 mmol/l), and SaO_2 86% (92-99%) with 10 l/min O_2. Body CT scan showed gas in and extensive destruction of the muscles of the legs, pelvis, abdomen and back and gas bubbles in the venous system (figure 2).

Piperacillin/tazobactam and clindamycin were started and emergency exploration, debridement and bilateral upper leg amputation were performed (figure 3). Radical resection was not possible due to the extent of the myonecrosis. Perioperatively hypotension and metabolic acidosis worsened as a sign of refractory toxic shock syndrome. Hyperbaric oxygen therapy was considered, but the patient was too unstable for transportation to a hyperbaric facility. Gram stains of muscle (figure 4) and blood (figure 5) showed Gram-positive rods. Cultures revealed Clostridium septicum, *confirming the diagnosis gas gangrene.*

Patient developed a progressive toxic shock and refractory hypotension and died approximately 30 hours after admission to the ICU.

DISCUSSION

Myonecrosis, or necrotising myositis, can be caused by Clostridium *species and group A Streptococcus.[1] Three types of clostridial soft tissue infection have been described: simple wound contamination, anaerobic cellulitis and myonecrosis.[2] Myonecrosis can occur in three different settings: traumatic, recurrent and non-traumatic or spontaneous. The third is most commonly caused by* C. septicum, *which produces four toxins (alpha, beta, gamma and delta toxin) and is aerotolerant. Typical is the acute invasion of healthy muscle, producing an anaerobic environment and acid pH which is optimal for growth of clostridial organisms. The mean incubation time is less than 24 hours. The first symptom is usually acute onset of severe pain.[3] The skin over the infected area becomes tense, tender and*

Figure 1. *Progressive swelling of the legs and a purple skin discolouration on the right thigh*

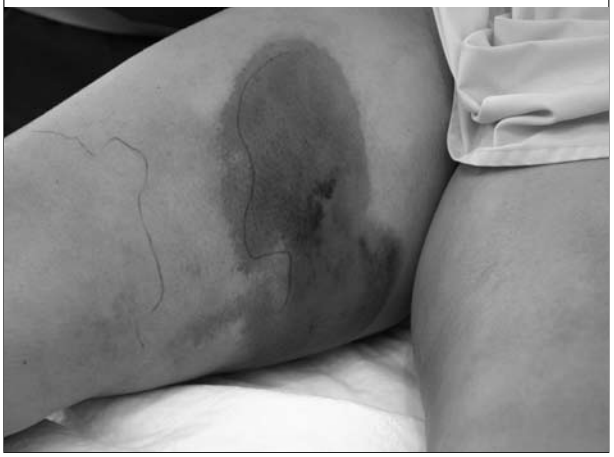


Figure 2. *CT scan with gas within the soft tissue and in the venous system and destruction of the muscles of the legs, pelvis, abdomen and back*

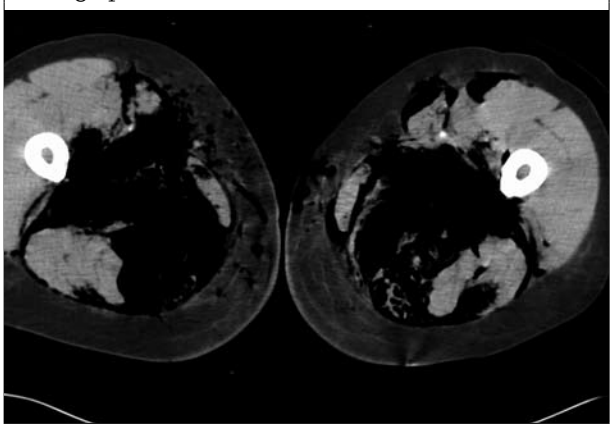


Figure 3. *Peri-operative illustration of right upper leg musculature: oedematous with a blue to black discolouration*

Figure 4. *Gram stain of muscle showing Gram-positive rods*

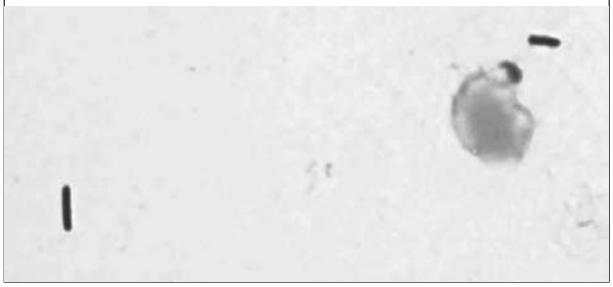
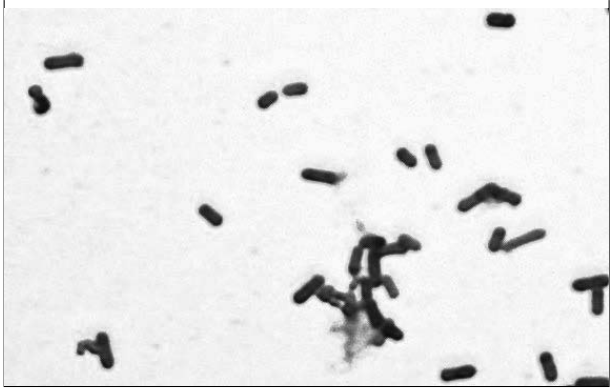


Figure 5. *Gram stain of the blood showing Gram-positive rods*



may appear purplish-red. Signs of systemic toxicity develop rapidly.[4] *Gas in the soft tissue supports the diagnosis. Definitive diagnosis is made by demonstrating large, Gram-positive rods. Debridement is mandatory and a combination of penicillin and clindamycin is warranted. The use of hyperbaric oxygen is controversial and the aerotolerance of* C. septicum *may reduce its efficacy.*[5]
Gas gangrene is a rapidly progressive and often lethal disease. Early recognition and aggressive surgical and antibiotic treatment are essential. The mortality of traumatic gas gangrene is greater than 25%, but mortality from spontaneous gas gangrene ranges from 67 to 100%, with the majority of deaths occurring within 24 hours of onset. Toxic shock syndrome with fatal consequences due to Clostridium *infection has been described after abortion.*[6]

ACKNOWLEDGEMENT

The authors would like to express their thanks to A. van Die, MD, for reviewing the CT-scan images.

