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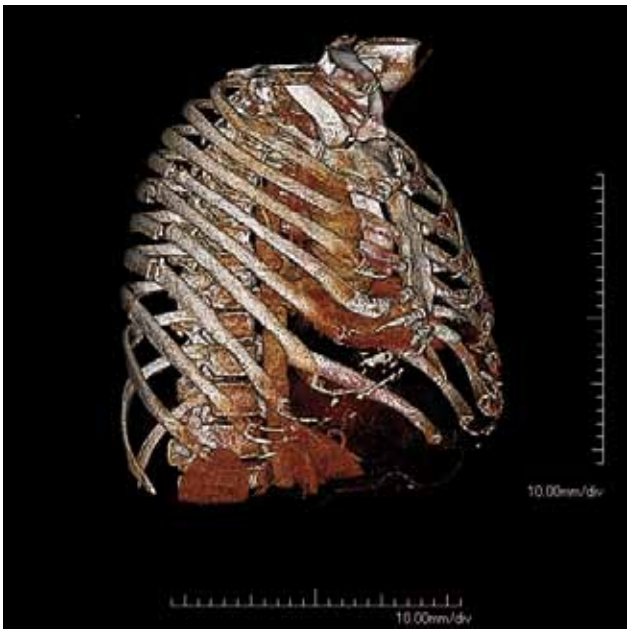

PHOTO QUIZ: A productive cough, see page 154

The Netherlands Journal of Medicine: THE NEXT EPISODE

AIR POLLUTION AS NOXIOUS ENVIRONMENTAL FACTOR

THE EMERGENCY CARE OF COCAINE INTOXICATIONS

ESTABLISHMENT OF REFERENCE VALUES FOR ENDOCRINE TESTS

IMMUNOPHENOTYPING OF MAST CELLS

DRESS SYNDROME CAUSED BY NITROFURANTOIN

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EDITORIAL

The Netherlands Journal of Medicine: the next episode

M. Levi

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This is the first issue of *the Netherlands Journal of Medicine* under the Amsterdam editorship that started in January 2009.[1] It should be mentioned, however, that many of the contributions in this issue were commissioned, reviewed, and edited under the previous editorship from the successful Nijmegen team.[2] Nevertheless, due to our active publisher we can guarantee a very short lag time between accepting manuscripts and publishing these papers in print, which means that already the next issues of the Journal will mostly come from papers just submitted in 2009 and therefore edited in Amsterdam. We hope prospective authors may appreciate the rapid turnaround time for editorial decision, eventual acceptance, and subsequent publication of their manuscripts and submit their contributions to our Journal. The time from acceptance to publishing papers on the internet, accessible through major medical research search engines, including PubMed, is even shorter. Indeed, many colleagues worldwide find this way to the papers published in the *Netherlands Journal of Medicine*, as evidenced by the large number of 'hits' on our internet pages and as reported in every issue of the Journal some months after publication. In addition, the number of citations of our manuscripts is growing every year as well, contributing to an improvement in the impact factor. The 'top 3' most cited papers in the *Netherlands Journal of Medicine* in the last three years[3-5] compare favourably with the number of citations in major international journals and we hope the number of papers achieving this attention will steadily grow. Interestingly, especially our review papers attract a lot of citations and we will try to build on this by further improving the quality of these manuscripts in the coming years. In addition, case reports, in particular those that present new and original observations, seem to be interesting for many readers worldwide.[6] Apart from that, the *Netherlands Journal of Medicine* may be a good forum for our residents in training and other colleagues to submit their interesting clinical observations in case series or case report format or as a photo quiz. In addition, the Journal may be a platform to publish novel guidelines of particular interest for Internal Medicine.[7] Taken together, the new editorial team is proud to have taken over the editorship of the Journal and we are looking forward to productive years and very interesting publications.

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REVIEW

Air pollution as noxious environmental factor in the development of cardiovascular disease

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ABSTRACT

A strong epidemiological association has been revealed between air pollution and the occurrence of cardiovascular disease (CVD). Deleterious consequences of such pollution, including myocardial infarction and coronary ischaemia, have occurred after both acute as well as chronic exposure to air pollution. The causal pathophysiological mechanisms through which these effects occur have not been identified but potential pathways include endothelial dysfunction and systemic reactions such as inflammation and oxidative stress. Because of increasing urbanisation and associated anthropogenic activities, air pollution is considered an important topic in public health and it remains challenging to translate these epidemiological observations into clinical consequences and guidelines. Nevertheless, for the high cardiovascular risk population, air pollution might have direct clinical relevance. In the future, more knowledge is required about the absolute risk of air pollution in specific high-risk populations and the pathophysiological mechanisms behind this relationship.

KEYWORDS

Air pollution, atherosclerosis, cardiovascular

INTRODUCTION

Cardiovascular disease remains the principle cause of morbidity and mortality in Western countries as well as in the developing world. In the Netherlands, cardiovascular disease causes more than 40,000 deaths each year.[1] In addition to well-established risk factors (such as dyslipidaemia and hypertension), exposure to air pollution has attracted a lot of attention in the media for its relationship with coronary ischaemia. A number of reports have noted associations between air pollution and the development of cardiovascular disease, pulmonary cancer and chronic obstructive pulmonary diseases.[2-4] In line with these observations, the World Health Organisation (WHO) estimated that each year approximately 800,000 people die prematurely which could be attributed to air pollution worldwide.[5] Air pollution is thought to predominantly exacerbate cardiopulmonary disease that causes death. Dutch national authorities estimate that 2300 to 3500 individuals a year will exhibit premature all-cause mortality due to fine particles. In case of long-term exposure, these effects are even more pronounced with a one-year life reduction for 18,000 individuals.[6] Against this background, various epidemiological studies have shown that increased levels of air pollution could augment cardiovascular morbidity and mortality due to ischaemic events, more frequent hospitalisations, worsening of heart failure and (ventricular) arrhythmias and that these effects occur both due to daily changes in air pollutant levels as well as due to lifetime exposure.[7]

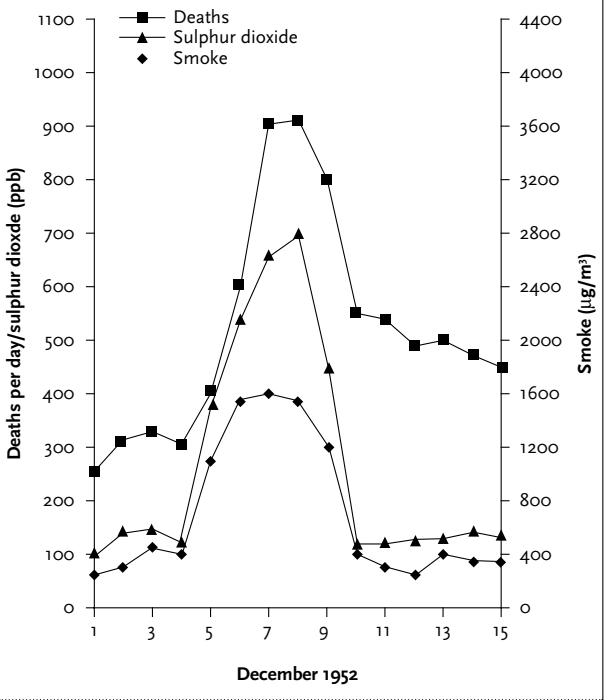
If air pollution has indeed become a relevant factor in the occurrence of cardiovascular disease, this should have worldwide consequences. Progressive dense urbanisation of distinctive regions is emerging globally with a concomitant increase in cardiovascular morbidity and mortality. This is also true for the urban regions on the African continent.[8] With this review, we aim to give an outline of current knowledge with regard to the relationship between air pollution and cardiovascular disease.

THE HISTORY OF AIR POLLUTION AND CARDIOVASCULAR DISEASE

The fact that air pollution could negatively affect human health is not just a current observation. In the past, various events have provided the first observations that air pollution

might have harmful effects on human health. The most well-known example is the impressive London fog episode in 1952 (*figure 1*). Between 5 and 9 December, an unusual dense fog covered the London area for four days. This was probably due to burning of large quantities of coal because of low temperatures combined with a diminished dispersing into the atmosphere of combustion products. Because of the subsequent high levels of atmospheric pollutants, about 4000 people died in the next two weeks as a consequence of both pulmonary and cardiovascular causes.[9] Similar events also occurred elsewhere. For example, in 1930, in the Meuse Valley in Belgium, one of the heaviest industrialised areas of that time, a period of severe fog led to a high prevalence of respiratory symptoms, retrosternal pain and early death among the population of the valley. A total of 60 deaths in the following three days were attributed to the fog and the subsequent marked rise in atmospheric pollutants.[10] These two different episodes became an important inspiration to execute additional studies into this phenomenon.

Figure 1. *The London fog 1952*



A four-day episode of severe fog led to increased levels of atmospheric pollutants and a subsequent rise in mortality during these days in London in 1952.
Source: http://www.ace.mmu.ac.uk/Resources/Fact_Sheets/Key_Stage_4/Air_Pollution/images/03a.jpg.

AIR POLLUTION AND CARDIOVASCULAR DISEASE

Exposure to air pollution has been associated with several adverse effects on human health including chronic obstructive pulmonary disease and pulmonary cancer. In the next paragraph we will focus on the cardiovascular effects of air pollution. Furthermore, we will limit our discussion to recently published studies on this topic with a special attention to the Western European region.

Short-term exposure

Several studies have shown a strong association between acute exposure to high levels of air pollution and the occurrence of acute coronary ischaemia. Within this perspective, short-term exposure refers to a few hours' exposure to high levels of air pollution, for instance a day with bad air quality day or a walk near a busy road.

Short-term exposure to various air pollutants could trigger the onset of ischaemic events. For instance, Peters *et al.* showed that exposure to high levels of $PM_{2.5}$ (particulate matter with diameter <2.5 µm) as well as a high traffic volume for only one or two hours could trigger the onset of a myocardial infarction.[11,12] In a study by Mills *et al.*, 20 male patients with a prior myocardial infarction were exposed to either dilute diesel exhaust (particulate matter 300 µg/m³) or filtered air in a controlled exposure chamber, while performing moderate exercise.[13] Heart rate increased equally during exercise in both groups. However, in the diesel-exposed group they documented significantly more ischaemic burden (defined as the depth multiplied by the duration of ST-segment depressions on 12-lead ECGs). The exposure in this study of 300 µg is not exceptional since concentrations of particulate matter can reach this level in heavy traffic, in occupational settings or in large cities. Within the Netherlands, the maximum peak levels of particulate matter that are measured by air quality stations are about 120 µg/m³. However, it is noteworthy that this peak level is a mean concentration over 24 hours. To translate this condition to that measured in the Mills' study one may assume that a one-hour exposure to 300 µg/m³ will increase your 24 hour accumulated exposure by only 12.5 µg/m³. Of importance, the day-to-day variation of particulate matter levels can vary between 10 and 15 µg/m³ on ordinary days. Thus, within this perspective, one hour exposure to 300 µg/m³ (Mills' study) is not excessively high, but apparently sufficient to induce coronary ischaemia.[13]

In addition to morbidity, short-term exposure is also associated with cardiovascular mortality. In the Air Pollution and Health: a European Approach 2 (APHEA2), a large European time-series study in 29 cities, the authors showed that each 10 µg/m³ increase of PM_{10} (particulate matter with diameter <10 µm) concentration was associated with an increase in non-accidental mortality of 0.68% (95% confidence interval [CI] 0.6 to 0.8%).[14] Differentiation in cause-specific mortality showed an increase of 0.76% (95% CI 0.47 to 1.05%) in cardiovascular deaths and 0.58% (95% CI 0.21 to 0.95%) in respiratory

deaths for each 10 μg/m³ increase in PM_{10} concentration.[15] In the Dutch region, PM_{10} levels are acknowledged to have a day-to-day and inter-regional variation of up to 20 μg/m³ and this increase in PM_{10} levels may indeed contribute to differences in morbidity and mortality rates.

Long-term exposure

Not only short-term exposure but also chronic exposure could effect the development of cardiovascular disease. Within this perspective, chronic exposure refers, for instance, to subjects who lived for months or years near heavy traffic roads or in areas with enduring high levels of air pollution such as large cities. A German study showed that a higher prevalence of clinically manifest CVD was present in those subjects who lived within 150 metres of a major road.[16] After adjusting for background air pollution and other conventional cardiovascular risk factors, the relative risk of future CVD was 1.85 (95% CI 1.21 to 2.84). In support of these epidemiological observations, people who live near a major road displayed more coronary artery calcifications: living within 50 metres, 51 to 100 metres and 101 to 200 metres was associated with odd ratios of 1.63 (95% CI 1.14 to 2.33), 1.34 (95% CI 1.00 to 1.79), and 1.08 (95% CI 0.85 to 1.39), respectively, for marked coronary artery calcification (defined as more than the 75th percentile for age and gender assessed by electron-beam computed tomography).[17] In addition to cardiovascular morbidity, long-term exposure to air pollution is also associated with cardiovascular mortality. A large Dutch cohort study showed that living near a major road (within 100 metres of a freeway or within 50 metres of a major urban road) was related to total mortality (relative risk 1.41, 95% CI 0.94 to 2.12) and an even more significant relationship was found with cardiopulmonary mortality (relative risk 1.95, 95% CI 1.09 to 3.52).[18] Noteworthy, positive associations between background concentrations of air pollution and mortality were less pronounced than those for traffic-related air pollution and mortality indicating that traffic per se might be an important source of the occurrence of harmful health effects.

AIR POLLUTION IN THE NETHERLANDS

Although air quality in general has improved significantly over the past few decades,[19] harmful effects on human health (with major manifestations for lung and cardiovascular disease) have also been reported under current air pollution levels. With the aim to reduce those negative health effects, several initiatives under the aegis of the European Union were launched to set limit levels for distinctive components from which air pollution is composed (*table 1*). Nevertheless, upper limits are frequently surpassed in almost all large European cities, including the Dutch.

Table 1. *Air quality limit values in the European region*

	Current		2015-2020	
	Daily	**Annual**	**Daily**	**Annual**
PM10	50 μg/m³*	40 μg/m³	50 μg/m³*	40 μg/m³
PM2.5	-	-	-	25 μg/m³

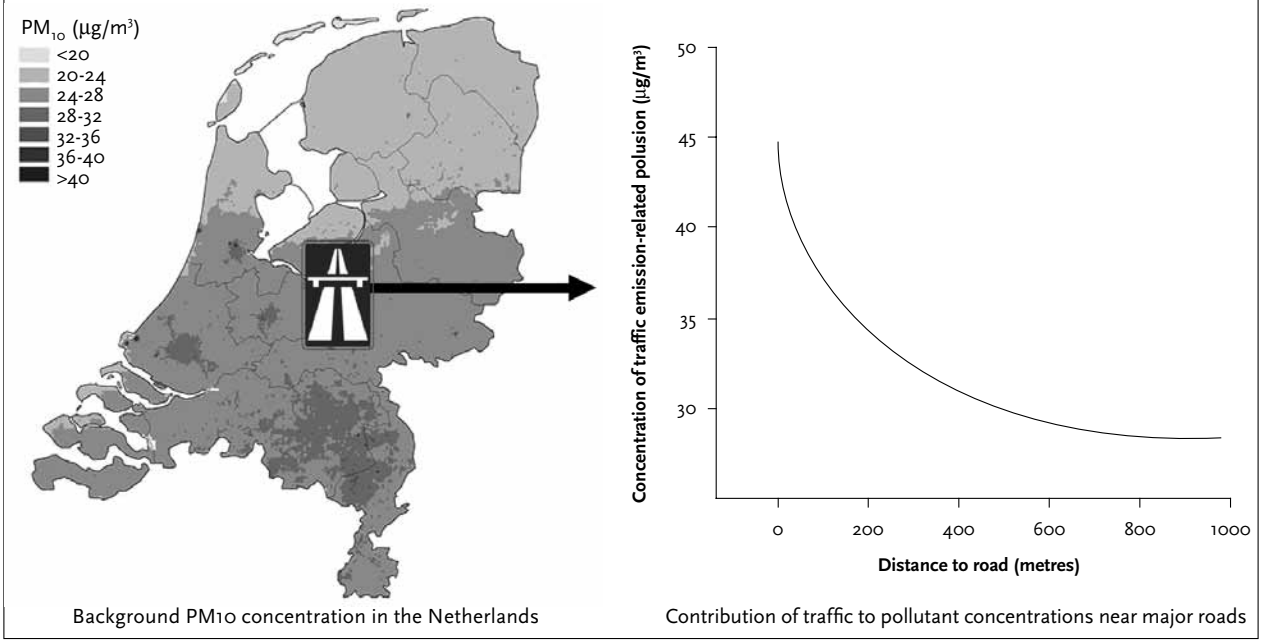
*Allowed to exceed on 35 days per year. Current and future daily and annual limit values as estimated by the European Union for particulate matter. Exceedence of daily limit value for PM10 is allowed on 35 days per year with an annual limit value of 40 μg/m³. In the current values there is no limit set for PM2.5.

Recent analyses have identified harmful environments with regard to air pollution densities. In heavy traffic, occupational settings and in the world's largest cities, particulate matter levels up to 400 μg/m³ have been documented.[19] Due to dense urbanisation, larger areas in the Netherlands, Belgium, Germany and Italy will suffer from higher levels of air pollution than areas in other parts of Europe. In most regions in the Netherlands, limits of air quality raise frequently with PM_{10} (particulate matter with diameter <10 μm) peak levels above 100 μg/m³.[20]

Interesting in the dilemma of air pollution is the aspect of its crossing of national borders. For instance, in the Netherlands, 30% of all airborne fine particles find their origin in nature (e.g. sea salt, soil dust), whereas 20% consist of anthropogenic origin that is generated in our own region (e.g. industries, refineries, agriculture). The remaining 50% have a foreign anthropogenic origin.[21] Only intensive international collaboration could therefore result in significant improvements in air quality and, as a consequence, health.

Furthermore, air pollution concentrations and the contribution of different sources can differ between different seasons; in the Dutch region, elevated particulate matter concentrations have been found in the winter, probably due to an increased contribution from traffic. In contrary, higher sulphate levels have been measured in the summer, because sulphate originates from oxidation of SO_3 by a photochemical process. Also, most days with severe smog (O_3 >240 μg/m³) have been recorded on summer days with a temperature of more than 25°C.[22] These observations are of special interest because in areas with high urbanised regions (for instance in the Netherlands), the average level of ambient air particles (as part of background pollution) is equally spread with only small variations with regard to different isolines. As a consequence, air samples taken from sites near a high volume traffic road most accurately reflect the long-term exposure to air pollution for an individual in that area (*figure 2*).

Figure 2. *Background and traffic-related air pollution concentrations in the Netherlands*



Background PM10 concentration in the Netherlands

Contribution of traffic to pollutant concentrations near major roads

BIOLOGICAL EFFECTS OF AIR POLLUTION

In order to explain these long- and short-term epidemiological observations regarding the relationship between air pollution and cardiovascular morbidity and mortality, the pathophysiology has not been elucidated as of yet. Potential mechanisms include increased oxidative stress, systemic inflammation, endothelial dysfunction, pro-atherothrombosis, hypertension and autonomic dysfunction.[23-27] Despite these numerous mechanisms, one may assume a different mechanism for the harmful biological effects of acute versus chronic exposure. Short-term exposure especially exerts endothelium-mediated processes (such as impaired vasodilatation and more coronary vasoconstriction) and an increase of sympathicotonus.[26] In healthy volunteers, these effects persist until 24 hours after exposure.[28] In long-term conditions systemic responses (e.g. oxidative stress, hypertension, inflammation) are more dominant.

It is unknown whether systemic effects of air pollution could be explained by pulmonary inflammation or by translocation of specific air borne particles from the alveolar space into the circulation.[29] In case of the last option, airborne particles could be part of micellar structures (lipophilic compounds), while hydrophilic airborne compounds circulate in aqueous solution. Currently, we are evaluating whether lipophilic airborne structures could be carried by large lipoproteins, such as chylomicrons and VLDL, and whether these loaded lipid particles could be deposited in the vessel wall with subsequent generation of atherogenic cascades (Trojan horse hypothesis).[30]

WHAT ARE THE LESSONS FOR CLINICAL PRACTICE?

Despite the strong relationship between acute and chronic air pollution levels and the occurrence of cardiovascular morbidity and mortality,[31] a major question remains as to the extent to which air pollution can account for significant changes in clinical conditions.

Nowadays, the estimated risk of cardiovascular disease for patients without previous CVD is based upon the presence of conventional risk factors including sex, age, smoking and systolic blood pressure.[32] In the near future, we should define to what extent air pollution adds to this individual cardiovascular risk profile and its ranking among the more conventional risk factors. From actual knowledge, it is plausible that a high exposure to air pollution might have an exaggerated effect on the development of complicated cardiovascular disease in those patients in whom one or more conventional risk factors are present or in those with previous CVD. Whether air pollution potentiates residual cardiovascular risk or has a positive interaction with conventional risk factors is not thoroughly elucidated yet.

Several clinical and epidemiological observations showed that the effects of air pollution might be more pronounced in patients with coronary artery disease, congestive heart failure or respiratory disease.[13,33] For instance, a recent observation in 12,865 North-American patients after a previous cardiac catheterisation showed that an increase of 10 µg $PM_{2.5}$ per cubic metre was associated with a 4.5% (CI 1.1 to 8.0) increased risk on acute ischaemic coronary

events.[34] Interestingly in their analysis was the fact that the most significant associations were found for that $PM_{2.5}$ exposure on concurrent and previous day, indicating a per acute effect of ambient fine particulate pollution on the presentation of ischaemic heart disease in patients with existing coronary heart disease. That study and a supportive one[11] convincingly show that a 10 to 25 $\mu g/m^3$ increase in $PM_{2.5}$ particles could be defined as a high-risk environment for patients after a (recent) coronary event or those with an increased cardiovascular risk profile. Indeed, the time subjects spent in their cars, on public transportation, or on their motorcycles or their bicycles was consistently linked with an increased myocardial infarction risk (onset of myocardial infarction one hour later) in patients who are susceptible for coronary heart disease.[12] In line with these observations, we recommend avoidance of environmental conditions in which an increase of 10 to 25 µm in $PM_{2.5}$ per cubic metre can be expected by regional weather forecasts for especially those patients after a (recent) ischaemic coronary event or those with an increased cardiovascular risk profile.

Discouraging these patients to travel outside in case of significant levels of air pollution or spending time in urban regions with a high dense fine particulate matter level in certain seasons (for instance the Athens region during summer time) will have several consequences for the clinical physicians and their advice for healthcare-related organisations. More initiatives concerning education should be developed for practising physicians to make them more aware of these associations, so they can advise their patients in creating the optimal living conditions. Complementary efforts should be expected from local and national governments with realisation of estate projects and measurements for appropriate inner house air quality. To support these last remarks, a Swedish study recently revealed these hazardous situations; increased levels of PM_{10} in a preceding two-hour exposure period gives rise to more ventricular arrhythmias in patients carrying an ICD.[35] Indeed, Berglind and colleagues have just published the results from a European multicentre study which showed that increased ambient air pollution was related with increased daily mortality in survivors from a myocardial infarction.[31] Of subsequent interest is whether patients with a specific profile (prone to develop ventricular arrhythmias for instance, first weeks after MI or in the presence of unstable angina), presence of certain weather conditions, living in the proximity of a road or being female with an age >60 years should be advised to change environment for a stay in a health resort for a certain period of time. Comparable beneficial effects on cardiovascular health have previously been attained in other conditions linked to air pollution; partners who stopped smoking reduced cardiovascular morbidity and mortality in passive (second hand) smokers, urban regions at the African continent had higher numbers for cardiovascular disease compared with their rural regions and living in a natural (green) environment decreased all-cause mortality, including from cardiovascular causes.[36] Finally, a recent analysis based on the correlation between reductions in particulate air pollution over the past few decades and increased life expectance in 217 counties in 51 metropolitan areas in the United States showed that a decrease of $PM_{2.5}$ of 10 $\mu g/m^3$ is associated with an increase in life expectancy of 0.61 (±0.20) year representing 15% of the overall increase in life expectancy in the study areas. These results were constant after adjustment for changes in socioeconomic, demographic, and smoking patterns over the same period.[37] Similar reductions in life expectancy have been estimated for the Netherlands[38] and these findings underline the importance of air pollution on human health.

CONCLUSION

Air pollution is an ecological and social dilemma in the Western world. In earlier times, several social movements, backed up by medical doctors, realised a change in environmental factors with, subsequently, a dramatic reduction in infectious disease. Currently, similar actions are required with regard to air pollution.

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REVIEW

The emergency care of cocaine intoxications

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ABSTRACT

Cocaine is frequently used, especially among adolescents and by men between the age of 25 and 44. Many of them are able to use cocaine in normal day-to-day life, without any problems.[1] Reduced prices of cocaine and other recreational drugs such as MDMA (ecstasy) and gamma hydroxybutyrate (GHB) has led to an increased incidence of intoxications with these drugs.[2] Since the production of cocaine is illegal, it may be impure and mixtures with other drugs such as atropine may occur. The treatment of patients with an acute cocaine intoxication can be complicated. Combination of cocaine with other drugs results in clinical pictures which are difficult to discriminate and that may have important consequences for treatment.

KEYWORDS

Cocaine, cocaine intoxication, emergency room

INTRODUCTION

Intoxications with drugs of abuse are part of day-to-day care at the emergency department (ED). Among these, intoxications with cocaine are especially challenging. Firstly, cocaine in itself may lead to life-threatening complications and is secondly frequently used in combination with other drugs, which may lead to a wide variety of clinical pictures with important consequences for treatment. In this review we describe four cases of patients who visit the ED because of a cocaine intoxication, with several different complications and co-intoxications. After some background information and a review of the literature about cocaine, we advise on the do's and don'ts in case of an acute cocaine intoxication.

CASE REPORTS

Patient A

A 39-year-old man with a history of alcohol abuse arrived at the ED with haematemesis after he had been found by a friend. He was very agitated, had a sinus rhythm of 126 beats/min and a blood pressure of 220/117 mmHg. Physical examination showed no abnormalities except for mydriasis. Electrocardiography (ECG) showed left ventricular hypertrophy, ST depression in the inferior (II, III and aVF), lateral (I, aVL, V_5 and V_6) and anterior leads (V_1 to V_4). Laboratory analysis showed a decreased haemoglobin level (3.6 mmol/l), and elevated troponin I (3.16 µg/l). Coagulation tests, arterial blood gas analyses, electrolytes, liver enzymes and creatinine were within the normal range. Urine tox-screen for cocaine was positive. Gastroscopy revealed a bleeding ulcer in the proximal duodenum, which was treated by local injection of adrenaline. Further initial management consisted of supplementary oxygen, acetylsalicylic acid, labetolol, nitroglycerin, diazepam and pantoprazole. The clinical course was uneventful and the patient could be discharged after one week in a good clinical condition.

Patient B

A 39-year-old man was found subcomatose in the lavatory of a care centre for drug addicts. He reported he had used cocaine and heroine, two bottles of strong liquor, five tablets of 5 mg diazepam and five tablets of 25 mg levopromazine. He complained of chest pain, pain in the epigastrium and in his left jaw region. He was taking naltrexone 1 x 50 mg, loperamide 8 x 2 mg, diazepam 2 x 5 mg, levomepromazine 3 x 25 mg, pantoprazole 1 x 40 mg and mirtazepine 1 x 30 mg. The physical examination showed a blood pressure of 93/50 mmHg and a heart rate of 110 beats/min. The ECG showed a sinus rhythm without signs of ischaemia, infarction, or left ventricular hypertrophy. Arterial blood gas analysis, while receiving oxygen (5 litres/min), showed

a respiratory acidosis: pH 7.25, pO_2 30.5 kPa, pCO_2 7.2 kPa, HCO_3 22.8 mmol/l. Further laboratory results showed an alcohol concentration of 1.2‰, but no other abnormalities. He lost consciousness in the emergency room and was intubated and mechanically ventilated. Neither naloxone nor flumazenil were administered. The patient was admitted to the intensive care unit (ICU). His vital functions were supported and treatment with acetylsalicylic acid and nitroglycerine was initiated. The next day his physical condition had improved and he was discharged after consultation of a psychiatrist.

Patient C

A 27-year-old man presented to the ED in the early morning. He was sweating, his hands were shaking, he complained of a sore throat and was agitated. During the last week he had had a flu-like illness. The evening before presentation he had snorted cocaine and consumed 15 alcoholic beverages. He used escitalopram because of depression. The physical examination showed a temperature of 40.3°C, heart rate of 130 to 160 beats/min, a blood pressure of 200/110 mmHg and a breathing frequency of 25/min. He had mydriasis, an inflamed throat and inspiratory wheezing. Blood analysis revealed an increased white blood count, elevated CRP and mildly elevated liver enzymes. Blood alcohol concentration was 2.2‰. The ECG showed sinus tachycardia, without further abnormalities. The patient was treated with oxazepam and metoprolol. He was seen by the ear, nose and throat specialist who concluded that the patient had a bacterial pharyngitis and prescribed amoxicillin/clavulanic acid. After five hours of observation his temperature decreased and the heart rate and blood pressure normalised. He was allowed to leave the ED and was further evaluated at the outpatient clinic.