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LETTER TO THE EDITOR

Gas gangrene spreading to the bone marrow

INTRODUCTION

Gas gangrene is a term reserved for fulminant soft tissue infections caused by Clostridium *species. The post-traumatic form of gas gangrene is caused by* Clostridium perfringens.[1] *Nontraumatic, or spontaneous, gas gangrene is even more rare and is usually caused by* Clostridium septicum*; underlying malignancy is often present.*[1] *Gas gangrene progresses rapidly and is often lethal.*

CASE REPORT

A 76-year-old man was sent to our emergency department with loss of sensibility and paresis of his left arm, which started two hours before arrival. In the ambulance, ten minutes before arrival, small bullae appeared and his skin turned a coppery colour. Upon arrival in the emergency department, he had clinical signs of class III haemorrhage. On physical examination a pupillary asymmetry, decreased corneal reflex, and loss of sensibility and paresis of the left arm were found. Several haemorrhagic bullae and severe subcutaneous emphysema of the whole left arm and left chest wall were seen (figure 1). On conventional radiographs of the thorax and shoulder, massive subcutaneous emphysema was diagnosed. Laboratory studies revealed a raised white cell count (10.4 x 10^9/l) and C-reactive protein (180 mg/l), with a haemoglobin of 6.7 mmol/l and a creatine kinase of 11,500 U/l. Computed tomography scan revealed extensive myonecrosis and subcutaneous emphysema in the left chest wall, shoulder, and arm, as well as further gas embolisms, even in the bone marrow (figures 2 and 3).

Figure 1. *Diffuse swelling of the left upper arm, with a purplish skin and several haemorrhagic bullae*

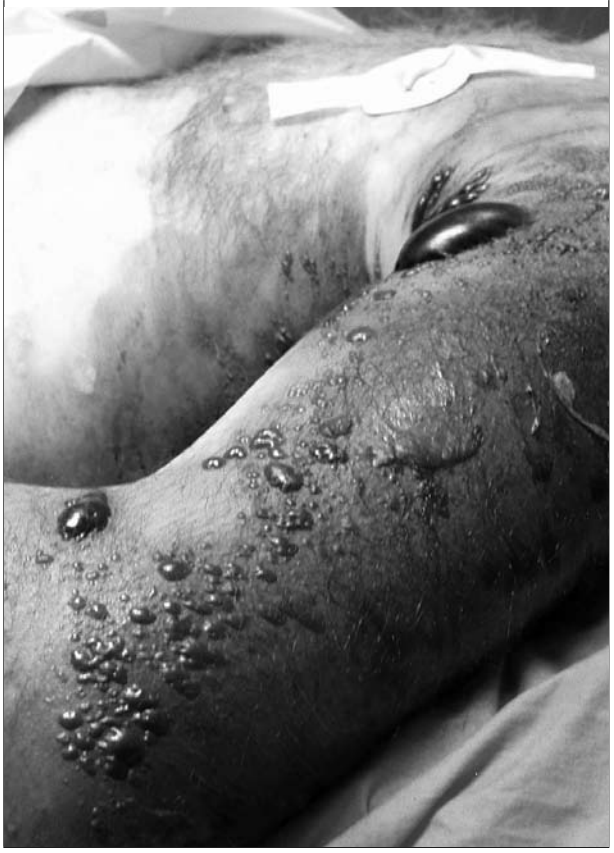


Figure 2. *A computed tomography scan shows massive myonecrosis and subcutaneous emphysema of the left chest wall, with gas in the arcus aortae*

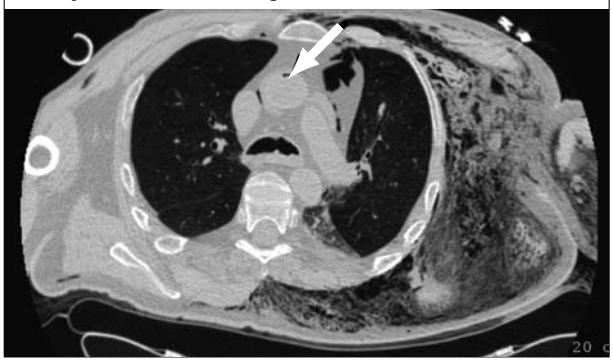
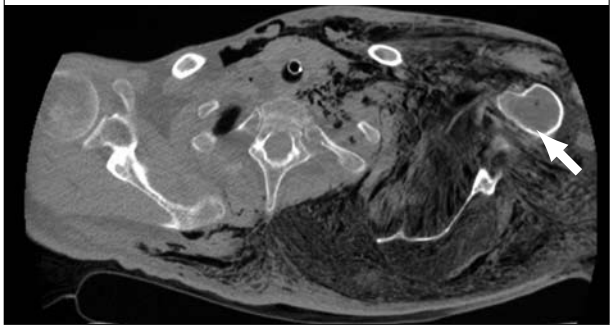


Figure 3. *A computed tomography scan shows gas diffusion into the bone marrow of the left humeral head*



The patient became asystolic 45 minutes after arrival. During cardiopulmonary resuscitation skin changes and subcutaneous emphysema were rapidly progressive. The patient died 1.5 hours after presentation. On the post-mortem examination, a tumour of the caecum was found with covered perforation. Mediastinal emphysema, pneumatosis coli, and extensive gas embolisms were found in the systemic circulation and in multiple organs. The blood cultures later grew C. septicum.