Patient D

A 35-year-old man became unconscious and in shock under obscure circumstances in a hotel room in the centre of Amsterdam. His friend phoned 112. At arrival of the ambulance the patient was in ventilatory and circulatory arrest and cardiac arrest without heart action on the ECG. After resuscitation in the ambulance the circulation was restored and the patient was brought to the ED. He was treated with naloxone and flumazenil, since a mixed intoxication with drugs of abuse was suspected. After intubation, the patient was admitted to the ICU for mechanical ventilation and for medical support to improve the circulation. For 24 hours, the patient was kept at 32 to 34°C to reduce post-anoxic cerebral damage. Urine tox-screen was positive for cocaine and heroin. In addition a toxic diazepam serum level was found (1200 µg/l; therapeutic level 125 to 750) and the blood ethanol concentration was 0.8‰.

The patient remained in a comatose state after normothermia. In addition, no cerebral activity was observed, including absence of activity on the electroencephalography (EEG) at several readings. Therefore the treatment was discontinued and the patient died the fourth day after he was admitted.

EPIDEMIOLOGY

Cocaine is a widely used drug, especially in North America (6.35 million people, 2% of the population >14 years), South America (2.74 million people, 1% of the population) and Western Europe (3.4 million people, 1% of the population.[3] Also in the Netherlands cocaine is used throughout society. In Amsterdam cocaine use is about four times higher than in the rest of the country.[1] The prevalence of people who had ever used cocaine has increased from 2001 to 2005, but first-time use of cocaine is decreasing. The percentage of recent and actual users was stable in this period.[1] The percentage usage in 12 to 18 year olds was also stable between 1996 and 2003. Between 2001 and 2005 the average age of recent users increased. Use of cocaine is most prevalent in 25- to 44-year-old men. It is especially used in trendy clubs and pubs.[1] Cocaine is also popular among users of heroin. About 70 to 90% of heroin addicts also smoke cocaine ('crack').[1]

PHARMACOLOGY

Cocaine, benzoylmethylecgonine ($C_{17}H_{21}NO_4$) is an alkaloid, extracted from the leaves of Erythroxylon coca.[4] Cocaine increases the activity of monoamine neurotransmitters in the central and peripheral nervous system by blocking reuptake pumps (transporters) of dopamine, norepinephrine and serotonin.[3] The concentration of these neurotransmitters in the presynaptic cleft is enhanced. In addition cocaine modulates preprodynorphin and the µ-, en κ-receptors of the endogenous opiate system.[5] All this leads to a feeling of increased energy, alertness, intense euphoria and decrease of tiredness, appetite and sleep.[3] Unwanted effects such as fear, irritation, panic attacks, paranoia, impaired judgement, delusions, disturbance of sleep, weight loss and hallucinations occur with increased doses, or a more efficient route of administration.[3] It also leads to an increase in heart rate and blood pressure, to mydriasis and diaphoresis as a consequence of stimulation of the sympathetic nervous system. Cocaine causes arrhythmias.[6] It acts as a local anaesthetic by inhibition of the membrane permeability of sodium ions during depolarisation. This leads to blocking of both initiation and transmission of electric signals.[5] Cocaine can be smoked, snorted or used

intravenously.[3,4] It is absorbed readily through all mucosae. The peak effect occurs between 1 to 90 minutes, depending of the route of administration. The initial half-life varies between seconds and 20 minutes after inhalation, intravenous administration or snorting respectively. After oral use the half-life is three hours. There are two forms of cocaine. Cocaine base ('crack', 'freebase') may be smoked. Because of its relatively low melting temperature (98°C), it evaporates before degradation takes place. It is relatively insoluble and therefore it cannot be used intravenously. The cocaine salt cannot be smoked, for it does not melt until 195°C. Therefore the molecule already degrades before evaporation takes place. The cocaine salt is well soluble and it may be administered intravenously or it may be snorted.[3,4] After intravenous administration the effect lasts for about 15 to 30 minutes, after snorting one hour and after oral use three hours.[7] Cocaine is hydrolysed in the liver by carboxyesterases. This results in the formation of the inactive benzoylecgonine.[3] The majority of metabolites are excreted in the urine.[3]

LIFE-THREATENING COMPLICATIONS OF A COCAINE INTOXICATION

Cocaine and the heart

Cocaine induces tachycardia, hypertension and an increased systemic vascular resistance due to an increase of sympathetic activity both directly on the heart and indirectly via the central nervous system.[3,4] This leads to an increased cardiac oxygen consumption, whereas oxygen supply decreases because of coronary vasoconstriction, eventually leading to cardiac ischaemia as was seen in patient A.[8] In addition activation of platelets results in thrombus formation.[9-11] The most frequently occurring cardiac complications of cocaine are angina pectoris, myocardial infarction, acute cardiac death, complicated acute arterial hypertension, myocarditis, cardiomyopathy, aortic rupture or dissection and left ventricular hypertrophy as was seen in patient B.[8] Blocking of sodium channels of myocytes results in decreased electric conduction and arrhythmias. Also without myocardial infarction, syncope or acute heart death may occur (patient D).[12] The typical patient with cocaine-related myocardial infarction is a young man, without cardiovascular risk factors other than smoking.[4] The relative risk of myocardial infarction is 23.7 times (95% CI 8.5 to 66.3) within 60 minutes after cocaine use and does not appear to be related to amount, route of administration or frequency of use.[13] At the ED most cocaine users complain about chest pain.[4,14] The incidence of myocardial infarction in these patients is about 6%. This suggests that these symptoms are not usually related to myocardial necrosis.[11,15]

In patient A the myocardial necrosis is also facilitated by the severe anaemia caused by a bleeding duodenal ulcer. This is infrequently seen after cocaine use and is caused by severe gastrointestinal vasoconstriction leading to mucosal ulceration.

Cocaine and hyperthermia

The autonomic adaptation of elevated body temperature consists of skin vasodilation and perspiration.[16] Normal behavioural adaptation results in evading heat and refraining from physical activities.[17] Cocaine results in an increase of the core temperature, decrease of heat perception and decrease of perspiration and skin circulation. Moreover the agitation hampers the normal behavioural response. All of these may lead to fatal hyperthermia.[16] Patient C had an elevated body temperature caused by an infection. However, cocaine-induced hyperthermia should always be considered in a patient with fever in the context of cocaine use. These patients should be aggressively cooled and one should be aware of complications such as rhabdomyolysis and diffuse intravascular coagulation.

CLINICALLY RELEVANT COMBINATIONS WITH COCAINE

Cocaine and alcohol

Most cocaine users also drink alcohol (patients B and C).[18] Cocaine has a sobering effect after ingestion of alcohol, leading to use of cocaine in the context of excessive alcohol consumption. Transesterification of cocaine and alcohol, catalysed by carboxyesterase 1, leads to the formation of the active metabolite cocaethylene, instead of the inactive hydrolysis product benzoylecgonine.[18] As cocaine, cocaethylene has a central stimulating effect. So co-ingestion of alcohol leads to an increase and longer duration of the effect of cocaine.[19] The combination leads to a further increase in heart rate and blood pressure and increased myocardial oxygen consumption.[20] Cocaethylene is associated with a 40-fold increase in the risk of cardiac events and 25-fold the risk of acute cardiac death.[21,22] Patients who die as a consequence of the combined use of cocaine and alcohol have lower blood cocaine concentrations than patients dying after the use of cocaine alone. This suggests that there is an additive or synergistic effect of alcohol on the severe cardiovascular effects of cocaine.[21,23] Lastly, the combination decreases the feeling of drunkenness and increases cocaine-induced euphoria.

Cocaine and heroin

Combined cocaine/heroin intoxications are not uncommon at the ED (patient B and D). In about 50% of the cases heroin is used in combination with cocaine,

alcohol or other drugs (usually benzodiazepines).[24,25] The combination of cocaine and heroin leads to clinical pictures which are difficult to interpret and have important consequences for treatment. Naloxone is an effective and potentially lifesaving antidote for opiate intoxications. However, it is not without side effects.[26] The administration of naloxone to a patient with a co-intoxication of an indirect sympathicomimetic, such as cocaine, can lead to severe complications. Firstly, it may lead to potentially life-threatening sympathicomimetic toxicity because the inhibiting effect of the opiate is suddenly reversed.[27] Secondly, the arrhythmogenic effect of naloxone may be synergistic with cocaine.[28] So, in the context of a co-intoxication with cocaine, the potential benefit of naloxone should be weighed against its possible harm. In many cases of comatose patients after heroin/cocaine overdose it is better to observe the patient and if necessary support the ventilation. The same is true for co-ingestion of cocaine with a benzodiazepine. Administration of flumazenil may precipitate seizures, agitation, sympathetic activity and thus cardiac complications.

THE TREATMENT OF AN ACUTE COCAINE INTOXICATION

In most instances treatment of a cocaine intoxication is supportive. The challenge for the physician in the ED is to identify patients at risk who would benefit from a specific intervention. All patients with an acute coronary syndrome, but especially young man without other risk factors than smoking, should be asked about cocaine usage. First-line treatment of a patient with cocaine-related chest pain compatible with myocardial ischaemia and ST-segment elevation consists of administration of oxygen, and sublingual nitroglycerin or verapamil. If there is no response, immediate coronary angiography should be performed. Both nitroglycerin and verapamil have been shown to reverse cocaine-induced hypertension, coronary arterial vasoconstriction, and tachycardia.[29] Beta-blockers (especially non-selective β-blockers) are relatively contraindicated in cocaine-associated acute coronary syndrome. However, in clinical practice they are frequently used in this situation as was the case in patients A (labetalol) and C (metoprolol). Beta-receptor blockade causes unopposed α-receptor stimulation which may lead to aggravation of coronary arterial vasoconstriction and systemic hypertension.[30] Some authors advise labetalol, a combined α- and β-blocker. However, labetalol is a non-selective β-blocker with only modest α-blocking properties. Thrombolysis should only be given when a thrombus has been shown on angiography or if pharmacological treatment has failed and angiography is not possible.[3] Administration of naloxone and flumazenil should be avoided, since they may lead to severe complications.

CONCLUSION

The emergency care of cocaine intoxications may be challenging. Cocaine is frequently used and its use is not always readily apparent, since users may deny cocaine usage. Moreover, it is frequently combined with other substances such as heroin and benzodiazepines, which may mask some of the cocaine effects. An acute cocaine intoxication may be lethal due to cardiovascular complications or hyperthermia. Alcohol increases the cardiovascular toxicity of cocaine. In the treatment of an acute cocaine intoxication administration of a benzodiazepine has a prominent place, whereas β-blockers are contraindicated. In the case of combined use of cocaine with heroin or benzodiazepine administration of naloxone or flumazenil may be dangerous.

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ORIGINAL ARTICLE

Establishment of reference values for endocrine tests. Part VII: Growth hormone deficiency

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ABSTRACT

Background: Plasma insulin-like growth factor (IGF-I) concentration can be used as a rough indicator of the growth-hormone status. However, for the diagnosis of growth hormone deficiency, dynamic tests are required. The growth hormone (GH) response in the insulin tolerance test (ITT) is considered to be the gold standard in this respect. An alternative for the ITT is the GHRH/GHRP-6 test, which has fewer side effects. In this study we established reference values for IGF-I levels and for the GH response in both dynamic tests.

Methods: We studied 296 subjects recruited from the general population, equally distributed according to sex and aged between 20 and 70 years. Serum IGF-I level was measured in all subjects and an insulin tolerance test (0.15 U/kg Actrapid iv) and GHRH/GHRP-6 test (1 μg GHRH/kg and 1 μg GHRP-6/kg) were performed in 49 subjects.

Results: In multivariate analyses both IGF-I and the GH response in the ITT were significantly influenced by age, whereas the GH response in the GHRH/GHRP-6 test was significantly affected by BMI. There was no sex difference in IGF-I and in the GHRH/GHRP-6 test, but in the ITT males had a higher GH peak. There was a significant correlation between the GH responses in both tests, and the GH response was significantly higher in the GHRH/GHRP-6 test than in the ITT. Age-adjusted reference values were established for each test.

Conclusion: We have established age-adjusted reference values for serum IGF-I and for the GH response in the ITT and GHRH/GHRP-6 test.

KEYWORDS

Growth hormone deficiency, insulin tolerance test, GHRH/GHRP-6 test, IGF-I

INTRODUCTION

Growth hormone deficiency (GHD) in adults is characterised by changes in body composition that include decreased lean muscle mass, muscle strength and exercise performance, and increased body fat mass. In addition, metabolic derangements and a decrease in bone mineral density are known to occur in this setting. There is also an association with a diminished quality of life. Administration of growth hormone replacement has shown to provide physical and psychological improvement.[1-5] A correct diagnosis in the evaluation of patients for suspected GH deficiency is essential. For, in case of a false-positive diagnosis, the patient will be subjected to a prolonged and expensive treatment, whereas a false-negative diagnosis will deprive the patient of treatment. To identify patients appropriately, there is a need for reliable tests to diagnose GHD.

Plasma insulin-like growth factor (IGF)-I is growth hormone (GH)-dependent and can be used as an indicator of GH status. IGF-I serum level is, however, affected by factors as age, nutritional status, thyroid function and lean body mass. There is significant overlap in IGF-I values between those with and without GHD, and the overlap increases with higher age. Serum IGF-I can be of some diagnostic assistance if levels are below the age-adjusted normal range, but a normal IGF-I result does not exclude a diagnosis of GHD.[4,6-10]

Other biochemical measurements such as IGF-binding protein-3 or mean 24-hour GH concentrations also have limited diagnostic value in adults, because of overlap between healthy individuals and those with a deficiency.[7,9,10]

For these reasons the diagnosis of GHD requires provocative tests of GH secretion.

The insulin tolerance test (ITT) is considered the test of choice. It allows evaluation of the complete hypothalamic-

pituitary-somatotroph axis, making it useful in patients with both hypothalamic and pituitary disease. The ITT has been found to have high sensitivity and specificity in all age groups.[11] However, there are several disadvantages to the ITT. It is associated with uncomfortable side effects and it is contraindicated in patients with seizure disorders or cardiovascular disease. In addition, it requires constant monitoring even in healthy adults, although it is quite safe in experienced hands under adequate supervision.[12] It is established that obesity, in the abdominal region in particular, is associated with a blunted GH response to stimulation. Thus, in overweight patients it may be difficult to distinguish GHD from the decreased responsiveness due to obesity.[13-15]

Although the ITT is considered the gold standard for the diagnosis of GHD, the limitations of this test suggest the need for additional tests, with appropriate cut-off levels, to diagnose GHD. Moreover, in patients with ≤2 known other pituitary hormone deficiencies, two independent stimulation tests are recommended to diagnose GHD in adults.[6,16] An alternative provocative test is the combined administration of GH-releasing hormone (GHRH) plus GH-releasing peptide-6 (GHRP-6). GHRP-6 is a synthetic GH secretagogue that is a very potent and reproducible stimulus of growth hormone secretion. It has previously been reported that the GHRH/GHRP-6 stimulation test was not confounded by body composition.[17] Also, the ability of the GHRH/GHRP-6 test to discriminate between normal GH state and GHD was reported not to be affected by age.[17,18]
The GHRH/GHRP-6 test is free from serious adverse effects, mild flushing being the only side effect encountered. There are no known contraindications to its use.[17,19]

The aim of this study was to establish reference values for the GH peak in the ITT and in the GHRH/GHRP-6 stimulation test. Reference values for IGF-I have previously been published by our laboratory in this journal.[20] However, at that time IGF-I was measured by a chemiluminescent immunoassay from Nichols (Nichols Advantage). Because the manufacturer stopped the production of this kit, we now report reference values for IGF-I on an Immulite 2000 system. This assay is used in the majority of the Dutch hospitals.