DISCUSSION

Clostridia are anaerobic, gram-positive, toxin- and spore-forming bacteria, normally found among gut flora and in the soil. The most common responsible pathogen for spontaneous gas gangrene is C. septicum, *which is aerotolerant.*[1,2]
Described predisposing factors for spontaneous gas gangrene are colon carcinoma, diverticulitis, and haematological diseases that cause immunosuppression.[2-4] *The nontraumatic form of gangrene has three patterns of spread of spontaneous infection, from local visceral cellulitis to metastatic infection.*[5] *The last form, which affected this patient, is extremely rare and often fatal.*[6,7]
The onset of nontraumatic gas gangrene is abrupt, with rapid progression. Excruciating pain is the most prominent first symptom.[1,3]
The treatment requires prompt antibiotic therapy with penicillin and aminoglycoside or piperacillin-tazobactam, and radical surgical débridement.[4,8] *The benefits of hyperbaric oxygen therapy remain unproven.*[2,4] *The mortality rate is about 70%.*[3]
To our knowledge, this is the first documented case of gas in the bone marrow.

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PHOTO QUIZ

A patient with sudden pain in the upper abdomen accompanied by vomiting

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CASE REPORT

An 82-year-old woman presented to the emergency department with pain in the upper abdomen. The pain had developed suddenly the day before and was accompanied by nausea and vomiting. The days beforehand, her oral food intake had been less because of decreased appetite. Although the patient had no recent complaints of heartburn or regurgitation, she had had documented stomach complaints accompanied by nausea and vomiting for a longer period. Furthermore, she had a history of chronic obstructive pulmonary disease, microcytic anaemia of unknown origin, osteoporosis, diverticulosis of the sigmoid and knee surgery. She was not on any medication at that moment. On physical examination she was moderately ill, drowsy and pale. Blood pressure was 208/100 mmHg, pulse rate 100 beats/min. and body temperature 36.4°C. Her abdomen was markedly distended and on prolonged auscultation we could not detect bowel sounds. There was tenderness in the upper abdomen. On rectal examination brown faeces, without blood or melaena, were collected. Blood tests revealed a C-reactive protein level of 13 mg/l, 11.9 x 10^9 leucocytes, 156 x 10^9 thrombocytes, haemoglobin level of 9.4 mmol/l, sodium 139 mmol/l, potassium 3.2 mmol/l, chloride 107 mmol/l, urea 8.1 mmol/l, creatinine 78 μmol/l, alkaline phosphate 115 U/l, ASAT 28 U/l, ALAT 13 U/l, γGT 24 U/l, LDH 411 U/l, glucose 13 mmol/l, bicarbonate 20.5 mmol/l and lactate 1.2 mmol/l.

A supine chest X-ray (*figure 1*) was taken. An attempt to put a transoesophageal drain into the stomach was not successful because the distal oesophagus could not be passed. On the second day after admission to the hospital the patient suddenly developed dyspnoea, a temperature of 38.2°C, low blood pressure and a drop in oxygen saturation. She frequently vomited small amounts of dark-brown fluid. A second attempt was made to insert a drain into the stomach, which fortunately was successful and about four litres of dark fluid stomach contents could be aspirated. After this episode, contrast was given through the drain and intravenously and a computed tomography (CT) scan of the thorax was carried out (*figure 2*).

WHAT IS YOUR DIAGNOSIS?

See page 260 for the answer to this photo quiz.

Figure 1. *Chest X-ray in bed*

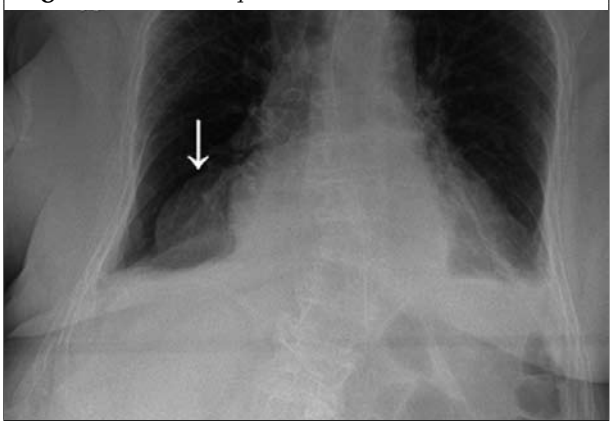

Figure 2. *A transverse contrast-enhanced CT scan of the chest*

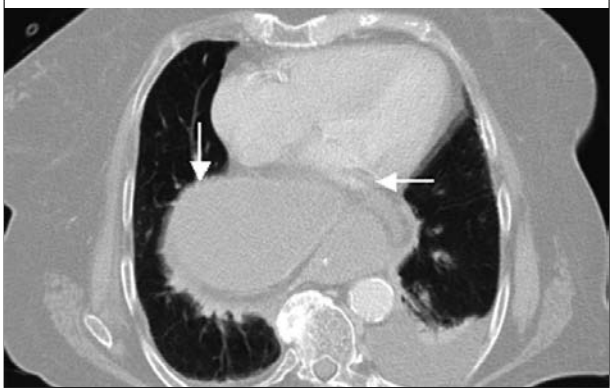

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PHOTO QUIZ

A woman with haemolytic anaemia

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CASE REPORT

A 73-year-old woman was admitted to the outpatient clinic of the Department of Internal Medicine for analysis of an anaemia.

For a few months she had been experiencing progressive fatigue and shortness of breath. During this period she also noticed peripheral oedema and had lost her appetite because of upper abdominal distension. Her medical history revealed atrial fibrillation and recurrent epistaxis. The patient's medication consisted of atenolol, digoxin, acenocoumarol and bumetanide. On physical examination her blood pressure was 140/55 mmHg, heart rate 50 beats/min, and the jugular venous pressure was elevated. There was a systolic murmur which was maximal at the third left intercostal space and a thrill was heard at the right upper abdominal quadrant with murmurs across the abdomen. Further examination of the abdomen showed hepatomegaly and she had pitting oedema up to the ankles.

Laboratory tests showed a haemoglobin concentration of 6.1 mmol/l, MCV 81, leucocytes 4.6 x 10^9/l, thrombocytes 118 x 10^9/l, LDH 627 U/l, haptoglobin 0.1 g/l, red blood cell fragmentation in the blood smear, Coombs direct and indirect negative, ferritin 25 µg/l, no signs of diffuse intravascular coagulation, creatinine 66 µmol/l and bilirubin 47 µmol/l.

So the diagnosis of mechanical haemolytic anaemia was made. On chest X-ray cardiomegaly with pronunciation of the hilar and pulmonary vessels was seen.

The echocardiography showed elevated right ventricular pressure and a severe tricuspid valve regurgitation. The vena cava inferior did not collapse with inspiration. The left ventricular function was intermediate. Liver veins were enlarged. Because of the unexpected finding of enlarged liver veins on echocardiography, computed tomography (CT) and subsequently an angiography of the abdomen were performed (*figures 1-3*).

WHAT IS YOUR DIAGNOSIS?

See page 261 for the answer to this photo quiz.

Figure 1. *Abdominal CT scan with intravenous contrast, enlarged liver veins filled with contrast*

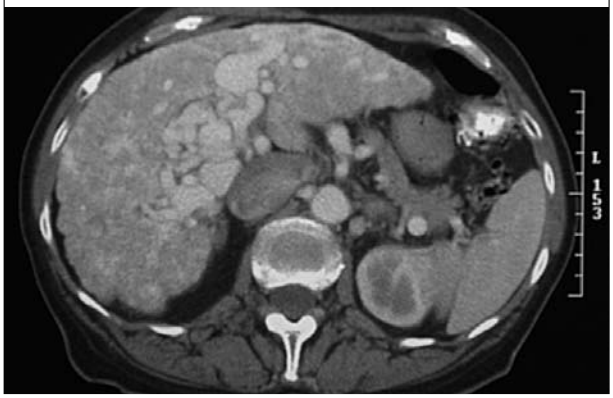


Figure 2. *Aneurysm of hepatic artery*

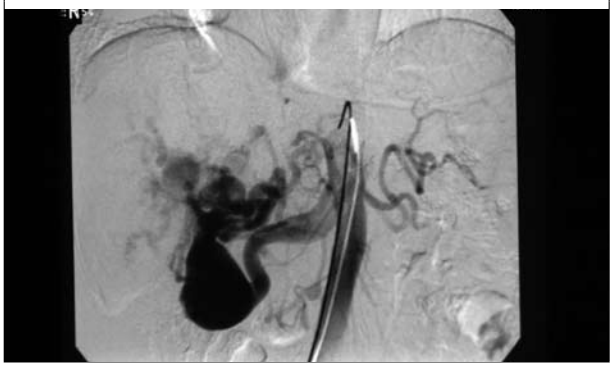
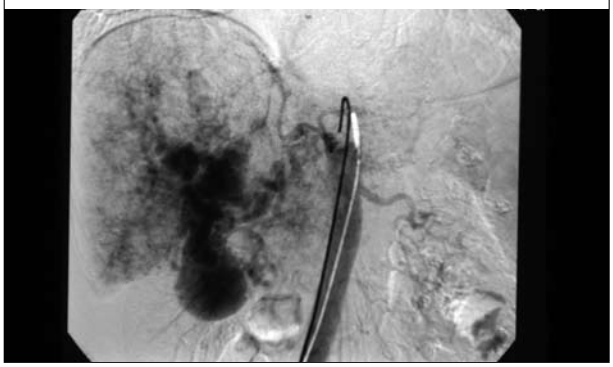


Figure 3. *Arteriovenous malformation*



ANSWER TO PHOTO QUIZ (ON PAGE 258)