SUBJECTS AND METHODS

Subjects

Volunteers were recruited by advertisement in a local newspaper with a free house-to-house distribution in the Amsterdam region and by advertisements in 'Status', the biweekly information bulletin of the University of Amsterdam. We placed an advertisement for subjects who allowed blood samples to be taken to establish reference values for IGF-I and an advertisement for subjects who were willing to undergo a GHRH/GHRP-6 test and an ITT. The subjects were screened for the inclusion and exclusion criteria by telephone and during the intake visit.
The inclusion criteria were age 20 to 70 years and self-reported good health. Exclusion criteria were a medical history of cardiac disease (angina pectoris or cardiac arrhythmias), cerebrovascular disease or neurosurgery, known diabetes mellitus, epilepsy, use of pharmacological doses of glucocorticoids, intravenous drug abuse and pregnancy. Pregnancy was excluded in all female subjects by a urine human chorionic gonadotropin (hCG) test.
IGF-I was measured in 296 subjects (148 women and 148 men, mean age 44.2 years (SD 14.3, range 20 to 70), mean BMI (measured in 246 persons) 25 (SD 4.7, range 18 to 44)).
From them, 49 subjects (25 women and 24 men, mean age 44.6 years (SD 14.6, range 20 to 68), mean BMI 25 (SD 4.6, range 18 to 43)) underwent an ITT and a GHRH/GHRP-6 test. In 36 subjects the ITT preceded the GHRH/GHRP-6 test, the others started with the GHRH/GHRP-6 test. At the intake visit, subjects were interviewed about general health and use of drugs and medication. Two females used oral contraceptives. Information was given about the study and the tests. Weight and height were measured. Informed consent was obtained from all subjects and the study was approved by the hospital's ethics committee.
The dynamic tests were performed at 8.30 am, after an overnight fast. Subjects were in a recumbent position. For all tests an indwelling venous catheter was inserted (t = -30 min) in an antecubital vein. IGF-I was measured in a basal sample.

Insulin tolerance test

Blood samples were taken at t = -15 min and t = 0 min for measuring GH. After the blood sample at t = 0 min, 0.15 U/kg Insulin (Actrapid Novo Nordisk, Mainz, Germany) was intravenously administered. Additional blood samples for measuring GH were taken at t = 15, 30, 45, 60 and 75 min. To reduce the inter-laboratory variation, GH assays have been harmonised in the Netherlands. A harmonisation sample from native serum with an assigned consensus value is used for this purpose.
Criteria for a valid test result were neuroglycopenic symptoms lasting for at least ten minutes and a blood glucose concentration of <2.2 mmol/l.

GHRH/GHRP-6 test

After three basal samples at t = -30, -15 and 0 min, the subjects underwent combined administration of GHRH

plus GHRP-6 as an intravenous bolus injection of 1 µg per kg bodyweight of GHRH (Ferring GmbH, Kiel, Duitsland), immediately followed by an intravenous bolus injection of 1 µg per kg bodyweight of GHRP-6 (Clinalfa Läufelfingen, Switzerland). Further blood samples were obtained at t = 15, 30, 45, 60, 90 and 120 min.

Analytical methods

GH was determined by time-resolved fluoroimmunoassay (Delfia, PerkinElmer, Turku, Finland) with a detection limit of 0.1 mU/l, an intra-assay coefficient of variation of 6.4% (3.4 mU/l) and 1.8% (20.1 mU/l), and an inter-assay coefficient of variation of 10.9% (3.0 mU/l) and 7.7% (21.7 nmol/l). Conversion factor GH: 1 µg/l = 3.67 mU/l.

IGF- I was measured on an Immulite 2000 system, (DPC, Los Angeles, USA) with a detection limit of 5 nmol/l, an intra-assay coefficient of variation of 2.5% (9.9 nmol/l) and 2.0% (89 nmol/l), and an inter-assay coefficient of variation of 5.2% (10.6 nmol/l) and 4.1% (55.8 nmol/l).

Statistical methods

The effects of gender, age and BMI on the IGF-I concentration were evaluated with linear regression analysis.

The peak serum GH response was used as the primary variable for analysis of the stimulation tests. The relation between the extent of the hypoglycaemia and the GH peak after insulin was tested with Pearson's correlation coefficient. The relations between age, BMI and gender and the GH peak in the insulin tolerance test and the GHRH/GHRP-6 were tested with linear regression analyses.

Square root transformations of the IGF-I concentration and the growth hormone peak (GH peak) concentrations after insulin injection and after GHRH/GHRP-6 were done to obtain normal distributions. The continuous variables age and BMI were included as linear terms in the model.

The correlations between IGF-1 and GH peak in the ITT and the GHRH/GHRP-6 test were tested with Pearson's correlation coefficient and the difference between the GH peaks in the ITT and the GHRH/GHRP-6 test with a paired *t*-test.

We modelled the IGF-I and GH concentrations as a function of age in a flexible way by means of restricted cubic spline functions. Spline functions are used to represent smoothly varying relationships between a predictor (age) and the response (IGF or GH peak), which can take on virtually any shape.[21] Reference values were based on the 95% prediction intervals as obtained from these models.

The SPSS 12.0.2 and R 2.7.0 (R Foundation for Statistical Computing, Vienna, Austria) statistical packages were used. In all analyses, p values <0.05 were considered statistically significant.

RESULTS

Basal plasma levels of IGF-I

The mean IGF-I concentration was 19.1 nmol/l (range 5.0 to 54.0 nmol/l). In a multivariate regression analysis, there was a significant negative influence of age on the IGF-I concentration (β = -0.030 change per year, 95% CI -0.036 to -0.024, p<0.001). The effect of BMI was borderline significant (β = -0.017, 95 % CI -0.035 to 0.000, p=0.05) and there was no effect of gender on the IGF-I concentration (β = 0.016, 95% CI -0.137 to 0.168, p=0.8). The reference values by age are given in *table 1* and *figure 1*.

Insulin tolerance test

No serious side effects were observed with the ITT. During this test all subjects had signs of neurohypoglycaemia; however, two subjects did not have a biochemical hypoglycaemia below 2.2 mmol/l (one 2.3 mmol/l and one 2.4 mmol/l). Nevertheless, they were included in the statistical analysis because their neurohypoglycaemia was evident. The depth of the hypoglycaemia varied from 0.5 to 2.4 mmol/l (mean 1.3, SD 0.4). Hypoglycaemia induced a GH release in all subjects. Most participants reached a GH peak 60 (35%) or 75 (43%) minutes after administration of insulin; 22% already reached the GH peak after 30 or 45 minutes. The GH peak height was not related to the nadir of the hypoglycaemia (r = -0.085, p=0.56).

In a multivariate regression analysis age had a significant negative effect on the GH peak (β = -0.053 change per year, 95% CI -0.099 to -0.008, p=0.02). There was also an effect of gender (β = 1.180, 95% CI 0.070 to 2.289, p=0.04), the GH peak was higher in men (mean 62.8 mE/l, SD 5.0) than in women (mean 46.4 mE/l, SD 4.3). After correction for age and gender, we did not find a significant effect of BMI (β = -0.091, 95% CI -0.236 to 0.053, p=0.2). The reference values are given in *table 1* and *figure 2*.

GHRH/GHRP-6 test

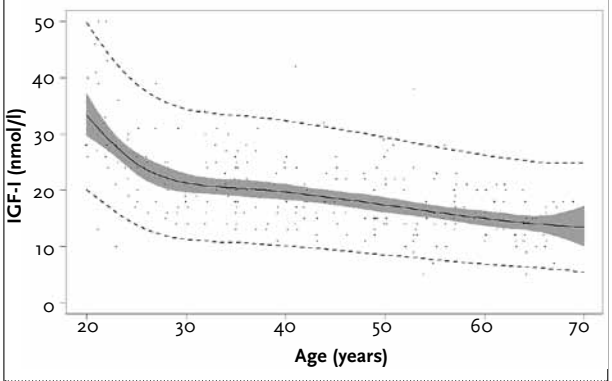
Most participants reached a GH peak after 15 (49%) or 30 (45%) minutes, three (6%) had a GH peak after 45 minutes. In a multivariate regression analysis, there was a significant negative effect of BMI on the GH peak (β = -0.529, 95% CI -0.730 to -0.329, p<0.001). The effects of age and of gender were not significant (β = -0.035, 95% CI -0.099 to 0.029, p=0.28 and β = -1.091, 95% CI -2.696 to 0.513, p=0.18 respectively). The mean GH peak was 135.6 mE/l, SD 15.0). The reference values by age are given in *table 1* and *figure 3*.

The IGF-I concentration was not correlated with the GH peak in the insulin tolerance test (r = 0.15, p=0.32). However, the correlation with the GH peak in the GHRH/GHRP-6 test just reached significance (r = 0.28, p=0.048).

Table 1. *Reference values for plasma IGF-I concentrations (nmol/l) and for GH peak concentrations (mE/l) in ITT and GHRH/GHRP-6 test*

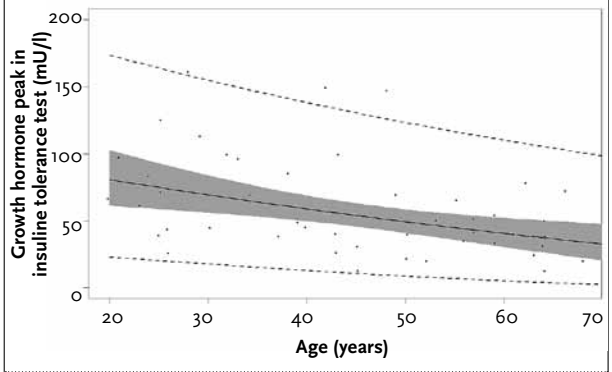
Age (years)	IGF-I P 2.5	IGF-I P 50	ITT P 2.5	ITT P 50	GHRH/ GHRP-6 P 2.5	GHRH/ GHRP-6 P 50	Age (years)	IGF-I P 2.5	IGF-I P 50	ITT P 2.5	ITT P 50	GHRH/ GHRP-6 P 2.5	GHRH/ GHRP-6 P 50
20	20.1	33.3	22.9	80.7	54.9	215.4	46	9.2	18.4	10.3	53.1	19.4	131.5
21	18.7	31.3	22.4	79.5	53.3	211.8	47	9.0	18.1	9.9	52.1	18.3	128.7
22	17.3	29.4	21.9	78.4	51.8	208.2	48	8.8	17.9	9.5	51.2	17.3	125.9
23	16.0	27.6	21.3	77.2	50.2	204.6	49	8.7	17.6	9.1	50.3	16.2	123.2
24	14.8	26.1	20.8	76.1	48.7	201.1	50	8.5	17.4	8.7	49.3	15.2	120.4
25	13.8	24.7	20.3	74.9	47.2	197.6	51	8.3	17.1	8.3	48.4	14.3	117.8
26	13.0	23.7	19.8	73.8	45.7	194.2	52	8.1	16.9	7.9	47.5	13.3	115.1
27	12.4	22.8	19.3	72.7	44.2	190.8	53	8.0	16.6	7.5	46.6	12.4	112.5
28	11.9	22.1	18.7	71.6	42.7	187.4	54	7.8	16.4	7.1	45.7	11.5	109.9
29	11.5	21.6	18.2	70.5	41.2	184.0	55	7.6	16.1	6.8	44.9	10.6	107.3
30	11.3	21.3	17.7	69.4	39.8	180.7	56	7.5	15.9	6.4	44.0	9.8	104.7
31	11.1	21.0	17.2	68.3	38.4	177.4	57	7.3	15.6	6.1	43.1	9.0	102.2
32	10.9	20.8	16.7	67.2	37.0	174.1	58	7.1	15.4	5.7	42.3	8.2	99.8
33	10.8	20.6	16.2	66.2	35.6	170.9	59	7.0	15.2	5.4	41.4	7.5	97.3
34	10.7	20.5	15.8	65.1	34.2	167.7	60	6.8	15.0	5.1	40.6	6.8	94.9
35	10.7	20.4	15.3	64.1	32.9	164.5	61	6.7	14.8	4.7	39.8	6.1	92.5
36	10.6	20.3	14.8	63.0	31.5	161.3	62	6.6	14.6	4.4	39.0	5.4	90.1
37	10.5	20.2	14.3	62.0	30.2	158.2	63	6.4	14.4	4.1	38.1	4.8	87.8
38	10.3	20.0	13.9	61.0	28.9	155.1	64	6.3	14.2	3.8	37.3	4.2	85.5
39	10.2	19.8	13.4	60.0	27.7	152.1	65	6.2	14.0	3.6	36.5	3.7	83.3
40	10.1	19.6	12.9	58.9	26.4	149.0	66	6.1	13.9	3.3	35.8	3.2	81.0
41	10.0	19.5	12.5	57.9	25.2	146.0	67	6.0	13.8	3.0	35.0	2.7	78.8
42	9.8	19.3	12.0	57.0	24.0	143.1	68	5.8	13.6	2.8	34.2	2.3	76.6
43	9.7	19.0	11.6	56.0	22.8	140.1	69	5.6	13.5	2.5	33.5	1.9	74.5
44	9.5	18.8	11.1	55.0	21.7	137.2	70	5.4	13.4	2.3	32.7	1.5	72.4
45	9.4	18.6	10.7	54.0	20.5	134.4							

Figure 1. *IGF-I concentrations (nmol/l)*



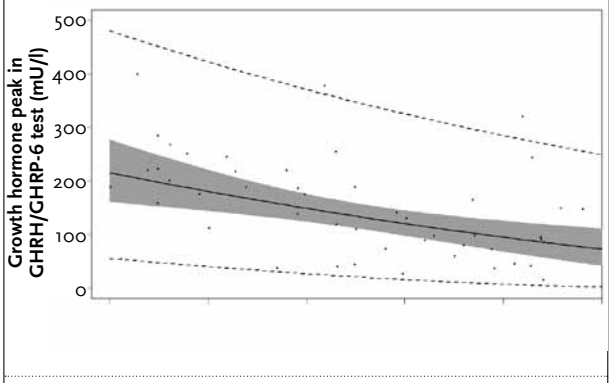
Black line: median. Shadow: 95% CI of mean. Dashed lines: 2.5 and 97.5 percentile.

Figure 2. *GH concentrations (mU/l) in the ITT*



Black line: median. Shadow: 95% CI of mean. Dashed lines: 2.5 and 97.5 percentile.

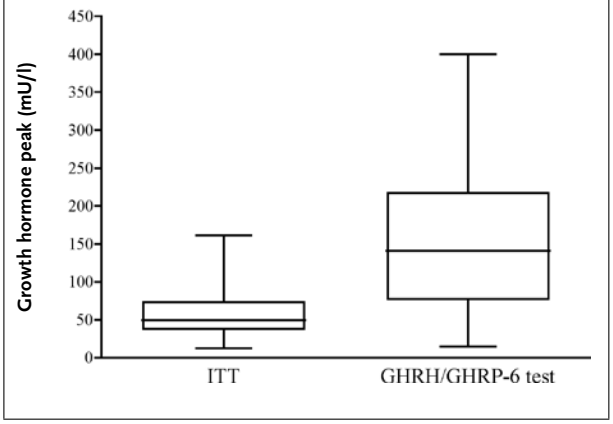
Figure 3. *GH concentrations (mU/l) in the GHRH/GHRP-6 test*



Black line: median. Shadow: 95% CI of mean. Dashed lines: 2.5 and 97.5 percentile.

There was a significant correlation between the growth hormone peak in both dynamic tests (r = 0.43, p<0.01), although the growth hormone peak in the GHRH/GHRP-6 test was significantly higher (mean 135.6 mE/l, SD 15.0) than in the ITT (mean 54.4 mE/l, SD 4.8, p<0.001) (*figure 4*). The number of days between both tests (median 12, range 1 to 112 days) did not effect the growth hormone peak.

Figure 4. *Box-and-whisker plot of the GH concentrations (mU/l) in the ITT and the GHRH/GHRP-6 test, showing the lowest value, 25th, 50th, 75th percentile and the highest value*



DISCUSSION

The present study provides age-adjusted reference values for plasma concentrations of IGF-I and for the GH response in the ITT and GHRH/GHRP-6 test. The recruited subjects were drawn from the general population.

We found no gender differences in IGF-I concentrations and GH response in the GHRH/GHRP-6 test. This is in line with the majority of other studies, although some found a sex difference in IGF-I concentration.[17,22-24] In our study there was an effect of gender on the GH response in the ITT, with a higher GH peak in men. This effect is small and of borderline significance. In the literature there is controversy about the effect of gender in the ITT. Some studies did not find a difference,[13,15] whereas others found a higher peak GH response in males.[25] If present at all, the differences will be negligible. Therefore, we did not establish gender-specific reference values.

The IGF-I concentration is inversely related to age in our study, which is in agreement with other studies.[22-24,26] The negative effect of BMI on the IGF-I concentration just reached significance in our study. In the literature, some other studies found an effect of BMI, although the majority did not.[24,26,27]

Age also had a negative effect on GH response in the ITT, the effect of BMI was not significant. Other studies showed a negative effect of age and BMI on the GH response.[27-29] Interesting is the effect of both of these predictors independently of each other. Biller *et al.* tested GH response in ITT in healthy subjects and found a significant inverse relationship between BMI and peak GH when controlling for age. In their study age had no significant effect. However, they only tested subjects under 55 years of age.[13] Qu *et al.* also found a negative correlation between BMI and GH response in 27 healthy subjects (age 20 to 49 years, mean BMI 24.7, range 16.0 to 32.5 kg/m^2), but they did not correct for age.[15]

We found no correlation between the degree of hypoglycaemia and the peak GH response, similar to findings reported earlier by Hoeck *et al.*[25]

In the GHRH/GHRP-6 test the GH peak occurred in all subjects within 45 minutes and in most of them already after 15 or 30 minutes. This is in line with the findings of other authors.[17,19]

The peak GH response in the GHRH/GHRP-6 test was negatively associated with BMI in a multivariate analysis. A negative effect of obesity was also found by Popovic *et al.*;[17,30] they did not find a correlation with age either.[17] Micic *et al.* studied adult, aged and very old subjects (19 to 96 years) and found no difference in GH response after GHRH/GHRP-6.[31] This is also in line with the findings of Haijma *et al.*, who did GHRH/GHRP-6 tests in elderly (mean age 74, SD 1.4 years), obese (BMI 40.6, SD 1.7 kg/m^2) and controls (age 51 ± 2.3, BMI 24.3 ± 1.0). They found a significant negative correlation between BMI and GH response, whereas age and GH response were not significantly correlated.[18]

Kelestimur *et al.* studied the GH peak after GHRH/GHRP-6 in subjects with different degrees of BMI (between BMI <20 and >40 kg/m^2). There was a significant

negative effect of adiposity. In this study, there was no correction for age.[32] Several studies showed that although the GH response is lower in obese patients, the GHRH/GHRP-6 test is able to distinguish the decreased GH secretion of obesity from GHD, at least in patients with a BMI lower than 35. In patients with a BMI exceeding 35, the cut-off values should probably be adjusted.[14,18,30,32]

In our study, the peak GH response in the GHRH/GHRP-6 test and in the ITT were correlated, although the peak was significantly higher in the GHRH/GHRP-6 test. This is in agreement with other studies.[17,19] A possible explanation therefore can be that GHRP-6 acts via a receptor (ghrelin receptor) that is different from the GHRH receptor. GHRH and GHRP-6 have a synergistic action on GH release.

We found no correlation between serum IGF-I and the GH peak in the ITT (p=0.32), but a borderline significant relation between IGF-I and the GHRH/GHRP-6 test (p=0.048). Petersenn *et al*. found a significant correlation between IGF-I and both dynamic tests[19] and Popovic *et al*. found a tendency to a significant correlation between IGF-I values and GH peak in the GHRH/GHRP-6 test (p=0.051)[17]

CONCLUSION

Serum IGF-I is used as an indicator of growth hormone status, although it is well known that it is influenced by many other factors and that a normal IGF-I cannot exclude growth hormone deficiency. For that diagnosis, a provocative test of GH secretion is necessary. The ITT is considered the gold standard, but has several limitations. A good alternative is the GHRH/GHRP-6 test. In our study we have established reference values for these tests.

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ORIGINAL ARTICLE

No modification of the beneficial effect of NSAIDs on colorectal cancer by *CYP2C9* genotype

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ABSTRACT

Background: CYP2C9 enzymes are involved in non-steroidal anti-inflammatory drug (NSAID) metabolism. Therefore, we investigated whether *CYP2C92 and *3 variant alleles, encoding for enzymes with lower activity, increased the protective effect of NSAIDs on colorectal cancer.**

Methods: Individual and combined associations of NSAIDs and *CYP2C92 and *3 variant alleles with colorectal cancer were studied in 7757 Caucasian individuals of The Rotterdam Study, a population-based prospective cohort since 1990. Additive and multiplicative effect modification models were used to examine drug-gene interactions.**

Results: There were 212 incident cases of colorectal cancer during follow-up. A reduced risk of colorectal cancer was observed in individuals who used NSAIDs for more than a year (HR 0.45; 95% CI 0.28 to 0.71), and in carriers of an *CYP2C9* variant allele associated with lower enzymatic activity (HR 0.67; 95% CI 0.47 to 0.96). The combination of both determinants was associated with a further risk reduction but without synergy.

Conclusion: Both NSAID use and *CYP2C92 and/or *3 carriage are associated with a reduced risk of colorectal cancer. However, no interaction between the determinants was found, which might indicate independent pathophysiological mechanisms.**

KEYWORDS

Anti-inflammatory agents, cohort studies, colorectal neoplasms, cytochrome p-450 enzyme system, interaction, non-steroid, polymorphism, single nucleotide

INTRODUCTION

Colorectal cancer is the second leading cause of cancer-related death in the Western world[1] and is considered to be the final stage of the sequence from adenoma to carcinoma by accumulation of genetic mutations in epithelial cells.[2] This may result from exposure to carcinogens and mutagens, which can be activated by xenobiotic-metabolising enzymes.[3] Furthermore, tumour development is dependent on vascularisation, cell proliferation and apoptosis.

Cytochrome P450 (CYP) enzymes, which metabolise most endogenous and exogenous substrates, are mainly expressed in the human liver, but also in normal intestinal epithelium and in colon adenocarcinoma.[4-7] The CYP2C subfamily accounts for 20% of all CYP in the liver, CYP2C9 being the main isoform.[8] The CYP2C9 isoform is capable of activating specific carcinogens and is related to the formation of DNA adducts.[9] Besides their role in (de)toxification, there is a physiological role for CYP2C9 in the metabolism of arachidonic acid, forming epoxyeicosatrienoic acids through epoxygenase.[10,11] An increase in arachidonic acid metabolism, resulting in decreased arachidonic acid levels, induces neo-vascularisation and cell growth and inhibits apoptosis,[12-15] conditions that might lead to tumour development. Additionally, CYP2C9 metabolises non-steroidal anti-inflammatory drugs (NSAIDs), which have been associated with a decreased risk of colorectal cancer (mortality).[16,17] In individuals of Caucasian descent, two allelic variants (*2 and *3) of the *CYP2C9* gene are relatively common. These *2 and *3 variants have been shown to result in a lower enzyme activity for several substrates, both *in vitro* and *in vivo*, compared with the activity of the wild-type allele.[5,18-20]

The objective of this cohort study was to investigate whether *CYP2C9*2* and *3 variant alleles are associated with an increase of the protective effect of NSAIDs on colorectal cancer risk in a Caucasian population.

METHODS

Setting

Data were obtained from The Rotterdam Study, a population-based prospective cohort study among inhabitants of the Rotterdam suburb of Ommoord. Between July 1989 and July 1993, all persons aged 55 years and older were invited (n=10,275). In total 7983 subjects (78%), including people living in one of the homes for the elderly, participated (4878 women and 3105 men). The design, ethical approval and rationale behind this study have been described earlier.[21]

At baseline, a home interview was performed followed by two visits to the research centre for clinical examinations. Blood samples were collected and DNA isolated. Baseline data collection was performed from October 1990 to July 1993. Since then, participants have been re-examined periodically. In addition, participants are continuously monitored for major events, including cancers, which occur during follow-up, through automated linkage with files from general practitioners. Information on medication use is available for all participants since January 1991. The seven computerised pharmacies that cover the research area are linked to one network. In this way, the date of prescription, the total amount of drug units per prescription and the prescribed defined daily dosage (DDD) are available per drug defined by an Anatomical Therapeutic Chemical (ATC) code.[22] Information on vital status is obtained regularly from municipal health authorities in Rotterdam and from general practitioners in the study district.

Cohort definition

Pharmacy data were available for 7857 (98%) subjects. Persons with a diagnosed colorectal cancer before 1 January 1991 (n=48) or who died or were lost to follow-up before this date (n=52) were excluded from the analyses. This resulted in a study cohort of 7757 (97%) individuals. Mainly due to a lack of blood samples, genotypes were determined in 6378 persons for whom genetic material was isolated and the assay was successfully performed. Follow-up time was defined as the period between 1 January 1991 and a diagnosis of colorectal cancer, death, or the end of the study period on 1 October 2004 whichever came first.

Exposure definition

The exposure of interest included both non-aspirin and aspirin NSAIDs. The following drugs were used by study subjects: acetylsalicylic acid, carbasalate calcium, diflunisal, sulindac, nabumetone, naproxen, ibuprofen, diclofenac, diclofenac/misoprostol, tolmetin, indomethacin, piroxicam, ketoprofen, dexketoprofen, flurbiprofen, azapropazone, meloxicam, celecoxib, etoricoxib, and rofecoxib. As previous studies showed that duration of use seemed more important than dose in reducing colorectal cancer risk, only cumulative time of use was considered in this study. Time-dependent exposure variables were defined by reference to the date of diagnosis of a colorectal cancer (index date) and to calculate cumulative duration for each case and the remainder of participants in the cohort until that date. Given this approach, subjects were eligible as controls as long as they were not a case or censored. Consequently, participants were used in several case-sets. Cases were censored at the date of diagnosis. The methodology of time-varying exposure has been described by Clayton and Hill.[23]

Genotyping

DNA was extracted using standard procedures and stored at -20°C until used for DNA amplification. *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) were determined using 2-ng genomic DNA with the Taqman Prism 7900HT 384 wells format allelic discrimination assay (Applied Biosystems, Foster City, California). Primer and probe sequences were optimised by using the SNP assay-by-design service of Applied Biosystems (for details, see http://store.appliedbiosystems.com). These allelic variants occur at appreciable frequency in the Caucasian population. The ALlele FREquency Database (ALFRED) reports allele frequencies of 13% for *CYP2C9*2* and 7% for *CYP2C9*3*.[24] The variants are the result of amino acid substitutions at position 144 (*CYP2C9*2*; $Arg_{(CGC)} \rightarrow Cys_{(TGC)}$) and position 359 (*CYP2C9*3*; $Ile_{(ATT)} \rightarrow Leu_{(CTT)}$). Since other variants are extremely rare among Caucasians, persons without *2 or *3 were considered as having the wild-type genotype (*1).

Case identification and validation

Three different databases were used for case identification. First, cases diagnosed by general practitioners in the research area were collected (International Classification of Primary Care (D75)). Second, the national registry of all hospital admissions was consulted to detect all malignancy-related hospital admissions for study participants. Third, regional pathology databases were linked to The Rotterdam Study to identify cases. Subsequently, colorectal cancer cases were validated by a physician (CS) on the basis of the general practitioners' medical records discharge letters, and pathology reports. The tenth edition of the International Classification of Diseases (ICD-10) was used to distinguish between the anatomical locations non-sigmoid colon (C18), sigmoid colon (C19) and rectal (C20) cancer. Because of their

low incidence and proposed different pathophysiology, anal cancers (n=3) were not included as cases. They were censored in the analyses at their date of diagnosis. Only pathologically confirmed cases were considered in the analyses. The index date was defined as the earliest date found in the pathology reports. Participants were not involved in any gastrointestinal screening programme.

Co-variables

On the basis of medical literature the following co-variables, assessed at baseline, were considered as potential confounders: age, gender, body mass index (BMI) (kg/m^2), total energy intake (kcal/day), alcohol (grams/day), vegetable (grams/day), fruit (grams/day), meat (grams/day), fibre (grams/day), and selenium intake (grams/day), hypercholesterolaemia (total cholesterol >6.5 mmol/l), physical activity (without difficulty, with some difficulty, with much difficulty, unable to do) and smoking (total pack-years). The method and validation of dietary assessment in The Rotterdam Study has been described elsewhere.[25]

Statistical analyses

Genotype proportions and allele frequencies were tested for deviations from the Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. Cox proportional hazard models were used to study associations between NSAID use or *CYP2C9* variant alleles and colorectal cancer risk. A first model adjusted for age and gender. A second model was made with those co-variables that changed the point estimate by more than 10% or which were independent risk factors for the outcome, according to the literature.