A PATIENT WITH SUDDEN PAIN IN THE UPPER ABDOMEN ACCOMPANIED BY VOMITING

DIAGNOSIS

The chest X-ray shows a paramediastinal shadow at the right lower thorax. This corresponds with a very large paraoesophageal hernia on the right side of the diaphragm. Paraoesophageal herniation is an uncommon disorder accounting for approximately 3 to 10% of all hernias of the oesophageal hiatus.[1] Ninety-five percent of hernias are on the left side, because of the congenital weakness on that side.[2] Paraoesophageal hernias can be distinguished from the more common sliding hiatal hernia by the relative preservation of the gastro-oesophageal junction in the abdomen.[1,3] In our patient, a CT scan confirmed that part of the stomach was extending into the right side of the thorax (arrow, *figure 1*). The CT scan (*figure 2*) showed a distended fundus of the stomach that was still situated under the diaphragm; the distal part of the stomach and the proximal part of the duodenum, however, were localised on the right side of the thorax. Due to sudden extension of the herniated distal part of the stomach into the right side of the thorax, we hypothesised that the right atrium was significantly compressed, causing obstructed inflow of the heart. Due to diminished ventricular filling, pulmonary and systemic circulation were seriously compromised explaining the hypoxaemia and low blood pressure. A reconstruction of the CT scan shows the flip-flopped stomach from the frontal view (*figure 3*). Support for our hypothesis was that the patient recovered after removal of four litres of fluid from her stomach.

The patient underwent median upper laparatomy to bring the stomach back into the normal position. The diaphragm was repaired and gastropexy was performed. After a short two-day postoperative period on the intensive care unit, she recovered well and was soon discharged home.

Figure 1. *Chest X-ray in bed*

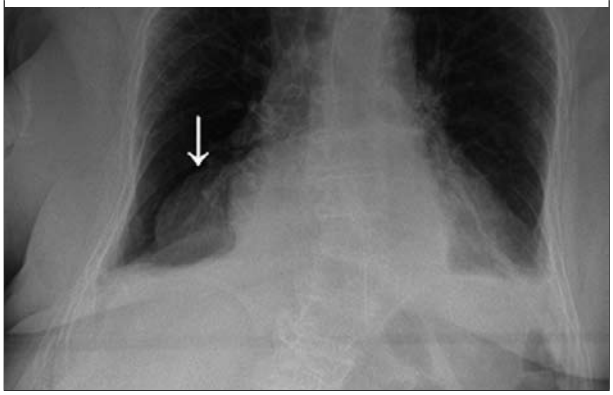


Figure 2. *A transverse contrast-enhanced CT scan of the chest*

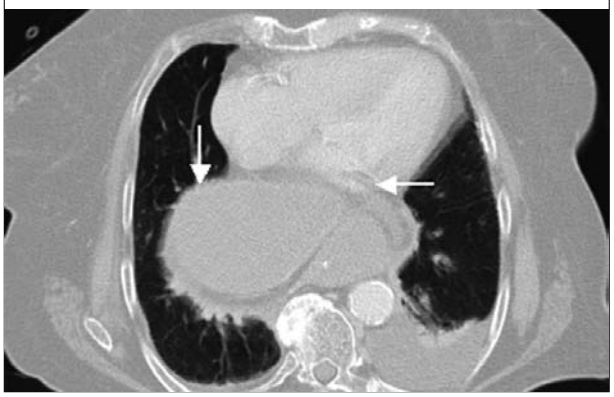
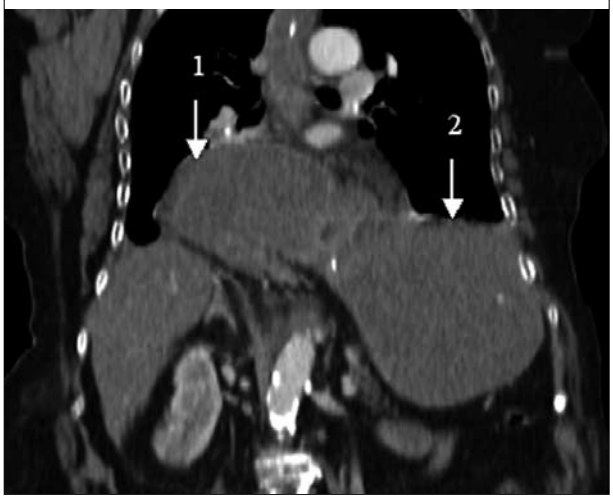


Figure 3. *Coronal reconstruction of the CT scan, showing the distal part of the stomach in the chest (1) and the proximal part of the stomach in the abdomen (2)*



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ANSWER TO PHOTO QUIZ (ON PAGE 259)

A WOMAN WITH HAEMOLYTIC ANAEMIA

DIAGNOSIS

The CT scan of the abdomen showed distended veins in the portal system (*figure 1*). An angiography of the abdominal arteries showed arteriovenous malformations of the hepatic artery and mesenteric artery. There was a large aneurysm at the shunting hepatic artery (*figure 2 and 3*). These vascular malformations are characteristic of Rendu-Osler-Weber disease.

Therefore, the diagnosis of Rendu-Osler-Weber disease with mesenteric arteriovenous fistula leading to severe high-output cardiac failure and secondary pulmonary hypertension was made.

The hypothesis for the cause of the haemolytic anaemia is turbulent blood flow. Turbulent flow is definitely present in arteriovenous malformation and possibly in tricuspid regurgitation. Recently, a case report described mechanical haemolytic anaemia in a haemodialysis patient with carotid-jugular arteriovenous fistula.[1]

Tricuspid regurgitation has never been described as a factor for haemolytic anaemia, only an artificial tricuspid valve can be a cause for haemolysis.

There are a few therapeutic options to improve arteriovenous malformations. The treatment of choice is embolisation of the malformation. If embolisation fails or can not be carried out, surgery can be performed.