NSAID use was studied both as a dichotomised variable (never / ever use) and in categories of cumulative duration (never use / 1-365 days use / >365 days use). The cut-off point of 365 days was chosen according to some previous studies that report a protective effect of NSAIDs after one year of cumulative use. The analyses were performed for total NSAID use and non-aspirin NSAIDs and aspirin NSAIDs separately. We defined five categories of medication exposure: never use of NSAIDs, 1 to 365 days of non-aspirin NSAID, >365 days of non-aspirin NSAID, 1 to 365 days of aspirin use and >365 days of aspirin use in which the 'never use' category served as a reference while the other categories could partly overlap when an individual used both aspirin- and non-aspirin NSAIDs during the study period. Trend analyses were performed to quantify a duration-effect response.

The association between genotype and colorectal cancer was studied in the total cohort, and in a subgroup of non-NSAID users to investigate the drug-independent effect of *CYP2C9* variant alleles. Carriers were defined as having at least one variant allele. The homozygous wild-type genotype (**1/*1*) served as the reference category. In addition to the association with the total group of colorectal cancers, the effect on anatomical subtypes (non-sigmoid colon, sigmoid colon, rectum) was investigated.

The combined effect of NSAIDs and *CYP2C9* genotype was studied by using the following groups: non-carriers without NSAID use (reference), variant carriers without NSAID use, non-carriers with NSAID use, variant carriers with NSAID use. Analyses were performed for the total colorectal cancer group as well as for anatomical subtypes. Drug-gene interactions were studied for the separate exposure subgroups (non-aspirin and aspirin NSAIDs) as well. When the interaction with non-aspirin NSAIDs was studied, the reference group was composed of only those without non-aspirin use and the analyses were adjusted for aspirin use. In a similar way, interaction of aspirin NSAIDs and genotype was studied. Trend analyses were performed on all these groups. The sequence for which the trends hold is based on the results of the separate analyses of NSAID use and *CYP2C9* variant allele carriage on colorectal cancer risk. Effect modification was studied with both additive (biological) and multiplicative interaction models. The relative excess risk reduction due to interaction (RERI) was used to evaluate departures from an additive scale. SAS software (Statistical Analysis Software version 8.2, Cary, NC) was used to derive regression coefficients (3) and covariance matrices (9). The numbers obtained were used to calculate RERIs ($RR_{combination} - RR_{exposure\ A} - RR_{exposure\ B} + 1$) and their corresponding 95% confidence limits.[26,27] If there is no biological interaction RERI is equal to 0. Interaction terms were added to the model to identify multiplicative effect modification. All analyses were performed with SPSS software (version 11.0.1; SPSS Inc., Chicago, USA). P values below the conventional level of significance ($p<0.05$) were considered statistically significant.

RESULTS

Individuals of whom the genotype was unknown (n=1379) were on average older, relatively more frequently female and smoker, and had a shorter follow-up time than those for whom genotype data were available. Baseline characteristics of the study group are presented in *table 1*. During a mean follow-up time of 9.8 years, 212 colorectal cancers (3 anal cancers not included) occurred. This was 3% of our cohort and corresponds with the incidence of colorectal cancer in the general Dutch population in persons aged ≥55 years.[28] The mean age was 68 years and 38% were males. Ninety-six percent of the population used an NSAID at any time during the study period. Non-aspirin NSAIDs were taken by 60% and aspirin NSAIDs by 30% of the population while 21% used both types during the study

Table 1. *Baseline characteristics of the total study population*

Total participants (genotyped)	7757 (6378)
Colorectal cancer cases (% of total participants) (genotyped)	212 (3%) (184)
Age, mean (SD)	68.1 (8.47)
Male gender, N (%)	2963 (38.2%)
Body mass index, mean (SD), N (%):	26.4 (3.7)
• Underweight (<18.5)	54 (0.7%)
• Normal weight (18.5-24.9)	2645 (34.1%)
• Overweight (25.0-29.9)	3367 (43.4%)
• Obesity (30-39.9)	1047 (13.5%)
• Extreme obesity (≥40)	23 (0.3%)
Smoking status, total pack years (SD), N (%):	26.7 (23.1)
• Never	2723 (35.1%)
• Former	3149 (40.6%)
• Current	1699 (21.9%)
Physical activity, N (%):	
• Without difficulty	4608 (59.4%)
• With some difficulty	1598 (20.6%)
• With much difficulty	496 (6.4%)
Unable to do	931 (12.0%)
Hypercholesterolaemia (>6.5 mmol/l), N (%)	3731 (48.1%)
Total energy intake (kcal/day), mean (SD)	1967 (501)
Vegetable intake (grams/day), mean (SD)	350 (137)
Fruit intake (grams/day), mean (SD)	230 (132)
Meat intake (grams/day), mean (SD)	108 (47)
Fibre intake (grams/day), mean (SD)	17 (5)
Selenium intake (grams/day), mean (SD)	33 (10)
Fat intake (grams/day), mean (SD)	40 (19)
Alcohol consumption (grams/day), mean (SD)	10 (15)
Genotypes, N (%):*	
• CYP2C9 *1/*1	4229 (66.3%)
• CYP2C9 *1/*2	1339 (21.0%)
• CYP2C9 *1/*3	593 (9.3%)
• CYP2C9 *2/*2	102 (1.6%)
• CYP2C9 *2/*3	89 (1.4%)
• CYP2C9 *3/*3	26 (0.4%)

***Hardy Weinberg χ^2 = 1.55 (p=0.34). Percentages do not sum up to 100% due to missing values. N = number; SD = standard deviation; CYP2C9 = cytochrome P450 2C9.**

period. Mean duration of non-aspirin and aspirin NSAIDs use was 90 and 280 days, respectively. Genotype data were in Hardy Weinberg equilibrium (χ^2 = 1.55; p=0.35). 33.7% of the study population carried at least one variant allele. Allele based frequencies of *CYP2C9*2* and *CYP2C9*3* were 12.8 and 5.8%, respectively.

Ever use of NSAIDs was associated with a 37% risk reduction of colorectal cancer (HR 0.63; 95% CI 0.47 to 0.85). Duration of use was inversely related to colorectal cancer incidence (p=0.001) (*table 2*). Both aspirin and non-aspirin NSAIDs were associated with a significant risk reduction for colorectal cancer, especially after more than one year of cumulative use. Total energy intake was the only potential confounder that changed the point estimate of NSAID use on the age and gender-adjusted colorectal cancer risk by more than 10%. The specified dietary factors did not change the risk when adjusted for the total intake. Other potential confounders were put into the model because they were considered as potential risk factors in the medical literature. Dietary data were not available for 23.1% of the population. Missing status of these and other factors had no effect on the association between NSAID use and colorectal cancer risk (p=0.11 to 0.51). Therefore, complete case analyses with a second model that consisted of age, gender, total energy intake, physical activity, body mass index, hypercholesterolaemia and the specified exposure were performed.

Carriage of a *CYP2C9* variant allele was also associated with a risk reduction (60%), primarily in the proximal parts of the colorectal tract (*table 3*). This risk reduction subsists for colon carcinoma in the analyses among non-NSAID users, although it is no longer significant. The number of cases was too small to investigate the individual effects of *CYP2C9*2* and **3* on cancer risk. Overall, the

Table 2. *Association between cumulative NSAID use and colorectal cancer*

Cumulative days of NSAID use	N	Model 1 HR (95% CI)	N	Model 2§ HR (95% CI)
Any NSAID use†#				
No use*	82	1.00 (reference)	63	1.00 (reference)
1-365 days use	90	0.72 (0.53-0.98)	69	0.65 (0.45-0.92)
>365 days use	40	0.48 (0.32-0.72)	32	0.45 (0.28-0.71)
Non-aspirin NSAID use‡				
No use*	82	1.00 (reference)	63	1.00 (reference)
1-365 days use	73	0.77 (0.55-1.06)	59	0.72 (0.50-1.05)
>365 days use	4	0.33 (0.12-0.91)	3	0.29 (0.09-0.95)
Aspirin NSAID use¥				
No use*	82	1.00 (reference)	63	1.00 (reference)
1-365 days use	17	0.48 (0.28-0.82)	10	0.34 (0.17-0.67)
>365 days use	36	0.55 (0.36-0.83)	29	0.51 (0.32-0.81)

***No use is defined as no use of non-aspirin or aspirin NSAIDs during the study period. §Complete case analyses. †Model 1: adjusted for age and gender, model 2: adjusted for age, gender, smoking, energy intake, physical activity, body mass index and hypercholesterolaemia. ‡Model 1: adjusted for age, gender and aspirin use, model 2: adjusted for age, gender, smoking, energy intake, physical activity, body mass index, hypercholesterolaemia and aspirin use. ¥Model 1: adjusted for age, gender and non-aspirin use, model 2: adjusted for age, gender, smoking, energy intake, physical activity, body mass index, hypercholesterolaemia and non-aspirin use. #Trend significant at 0.001 level.**
NSAID = non-steroidal anti-inflammatory drug; N = number; HR = hazard ratio; CI = confidence interval.

Table 3. *Association between CYP2C9 genotype and colorectal cancer*

	All participants				Non-NSAID users			
	N	Model 1 HR (95% CI)	N	Model 2 HR (95% CI)	N	Model 1 HR (95% CI)	N	Model 2 HR (95% CI)
Colorectal cancer	184		155		71		60	
CYP2C9	132	1.00 (reference)	115	1.00 (reference)	50	1.00 (reference)	44	1.00 (reference)
CYP2C9 variant	52	0.79 (0.57-1.09)	40	0.67 (0.47-0.96)	21	0.94(0.56-1.56)	16	0.78 (0.44-1.38)
Non-sigmoid colon	78		66		34	1.00 (reference)	30	
CYP2C9	61	1.00 (reference)	55	1.00 (reference)	27	0.59 (0.26-1.35)	25	1.00 (reference)
CYP2C9 variant	17	0.56 (0.33-0.96)	11	0.38 (0.20-0.72)	7		5	0.43 (0.16-1.13)
Sigmoid colon	65		52		21		16	
CYP2C9	46	1.00 (reference)	37	1.00 (reference)	14	1.00 (reference)	11	1.00 (reference)
CYP2C9 variant	19	0.82 (0.48-1.40)	15	0.79 (0.44-1.45)	7	1.09 (0.44-2.69)	5	1.00 (0.35-2.88)
Rectum	41		37		16		14	
CYP2C9	25	1.00 (reference)	23	1.00 (reference)	9	1.00 (reference)	8	1.00 (reference)
CYP2C9 variant	16	1.28 (0.69-2.41)	14	1.21 (0.62-2.36)	7	1.73 (0.64-4.66)	6	1.57 (0.53-4.62)

Model 1: Adjusted for age and gender. Model 2: Adjusted for age, gender, smoking, energy intake, physical activity, body mass index and hypercholesterolaemia. CYP2C9 = cytochrome P450 2C9; N = number; HR = hazard ratio; CI = confidence interval.

reduced risk of colorectal cancer associated with NSAID use seemed to be stronger than that associated with variant allele carriage.

Combinations of both variant allele carriage and NSAID use resulted in more protection than either of the factors alone (*table 4*). This effect was primarily seen in proximal parts with a significant trend for non-sigmoid colon cancer. However, significant effect modification on an additive or multiplicative scale did not occur (p>0.05). The results were similar for aspirin and non-aspirin NSAIDs.

DISCUSSION

This prospective population-based cohort study demonstrates associations between NSAID use, *CYP2C9**2 and *3 variant allele carriage and colorectal cancer incidence. Duration of NSAID use was inversely related to the incidence of colorectal cancer. Since both non-aspirin and aspirin NSAIDs have been associated with a decreased risk of colorectal cancer in former studies, we combined both types of NSAIDs and additionally

Table 4. *Combined effect of NSAID use and CYP2C9 genotype on colorectal cancer risk*

	Total NSAID use*		Non-aspirin NSAID use†		Aspirin NSAID use‡	
	N	HR (95%CI)	N	HR (95%CI)	N	HR (95%CI)
Colorectal cancer						
CYP2C9 wild-type, no use	50	1.00 (reference)	57	1.00 (reference)	100	1.00 (reference)
CYP2C9 variant, no use	21	0.92 (0.56-1.54)	25	0.94 (0.59-1.51)	39	0.79 (0.55-1.15)
CYP2C9 wild-type, use	82	0.64 (0.45-0.93)	75	0.87 (0.61-1.24)	32	0.62 (0.41-0.93)
CYP2C9 variant, use	31	0.47 (0.30-0.74)	27	0.60 (0.38-0.96)	13	0.50 (0.28-0.89)
Trend§	184	p=0.001	184	p=0.06	184	p=0.003
Non-sigmoid colon						
CYP2C9 wild-type, no use	27	1.00 (reference)	28	1.00 (reference)	51	1.00 (reference)
CYP2C9 variant, no use	7	0.57 (0.25-1.31)	10	0.77 (0.37-1.58)	11	0.44 (0.23-0.85)
CYP2C9 wild-type, use	34	0.50 (0.30-0.85)	33	0.81 (0.48-1.37)	10	0.38 (0.19-0.77)
CYP2C9 variant, use	10	0.29 (0.14-0.60)	7	0.33 (0.14-0.77)	6	0.45 (0.19-1.07)
Trend§	78	p<0.001	78	p=0.02	78	p=0.002
Sigmoid colon						
CYP2C9 wild-type, no use	14	1.00 (reference)	18	1.00 (reference)	32	1.00 (reference)
CYP2C9 variant, no use	7	1.10 (0.44-2.62)	8	0.95 (0.42-2.20)	15	0.94 (0.51-1.74)
CYP2C9 wild-type, use	32	0.93 (0.49-1.77)	28	1.02 (0.55-1.87)	14	0.88 (0.46-1.68)
CYP2C9 variant, use	12	0.67 (0.30-1.47)	11	0.76 (0.35-1.64)	4	0.50 (0.17-1.41)
Trend§	65	p=0.32	65	p=0.61	65	p=0.25
Rectum						
CYP2C9 wild-type, no use	9	1.00 (reference)	11	1.00 (reference)	17	1.00 (reference)
CYP2C9 variant, no use	7	1.73 (0.65-4.66)	7	1.38 (0.53-3.56)	13	1.57 (0.76-3.23)
CYP2C9 wild-type, use	16	0.63 (0.27-1.46)	14	0.77 (0.34-1.75)	8	0.85 (0.36-2.01)
CYP2C9 variant, use	9	0.68 (0.27-1.76)	9	0.96 (0.39-2.37)	3	0.63 (0.18-2.17)
Trend§	41	p=0.20	41	p=0.67	41	p=0.50

No use is defined as no use of the NSAID type of interest. For the total NSAID group this means no use of any non-aspirin or aspirin NSAID, for the non-aspirin NSAIDs group this means no non-aspirin NSAID use and for the group of aspirin NSAIDs this means no aspirin NSAID use §Sequence for which the trend holds is based on the results of the separate analyses that NSAID use seems to be more protective than carriage of a variant allele. *Adjusted for age and gender. †Adjusted for age, gender and aspirin use. ‡Adjusted for age, gender and non-aspirin use. NSAID = non-steroidal anti-inflammatory drug; CYP2C9 = cytochrome P450 2C9; N = number; HR = hazard ratio; CI = confidence interval.

performed separate analyses. No obvious differences were observed in the protective effect of aspirin and non-aspirin NSAIDs. Carriage of a *CYP2C9* variant allele was associated with a lower risk of non-sigmoid colon cancers, even in non-NSAID users, although for this last group no significance was reached, probably because of insufficient power. A combination of variant allele carriage and use of NSAIDs resulted in a larger reduction in colorectal cancer risk than one of the determinants independently. This seems to be due to independent pathophysiological mechanisms, since both additive and multiplicative interaction terms were not significantly different from the combined risk reduction. The influence on different regulatory pathways in the arachidonic acid metabolism and the influence CYP2C9 seems to have on the formation of DNA adducts might explain these independent mechanisms.[9]

Regular and long-term use of NSAIDs[17,29-35] and the effect of *CYP2C9* genotype[36-40] on colorectal cancer risk have both been studied before. Currently, there is increasing evidence that regular or long-term use of NSAIDs protects against malignancies in the gastrointestinal tract but final proof from randomised clinical trials is hardly available. In most observational studies and in line with our results, cumulative NSAID use is associated with a reduced cancer risk already after one to two years of cumulative use.[29-32] Other studies and trials, however, report a minimum required period of use of more than ten years.[33,34] Most trials were designed for their effect on adenoma risk reduction. The primary prevention of colorectal cancer, with at least ten-year latency of effect, is consistent with the understanding of the adenoma-carcinoma sequence.[41] The relatively short effect period found in a number of observational studies might be related to other mechanisms involving reduction of active tumour growth, bias by other health factors, stronger associations in smaller studies[41] and potential different effects of dose on COX-1 and COX-2 enzymes.[42] Despite the uniformity in chemopreventive effect of non-aspirin and aspirin NSAIDs, the inconsistency in desired duration of use between trials and observational studies needs further investigation. Furthermore, one most consider the effect of aspirin use on a more rapid manifestation by causing early bleeding from polyps that would therefore be identified in a less advanced stage. Nevertheless, this will most probably be random over genotypes.

More conflicting are the results of studies that investigated the CYP2C9-cancer relation. Two previously published studies were in line with our finding that *CYP2C9*2/*3* gene variant allele carriers had a decreased colon cancer risk.[36,38] Nevertheless, some others found inconsistent results.[37,39,40] The different risk per anatomical subtype might be the result of a different pathophysiological process that depends on a variety of environmental and genetic factors for proximal and distal cancers,[43] which would argue for performing studies by anatomical site.

The objective of our study was to investigate whether *CYP2C9*2* and **3* variant alleles are associated with an increase of the protective effect of NSAIDs on colorectal cancer risk due to a longer effective period of these drugs. The question whether there is synergism between *CYP2C9* variant alleles and NSAID use has been studied before in association with colon adenoma[44,45] and colorectal cancer.[37,46] Besides differences in outcome definition, the exposure definitions and methodology of these studies also vary. One of the previously published studies only presents results on the association with aspirin use,[45] while others report separate results for aspirin and non-aspirin NSAIDs[37,44] or aspirins and ibuprofen.[46] Similar to the first three studies, we did not find effect modification on a multiplicative scale. Additive modification of the drug effects was found in the first published study in approximately 500 cases and a similar number of controls.[44] The protective effect of aspirin on colorectal cancer risk appeared to be absent in those who carried a variant allele. Our results indicate the opposite with the lowest risk in both aspirin and non-aspirin NSAID users who are variant carriers. The different study outcome (adenoma *vs* carcinoma) or techniques to study additive effect modification might explain this contradiction.

Unlike earlier studies, we studied potential effect modification on an additive and multiplicative scale in both non-aspirin and aspirin users in one cohort to investigate whether there was synergism between *CYP2C9* variant alleles and NSAID use on colorectal cancer. For additive interaction we provided information about significance by using RERIs. None of the interaction terms were significant and consequently, based on our population-based study, there is no strong evidence that there is synergism and that *CYP2C9* variant carriage enhances the potential protective effects of NSAIDs on colorectal cancer risk. This seems in contrast with results from previous studies that *CYP2C9* variant allele carriers may accumulate NSAIDs and enhance their activity by a decreased metabolism.[47-49] However, these studies focussed on serum levels of the drug and not the protective effect of this accumulation in the human body. Although we can not be entirely certain about it as we have seen before, it is more or less established that duration of use is more important than dose in the prevention of colorectal cancer.[31] It is therefore possible that accumulation of an NSAID, resulting in higher serum levels of the drug, does not add so much extra protection against colorectal cancer. The observed reduced risks in persons with both factors present might therefore be considered as the sum of two independent risk factors. Hence, these factors most probably act primarily through different pathophysiological pathways. Nevertheless, enhancement of the effects of

NSAIDs on colorectal cancer by *CYP2C9* variants can still be present for specific NSAIDs, as was observed in a recently published American case-control study.[46] Due to a lack of power, we were not able to study the interaction between *CYP2C9* variant alleles and NSAIDs for individual products.

Observational studies might have some limitations. Selection bias due to including only those for whom blood samples were available seems unlikely since genotype data were in Hardy Weinberg equilibrium. Even though this group was slightly younger and possibly healthier, selection bias would not explain the risk reduction that we found in NSAID users. Next, 12% of persons were not able to do any physical activity. Most of these persons lived in one of the homes for the elderly. Although physical activity and colorectal cancer incidence are known to be associated, it was adjusted for in the analyses and this will probably not have led to any spurious results in the analyses on interaction, as there are no suspicions that physical activity is associated with genotype. As data on both disease status and medication use were prospectively gathered without knowledge of the research hypothesis, information bias is unlikely as well. However, NSAIDs have been available over-the-counter in the Netherlands since 1996 but to a limited extent and in a relatively low daily recommended dose. Moreover, it is unlikely that persons with a prescribed NSAID also use them over-the-counter, since all NSAIDs on prescription – including long-term use – are fully reimbursed. One of the strengths of this study was the use of pharmacy data on a day-to-day basis with little room for misclassification of the drug exposure due to recall bias. Misclassification of the anatomical subtypes would, if present, be random and lead to underestimation of the true estimates as well. Confounding of specified factors was adjusted for in the analyses. Furthermore, one must always consider the impact of insufficient power. For gene-environmental interactions the numbers of cases and controls required might be much higher than used in our analyses. However, the insignificant results of our interaction analyses could suggest that there might not be a very strong interaction. Nevertheless, it does not exclude a weaker interaction.

CONCLUSION

NSAID use and *CYP2C9* variant alleles are both associated with a reduced risk of colorectal cancer, primarily of the non-sigmoid colon. Both aspirin and non-aspirin NSAIDs account for this effect. Variant allele carriers who used NSAIDs experienced the strongest reduction in risk. This seems to be due to independent mechanisms, and not as a consequence of interaction. Nevertheless, interaction might be present for specific NSAIDs and future studies must include more cases to be able to identify differences in multiple subgroups, include longer follow-up times and include information on tumour stage, differentiation, treatment and use of other potentially interfering medications.

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ORIGINAL ARTICLE

Immunophenotyping of mast cells: a sensitive and specific diagnostic tool for systemic mastocytosis

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ABSTRACT

Introduction: The diagnosis of systemic mastocytosis (SM) is based on a combination of major and minor criteria. Flow cytometric detection of aberrant expression of CD2 and/or CD25 on CD117-positive mast cells is one of the minor criteria used. In the present study we examined the sensitivity and specificity of mast cell immunophenotyping in the diagnosis of SM.

Material and methods: Patients were 36 persons with systemic mastocytosis diagnosed according to WHO criteria. Controls were 31 patients without SM. Immunophenotyping was performed according to published guidelines.

Results: All patients with SM were positive for CD2 and/or CD25. All patients without SM, except one, were negative for these markers. The sensitivity for immunophenotyping was 100%, the specificity 91%. The positive and negative predictive values were 97% and 100% respectively.

Conclusion: Immunophenotyping of bone marrow derived mast cells is not only a very sensitive but also a very specific method to diagnose SM with high positive and negative predictive value.

KEYWORDS

Immunophenotyping, mastocytosis, sensitivity, specificity, tryptase

INTRODUCTION

Systemic mastocytosis (SM) is a disease characterised by an accumulation of mast cells in one or more organs with a variable clinical course. Manifestations of the disease are largely provoked by the resultant increase in mast cell-derived mediators, which have a variety of local and systemic effects. Diagnosis is based on a combination of major and minor criteria (*table 1*). If at least one major and one minor or at least three minor SM criteria are fulfilled, the diagnosis of SM is made.[1]

Flow cytometric immunophenotyping offers a rapid, objective, and sensitive multiparameter analysis of high numbers of cells from a sample, with information being provided on a single-cell basis. Recent flow cytometric studies have demonstrated that normal human mast cells exhibit a myeloid immunophenotype characterised by the expression of CD117 and CD33 in the absence of reactivity for CD34, CD14, CD15 and lymphoid-lineage-associated markers.[1-3] Mast cells from mastocytosis patients display unique immunophenotypic characteristics, including lowered expression of CD117, aberrant expression of CD2 and/or CD25, and increased expression of CD11c, CD35, CD59, CD63 and CD69.[1-3] Particularly the aberrant expression of CD2 and/or CD25 is of great relevance in the diagnosis and differential diagnosis of SM and consequently flow cytometric detection of CD2 and/or

Table 1. *Diagnostic criteria for systemic mastocytosis[1]*

Major criteria
- Multifocal dense infiltrates of MC detected in sections of bone marrow and/or of other extracutaneous organ(s)

Minor criteria
- In MC infiltrates detected in sections of bone marrow or other extracutaneous organs, >25% of MC are spindle shaped or: in bone-marrow smears, atypical MC comprise >25% of all MC
- Detection of a *c-KIT* point mutation at codon 816 in bone marrow or blood or other extracutaneous organs
- Kit+ MC in bone marrow or blood or other extracutaneous organ(s) coexpress CD2 and/or CD25
- Serum total tryptase concentration persistently >20 µg/l

If one major and one minor or three minor criteria are fulfilled, then the diagnosis is systemic mastocytosis

CD25 on CD117 positive mast cells is now one of the minor criteria used to diagnose SM.[1]
In this study, we examined the sensitivity, specificity and positive and negative predictive value of immunophenotyping for the diagnosis of SM.

MATERIAL AND METHODS

Patients

The study was conducted at the Department of Internal Medicine and the Department of Immunology of Erasmus University Medical Centre, the Netherlands. Included in the present study are all 36 consecutive patients seen between January 2003 and May 2007 for whom immunophenotyping was performed as part of the diagnostic work-up for SM in our centre. Immunophenotyping is performed routinely since January 2003 in all patients presenting with a suspicion of SM and for whom a bone marrow examination is regarded necessary. Characteristics of the patients are listed in *table 2*. Based on the presence or absence of so-called B- and C-findings, and the presence or absence of other haematological non-mast cell disease in the bone marrow, the patients were subdivided.[1] The majority of patients (n=31) suffered from indolent SM (no B- or C-findings present). Two

Table 2. *Characteristics of patients with systemic mastocytosi*

Age	Sex	Diagnosis	Biopsy	Smear	c-KIT D816V	Tryptase	CD2	CD25	% mast cells in BM
58	F	ISM	+	+	+	141	+	+	0.1
57	F	ISM	+	+	+	25.5	+	+	0.03
45	F	ISM	+	+	+	182	+	+	0.04
50	F	ISM	+	+	+	221	-	+	0.3
42	F	ISM	+	+	+	179	+	+	0.3
50	F	ISM	+	+	+	27.2	+	+	0.1
62	F	ISM	+	+	+	47.4	+	+	0.03
53	F	SSM	+	+	+	244	+	+	0.8
60	F	ISM	+	+	+	29	+	+	0.08
76	F	ISM	+	+	+	292	+	+	0.04
50	F	ISM	+	+	.	29.4	+	+	0.06
51	F	ISM	+	+	-	32.5	+	+	0.02
64	F	ISM	+	+	-	123	+	+	0.08
36	F	ISM	+	+	-	33.4	+	+	0.05
76	F	ISM	+	-	+	20.7	+	+	0.11
51	F	ISM	.	+	+	22.3	+	+	0.06
41	M	ISM	+	+	+	26.9	+	+	0.2
65	M	ISM	+	+	+	42.9	+	+	0.8
49	M	ISM	+	+	+	65.6	+	+	0.07
53	M	ISM	+	+	+	113	+	+	0.5
45	M	ISM	+	+	+	14.1	+	+	0.06
64	M	ASM	+	+	+	36	+	+	0.1
49	M	AHNMD	+	+	+	24.4	+	+	0.07
49	M	SSM	+	+	+	453	+	+	0.6
35	M	ISM	+	+	+	81.3	+	+	0.13
45	M	ISM	+	+	+	49	+	+	1
50	M	ISM	+	+	+	115	+	+	0.8
52	M	ISM	+	+	.	106	+	+	0.06
43	M	ISM	+	+	-	22.9	+	+	0.02
76	M	ASM	+	-	+	212	+	+	0.01
59	M	ISM	+	-	+	30.1	+	+	0.02
47	M	ISM	-	+	+	28.8	+	+	0.05
61	M	ISM	-	+	+	24.8	+	+	0.06
66	M	ISM	-	+	+	24.2	+	+	0.14
41	M	ISM	-	+	+	14.4	+	+	0.1
62	M	ISM	-	+	-	44.9	+	+	0.08

M = male; F = female; ISM = indolent systemic mastocytosis; ASM = aggressive systemic mastocytosis; SSM = smouldering systemic mastocytosis; AHNMD = associated haematological non-mast cell disease. + = positive; - = negative; . = missing.
For the column 'biopsy' + means: multifocal dense mast cell infiltrate present. For the column 'smear' + means: >25% spindle shaped mast cells present.

patients had a smouldering SM (B-symptoms present but C-findings absent), two patients presented with aggressive SM (C-findings present) and one patient presented with associated haematological non-mast cell disease.

Controls

Controls were 31 patients from the same university hospital without SM, undergoing bone marrow examination for various reasons between March and May 2007. In most cases the control subjects were diagnosed with a haematological disorder (n=20). Four controls were suspected to be suffering from a malignant haematological disorder, but no diagnosis could be made. Three controls had no underlying disease. They underwent bone marrow examination prior to potential bone marrow donation. One of the controls underwent bone marrow examination for reason of an elevated serum tryptase following an allergic reaction. The other controls had various diseases such as pulmonary hypertension, AIDS and liver cirrhosis.