Figure 1. *Abdominal CT scan with intravenous contrast, enlarged liver veins filled with contrast*

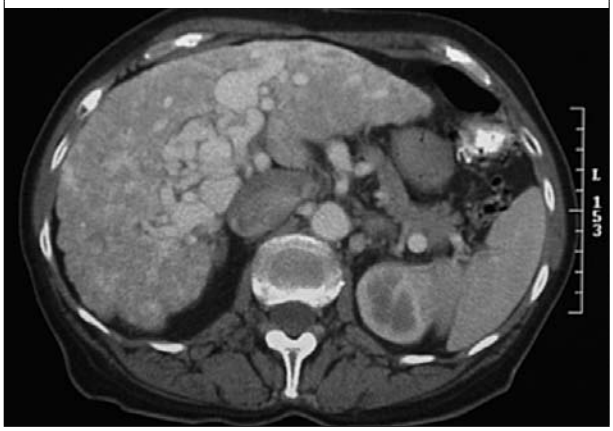


Figure 2. *Aneurysm of hepatic artery*

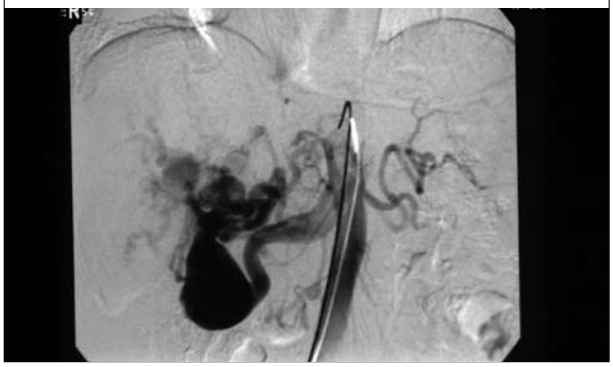
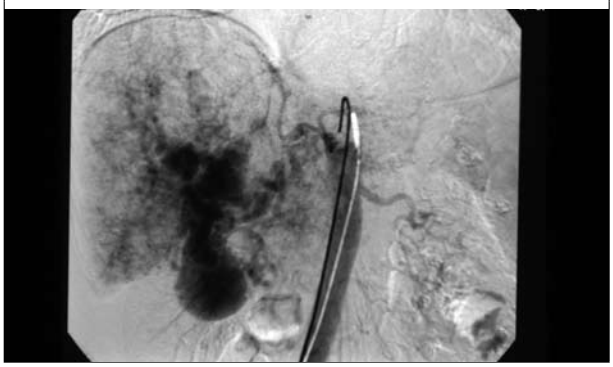


Figure 3. *Arteriovenous malformation*



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SPECIAL REPORT

The case for case reports in the Netherlands Journal of Medicine

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INTRODUCTION

Case reports are probably one of the most accessible forms of medical literature and when well written are a joy to read. They probably reflect clinical practice most accurately and give insight into the thoughts of the internist during his/her daily work collecting clinical information and making deductions in order to reach the diagnosis. The connection to clinical practice perhaps explains why case reports are so well liked by readers. In addition, they can be very useful for establishing the right diagnosis in patients with rare diseases. By nature, a case report is also one of the first papers that a resident, as a novice inexperienced author, will write.

Despite the popularity among readers, the status of the case report ranks low on the hierarchical ladder in this age of evidence-based medicine. This may help to explain why editors often shy away from publishing case reports.[1] On the other hand, many Journals do still publish case reports and a PubMed search (http://www.pubmed.gov) revealed that over 200,000 articles in the medical field have been published as case reports over the last five years.

We believe in the value of a good case report in that it is educative, enticing, and even entertaining. Furthermore, and this is reflected by our mission statement, we aim to provide the practising clinician with up-to-date medicine and to inform him/her on important issues in topical health care. The Netherlands Journal of Medicine usually publishes two to three case reports per issue. The competition for case reports is rather stiff. The Journal receives many case reports for consideration and although they probably all represent hard work we have to make choices. We would like the Netherlands Journal of Medicine to be your first port of call for publishing your case report, and we want to shed some light on what we expect from our prospective authors and how we, as Editors, deal with case report submissions.

WHAT TYPES OF CASE REPORTS ARE THERE?

We have not issued any specific guidelines or given advice to prospective authors in our 'Information for authors' section and we wish to change that. Generally speaking, when selecting articles the Editorial Board mainly focuses on four different items:

- Is the science correct?
- Is the material new and will it have any impact on clinical practice or add substantially to current knowledge?
- Is the message appropriate for the practising internist?
- Has the manuscript been prepared carefully or will major revisions be required to bring it up to par with the required standards?

For case reports we delve deeper as we want to publish case reports which discuss new aspects on clinical presentation, diagnosis or treatment. These elements describe our wishes in relatively general terms and each author always points to some aspect of novelty. So, we need to obtain more detail. Others have tried to classify case reports and have come up with a classification that divides case reports into various subcategories depending on the main focus of the article (*table 1*).[2] We have analysed the types of case reports that were published in the Journal in 2004 and 2005. *Table 1* lists the various categories along with the number of case reports published in the Journal in this period.[3,4] As can be seen, most case reports (40%) focused on an unusual aspect or presentation of a relatively common case. For example, while prostate cancer is a common disorder, it rarely presents with severe hypocalcaemia associated with extensive osteoblastic metastases.[5] Frequently, case reports describe unexpected rarely reported complications of therapeutic procedures. Some 26% of case reports cover this aspect, and Stridor and Horner's syndrome after attempted right subclavian vein cannulation is a good example.[6]

Table 1. *Types of case reports published in the Journal in 2004 and 2005*

Main focus of case report	Number per category	Example reference
Rare and previously sparsely reported condition	6	7
Unusual presentation/symptom of a (common) disease	17	8
Unexpected association between two relatively uncommon symptoms/signs	2	9
Impact of one disease process on another	1	10
Unexpected event in the course of observing or treating a patient	1	11
Novel insight into pathogenesis of disease	1	12
Unexpected rarely reported complication of treatment or procedure	11	13
New and unique treatment	3	14
Honest mistakes in management	0	
Totally original condition/new disease	0	
Impact of a treatment regime of one condition on another disease	0	

WHAT TYPES OF CASE REPORT ARE WE INTERESTED IN?

While it is exciting to be the first to diagnose a patient with an aortic regurgitation murmur in endocarditis and you feel an imminent urge to run it to press, first ask yourself a set of questions.[15] What is novel, original, or unique about this report? A case report is a work of science and it should contain a novel point either in the form of a new problem, a new solution or a new idea. Ideally, you should be able to make this point in a single line. Try to define why you think this case or observation is important at all. Next, you should check whether the point you want to make is in fact unique. PubMed is an excellent resource to check this out. Taking the above-mentioned example will give you numerous articles, so you might want to reconsider. Next, why is this case important and clinically relevant to the audience of the Netherlands Journal of Medicine, internists? In other words: what does it teach us? For instance, the Journal is not looking for articles on paediatric subjects, such as a case of neonatal jaundice.
The facts from the report should speak for themselves. As stated by Vandenbroucke: 'The writer should lay bare his/her thought process, as crisply and pointedly as possible, because that it the only way to impress and strike a chord with the reader'.[4]

Our mailbox is well stocked with papers describing a rare manifestation of relatively common conditions. Although these case reports are welcome, we prefer case reports describing novel treatment options. Similarly, we have our share of papers describing a complication of treatment or a procedure, but we could use those that deal with biologically plausible but unexpected associations between two relatively uncommon symptoms or signs.
On the other hand, if you have performed a thorough literature review on a case, why not consider submitting this as a mini review to the Journal? You avoid the crowded case reports box and improve your chances of getting into the Journal.

THE ANATOMY OF A CASE REPORT

While we do not aim to give an introductory writing course, we do want to provide some guidelines on how to write a case report. A case report can be broken down in several components: Title, Introduction, Case report, Discussion and References.[16]

Title

The title should be informative, and it is important that it contains the key elements of the case. With the title as input line you should be able to obtain optimal retrieval with electronic searching. As a test of principle, use your title as a search item on PubMed and check what you get. You should be able to get one or more references that have been included in your reference list. Lastly, it should be interesting enough to attract the reader's attention. Remember, most readers will only see your title and decide on the basis of that whether they should go on reading.

Introduction

The introduction should contain no more than 200 words. The introduction contains reference to the clear and compelling rationale for the 'uniqueness criteria' that justify publication of the work. It should describe why the case is unique. If not, does the case contain unusual elements with respect to diagnosis, prognosis or therapy? Case reports educate, and we want to see whether the author is able to establish instructive or teaching points that add value to this case. Lastly, we would like to see a line on how the case expands scientific knowledge.

Case report

The case report (300 words maximum) should give a meticulous description of the history, examination and investigations pertinent to the point the report wants to make. Do not elaborate on irrelevant details that distract from the message of the paper. Is the cause of the patient's illness clear-cut? Are there any other plausible explanations? Describe the treatment in enough detail. Have all available therapeutic options been considered? If not explain why. You should be able to describe whether the outcome is related to the treatment. If the patient would

have improved regardless of treatment, how important was that therapy? Lastly, the description of a single patient is fine, but the description of more patients that support your point is better. Case series are more convincing than a single case report.

Discussion

The discussion is the hardest part for many authors and, in our view, should be limited to 500 words. Many authors end up writing a prosaic free-floating story rather than describing the merits of the case in a concise manner. Structure improves the quality and readability of the discussion. The discussion starts by highlighting the most pertinent findings from the case. The key question here is 'Does our case provide sufficient detail and documentation to support the conclusion?' Next, explain the rationale for reporting the case. What is unusual about the case? Does it challenge prevailing knowledge, or provide an opening to novel insights into a disease pathogenesis? If you find an unexpected association first explain what you expected, and then try to explain your finding in precise terms. Is the association contrary to common thinking? If so, explain how and why the well-accepted 'truth' is challenged? The discussion on each case report should contain a thorough, if not exhaustive (your case is unique so there will only be few other cases) literature review of other similar cases. Then go on to describe how your case is different, or whether you have recognised a common pattern that can be tested in future cases. You have seen the patient and performed the literature review so you are the person to give recommendations on how things can be done differently in a similar case in the future. Finally, the conclusion should be in line with the report. Case reports do not establish cause-and-effect relationships between interventions and outcomes, but might open the door to new (testable) hypothesis.

CONCLUSION

Case reports describe practice. As such they appeal to the readers and the Editorial board would like them to remain this way. However, we feel that handing out a set of guidelines could improve the standard of case reports in the Journal. The Journal is improving and it is important that the case reports show similar improvements.[17] We hope that prospective writers for the Journal will benefit from these guidelines and welcome any comments.

ACKNOWLEDGEMENT

The author wishes to thank the faculty of the Young Investigator Meeting course hosted by the Association of National European and Mediterranean Societies of Gastroenterology for insightful discussions.

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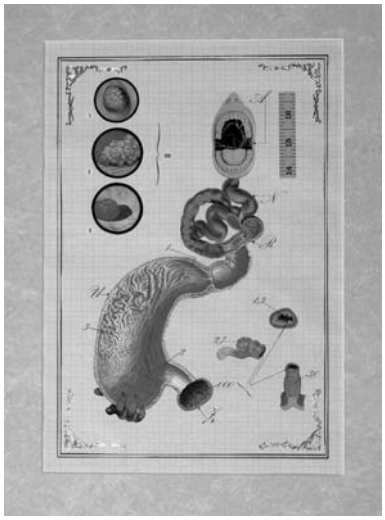
ABOUT THE COVER

The never-ending story

Helmie van de Riet



Helmie van de Riet attended the Academy of Graphic Art in Maastricht (1995-2000). Since 2000 she has exhibited her work in many group and solo expositions in the Netherlands and abroad. In 2006 she will participate in the Romanian Textile Arts Triennial in Boekarest. Her work can also be seen in exhibitions at the Graphic Centre in 's Hertogenbosch, Winterswijk and Utrecht, for example.

This month's cover, made using the collage technique, is called 'The never-ending story' because it is about fertilising the land. Because of this, vegetables, plants and grass will grow for the cattle and will eventually serve as food for people. After consumption and digestion, this will lead to manure to fertilise the land once more. This circle, the never-ending story, always occurs within the digestive tract which is a circle in its own right. This is symbolic for the 'never-ending story' of artists visualising processes: I am being fed by stimuli from society, these impulses activate my thinking process and by creativity the images will be filtered into 'food' for society.

An original print (with passe-partout 40 x 50 cm, without passe-partout 27 x 39 cm) is available in a limited edition of three at a price of € 250 and can be ordered from Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands, e-mail: Galerie-Unita@planet.nl, www.galerie-unita.com.

INFORMATION FOR AUTHORS

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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.

Manuscripts
Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Language
The language of the Journal is English. English idiom and spelling is used in accordance with the Oxford dictionary. Thus: Centre and not Center, Tumour and not Tumor, Haematology and not Hematology.

Submission
Starting February 2006 all submissions to the *Netherlands Journal of Medicine* should be submitted online through Manuscript Central at http://mc.manuscriptcentral.com/nethjmed. Authors should create an account and follow the instructions. If you are unable to submit through Manuscript Central contact the editorial office at g.derksen@aig.umcn.nl, tel.: +31 (0)24-361 04 59 or fax: +31 (0) 24-354 17 34.

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Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process, number the lines in the margin and the pages.
Subheadings should not exceed 55 characters, including spaces.
Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for generic names of substances and materials.
A *Covering letter* should accompany the manuscript, identifying the corresponding person (with the address, telephone number, fax number and e-mail address). Conflicts of interest, commercial affiliations, consultations, stock or equity interests should be specified. In the letter one to three sentences should be dedicated to what this study adds. The letter should make it clear that the final manuscript has been seen and approved by all authors. All authors should sign the letter. The letter should either be submitted through http://mc.manuscriptcentral.com/nethjmed or faxed to the editorial office (+31 (0)24-354 17 34).

Divide the manuscript into the following sections: Title page, Abstract, Keywords, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

The *Title page* should include authors' names, degrees, academic addresses, correspondence address, including telephone number, fax number, e-mail address and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

The *Abstract*, not exceeding 250 words, should be written in a structured manner and with particular care. In original articles, the Abstract should consist of the following paragraphs: Background, Methods, Results and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

Keywords: Include three to five keywords.

The Introduction should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely, without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

Acknowledgement: All funding sources should be credited here. Also a statement of conflicts of interest should be mentioned.

References should be numbered consecutively as they appear in the text (after the punctuation and in square brackets). Type the reference list with double spacing on a separate page. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med 1991;324:424-8).

Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.
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Please note that all authors should be listed when six or less; when seven or more, list only the first three and add et al. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against the reference list after your manuscript has been revised.
The use of bibliographic software programmes that are designed to generate reference lists such as Reference Manager© or Endnote© is highly encouraged. Authors can use the predefined output 'Vancouver' style from these programmes.

Tables should be typed with double spacing each on a separate page, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction (>300 DPI). Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. Colour figures are occasionally possible and will be charged to the authors.
Legends for figures should be typed, with double spacing, on a separate page.

Case report
Case reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Selection of case reports will be based on criteria as outlined in a special report by the editors (Drenth et al. The case for case reports in the Netherlands Journal of Medicine. Neth J Med 2006;64(7):262-4). We advise potential authors to take notice of the instructions in this report. Articles published in this section should be no longer than 1000 words, and supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references.

Mini review
Mini reviews are concise notes that bring the reader up to date with the recent developments in the field under discussion. The review article should mention any previous important reviews in the field and contain a comprehensive discussion starting with the general background of the field. It should then go on to discuss the salient features of recent developments. The authors should avoid presenting material, which have already been published in any previous review. The manuscript should be divided as follows: title page, abstract and main text. The text may be subdivided further according to the areas to be discussed. The length of the text can be a maximum of 2500 words.

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Letters to the editor will be considered by the editorial board. Letters should be no more than 400 words. Please mind the use of SI units for measurements and provide the references conform the Vancouver style (N Eng J Med 1991;324:424-8). No more than one figure is allowed. For letters referring to articles previously published in the journal, the referred article should be quoted in the list of references.

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Submission of a photo quiz should not exceed 500 words and include no more than two figures and four references conform the Vancouver style. Abbreviations of measurements should be quoted in SI units.

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