Immunophenotyping

Immunophenotyping was performed using flow cytometry according to guidelines published by the Spanish mastocytosis network.[1] Briefly, bone marrow aspirates were collected in heparin tubes and processed within 24 hours. After lysis of erythrocytes using ammonium chloride (pH 7.4) (10 minutes at room temperature (RT)), leucocytes were washed with PBS/BSA and a cell suspension of 60 million/ml was made. Fifty microlitres of this suspension was subsequently stained with antibodies (10 minutes at RT). Antibodies used are: CD117-PE (104D2), CD45-PerCP (2D1), CD25-APC (2A3), CD117-PE-Cy7 (104D2; custom conjugated), CD34-APC-Cy7 (8G12; custom conjugated) (all from BD Biosciences), CD2-FITC (T11), CD2-PE (T11), CD33-PE (My9) (all from Beckman Coulter), and CD117-APC (104D2; Dako Cytomation, Glostrup, Denmark). After incubation, cells were washed with PBS/BSA and resuspended in FACSFlow solution (BD Biosciences). Data were acquired on a FACSCalibur or FACS Canto B; one million cells were acquired per tube. Data were analysed using Paint-a-Gate Pro or FACS Diva software. Mast cells were gated based on strong CD117 expression; in the six-colour analysis additional gating was performed using CD33 positivity and CD34 negativity.

Other diagnostic tests

c-KIT mutations (particularly the D816V mutation) were analysed as described previously.[4] Serum tryptase levels were determined using a commercial fluorescent enzyme immunoassay (UniCAP assay and UniCAP 100 instrument; Phadia, Nieuwegein, the Netherlands) according to the manufacturer's instructions. Morphological analysis of bone marrow smears and iliac crest biopsies was performed using standard methods.

RESULTS

Immunophenotypic analysis of mast cells

In all patients with SM, mast cells were detected in the bone marrow aspirate (mean 0.21%; range 0.01 to 1.0%). The number of mast cells in SM patients was significantly higher than in control samples (mean: 0.05%; range 0.00 to 0.72%; p value for difference 0.005).

All patients with SM showed expression of either CD2 or CD25 or both on CD117-positive bone marrow mast cells. CD25 was present on mast cells of all SM patients. CD2 (either conjugated with FITC or PE) was absent on mast cells from one patient with SM. One control patient diagnosed with chronic myelomonocytic leukaemia was positive for CD25, but negative for CD2. Although this patient had a slightly elevated serum tryptase (16.0 µg/l) and an increased number of aberrant mast cells in the bone marrow aspirate, this patient did not fulfil the criteria for a diagnosis of SM, as he had no major and only two minor criteria. D816V mutation analysis was negative in this patient.

Two patients had three positive criteria only after including immunophenotyping as a diagnostic criterion. For the calculation of sensitivity and specificity these two patients were left out of the analysis.

In our patient group sensitivity for CD25 positivity was 100% and specificity was 91%. Negative and positive predictive value were 100 and 97% respectively (*table 3*).

Table 3. *Sensitivity, specificity, positive and negative predictive value of immunophenotyping*

	Immunophenotyping		
	CD25+	*CD25-*	*Total*
SM	34	0	34
Not SM	1	30	31
Total	37	30	65
Sensitivity	*Specificity*	*Positive predictive value*	*Negative predictive value*
100%	91%	97%	100%

Other diagnostic criteria for SM

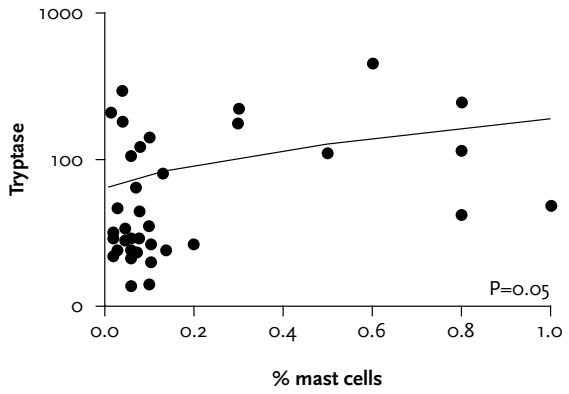
c-KIT mutations could be analysed in 34 SM patients and in 29 patients (85%) the D816V mutation was detected. c-KIT mutation analysis was not routinely performed in control subjects.

All but two SM patients had a serum tryptase level above 20 µg/ml. Serum tryptase was not routinely measured in control subjects, but two controls had an elevated serum tryptase although lower than 20 µg/ml.

A bone marrow biopsy showed multifocal clusters of aberrant mast cells in 30 SM patients (83.3%). Five patients (13.8%) had no clusters of aberrant mast cells. In one

patient the bone marrow biopsy was of insufficient quality for reliable interpretation.
The bone marrow smear showed spindle shaped mast cells in 33 SM patients (91.6%). As mentioned above, one control subject also had an increased number of aberrant mast cells. However, in the control group there was no active search for an increase in aberrant mast cells.
There was a weak association between serum tryptase and the percentage of aberrant mast cell in the bone marrow aspirate assessed by flow cytometry (*figure 1*).

Figure 1. *Association between % mast cells and serum tryptase (µg/ml)*



DISCUSSION

The results of this study indicate that in most cases SM can accurately be diagnosed based solely on immunophenotyping of mast cells in a bone marrow aspirate. In a recent study by Akin *et al.* aberrant mast cells were also found in a bone marrow aspirate of patients with 'idiopathic anaphylaxis' initially not fulfilling sufficient criteria for the diagnosis of SM.[5] Nevertheless, all five patients with aberrant expression had at least one other minor criterion for SM suggesting that SM might be present in these patients as well. In fact, in three of these patients the diagnosis of SM could be confirmed based on the presence of three minor criteria.
Perhaps more importantly our results show that SM can be ruled out if immunophenotyping does not show aberrant expression of either CD2 or CD25 on CD117-positive mast cells.
This study has several drawbacks. First of all the number of controls is relatively small. Although this will not influence sensitivity and negative predictive value, specificity and positive predictive value may be overestimated. Furthermore, theoretically the results of this study may have been hampered by misclassification, as many of the diagnostic criteria for SM (serum tryptase, mutation analysis, bone marrow biopsy) could not be measured routinely in controls. However, this seems unlikely since SM is a very rare disease. Therefore the chance that a control subject is suffering from systemic mastocytosis is negligible.

Our study shows that immunophenotyping may be superior to other subcriteria in the diagnosis of SM. First, a bone marrow biopsy showing clusters of aberrant mast cells, the sole major criterion for SM, was negative in 13.8% of SM patients. This finding is in agreement with the results from Butterfield *et al.* who showed that in four out of 21 patients undergoing bilateral iliac crest biopsy, one of the biopsies was negative for SM.[6] Secondly, sensitivity and specificity of bone marrow smears are unknown but likely to be inferior to bone marrow biopsy and prone to be influenced by the experience of the laboratory technician.
Third, although none of our patients had a serum tryptase within the normal range, in two patients (6%) serum tryptase levels did not exceed 20 µg/ml, the minimum level to be regarded as a criterion for SM. In line with this observation, Sperr *et al.* reported that in eight out of 43 patients with SM, serum tryptase was below 20 µg/ml.[1] Furthermore, serum tryptase can also be elevated in various other conditions, including haematological malignancy and following an allergic reaction as shown in our control group.
Fourth, D816V mutations were found in 85% of patients screened for these mutations, but in five patients no such mutations were detected. These data are in agreement with the finding that the presence of the D816V mutation, found in more than 80% of patients, is a strong argument for the diagnosis of SM.[7,8] However, other c-KIT mutations (that are not looked for routinely) may occur as well.[1,9,10] In addition c-KIT mutations can also be found, although rarely, in patients with germ cell tumours and other neoplasms without coexisting SM.[11-13]

One might correctly argue that based on the data presented a diagnosis can also be made without immunophenotyping in the vast majority of patients (34 out of 36). The strength of immunophenotyping, however, lays in its high negative predictive value, obliviating the need to do further tests if negative.

CONCLUSION

Based on the results of this study and given that immunophenotyping is fast and straightforward, we argue that immunophenotyping should routinely be used as first-line diagnostic tool to confirm or rule out SM.

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CASE REPORT

DRESS syndrome caused by nitrofurantoin

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ABSTRACT

Systemic side effects of nitrofurantoin are rare but can be life-threatening. Serious side effects are pulmonary involvement and Stevens-Johnson syndrome. We report a case of a patient developing circulatory and renal failure together with eosinophilia and a rash. This syndrome of drug rash, eosinophilia and systemic symptoms is called DRESS syndrome.

KEYWORDS

DRESS syndrome, nitrofurantoin, erythema multiforme, eosinophilia

INTRODUCTION

Nitrofurantoin is an antibiotic often prescribed for urinary tract infections. Side effects are usually mild, but may also be life-threatening. Pulmonary involvement and Stevens-Johnson syndrome are well known. We describe a patient who developed circulatory and renal failure together with eosinophilia and a rash. This is called DRESS syndrome (drug rash, eosinophilia and systemic symptoms). An overview of this syndrome is presented, together with the associated drugs.

CASE REPORT

A 77-year-old woman was admitted to our emergency room because a one-day history of confusion. Besides Meniere's disease and previous pyelonephritis she had no medical history of note. She experienced some pain in her upper abdomen and felt very weak. Anuria had been present for more than 12 hours. Four days earlier her general practitioner prescribed her nitrofurantoin for a urinary tract infection. She had also been taking betahistine for many years. Two days later she developed a rash, nevertheless she continued using this antibiotic.

On physical examination she was tachypnoeic and febrile (38°C). Blood pressure was 109/63 mmHg. The abdomen was tender. The skin showed red macules with target-shaped lesions on chest, back and extremities, including soles of her feet and palms of her hands.
Blood tests showed leucocytosis (31.4 x 10^9/l) partly based on an elevated eosinophil count (11.3 x 10^9/l), furthermore the level of creatinine was elevated (131 µmol/l). Alkaline phosphatase, gamma glutamyl transpeptidase, alanine aminotransferase and lactate dehydrogenase were slightly elevated. The arterial blood gas analysis showed a respiratory alkalosis due to hyperventilation (*table 1*). The urine showed some leucocytes, bacteria and granular casts. Electrocardiogram showed atrial fibrillation with a ventricular response of 181/min. Digoxin and metoprolol were administered. Blood pressure decreased to 85/50 mmHg. She was admitted to the intensive care unit.

Table 1. *Laboratory data at time of admission*

Haematology			**Chemistry**		
ESR	6	mm/hour	Sodium	132	mmol/l
Haemoglobin	9.7	mmol/l	Potassium	3.9	mmol/l
Thrombocytes	163	x 10^9/l	Creatinine	131	µmol/l
Leucocytes	31.4	x 10^9/l	Urea	15.6	mmol/l
Neutrophils	16.6	x 10^9/l	CRP	101	µmol/l
Eosinophils	11.3	x 10^9/l			
Liver enzymes			**Blood gas analysis (with 4 litres oxygen)**		
Bilirubin	13	µmol/l	PH	7.52	
ALP	177	IU/l	PCO_2	24	mmHg
γGT	42	IU/l	pO_2	139	mmHg
ASAT	27	IU/l	HCO_3^-	19.5	mmol/l
ALAT	67	IU/l	BE	-1.5	mmol/l
LDH	315	IU/l	sO_2	99	%

ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; AP = alkaline phosphatase; γGT = gamma glutamyl transferase; ASAT = asparate aminotransferase; ALAT = alanine aminotransferase; LDH = lactate dehydrogenase.

Broad-spectrum antibiotics for a possible sepsis and norepinephrine were started. In addition corticosteroids were administered in a high dosage for the diagnosis of erythema multiforme. Urine production increased and the maximum creatinine concentration was 199 µmol/l. The eosinophil count increased to a maximum of 23.2 x 10^9/l after eight days. Bone marrow biopsy showed a high eosinophil count and normal haematopoiesis without signs of malignancy. Renal function normalised and the skin lesions disappeared in the following days. Cultures of urine, blood and faeces remained negative. One week after admission a pacemaker was implanted for a sick sinus syndrome. Creatinine concentration returned to normal level (54 µmol/l). She made an uneventful recovery and was discharged after 12 days.

DISCUSSION

To our knowledge this is the first reported case of DRESS syndrome caused by nitrofurantoin. DRESS syndrome, previously called hypersensitivity syndrome, describes a collection of symptoms and signs occurring at the severe end of a spectrum of drug hypersensitivity reactions.[1,2] Symptoms occur within eight weeks after starting the drug, usually after more than one week.[3] Various diagnostic criteria have been suggested. The criteria most often used are: (1) cutaneous eruption; (2) absolute eosinophilia (≥1500/µl) with or without atypical lymphocytes; and (3) systemic involvement (lymphadenopathy ≥2 cm, *aspartate* aminotransferase ≥2x upper limit, interstitial nephritis, interstitial pneumonitis, or carditis). Diagnosis can be made if all three of the criteria are present. Skin biopsy is widely used to confirm the diagnosis but is non specific.[4]

DRESS syndrome has recently been classified under a delayed type IV b hypersensitivity reaction with T helper type II cells playing a significant role. Furthermore there are some studies suggesting that viral infections, especially reactivation of human herpes virus type 6, commonly occur. Whether this plays a causal role or represents a consequence of DRESS syndrome is not clear.[5,6] Eosinophil accumulation is thought to account for internal organ involvement.[1]

All kinds of drugs can be involved. The syndrome is most frequently seen in association with anticonvulsants and antibiotic agents. An overview is shown in *table 2*.

Other diagnoses for skin abnormalities after drug use include Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN), pustular drug eruptions and erythematous drug eruptions in general. The causative drugs for SJS/TEN are for the greater part similar to those of DRESS syndrome. SJS has also been reported after taking nitrofurantoin.[7] One of the most frequently seen pustular drug eruptions is generalised exanthematous pustulosis, which is caused by β-lactam antibiotics and usually occurs within a few days in contrast to DRESS syndrome. Also erythematous drug eruptions tend to present within days.

It can be difficult to differentiate between the above-mentioned diagnoses. Important is that patients with SJS/TEN always develop bullae (SJS <10% and TEN >30% of body surface area). In most patients with SJS/TEN the mucosa of mouth, genitalia and/or conjunctivae are involved in contrast to those with erythema multiforme, which presents with target lesions. In TEN internal epithelial surfaces (lung, gastrointestinal tract) may also be affected and multiorgan failure can occur as in DRESS syndrome. The most important laboratory finding to differentiate DRESS syndrome from other diagnoses is eosinophilia. Mortality in DRESS syndrome is 10%, with hepatic involvement being a bad prognostic factor.[8] Earlier discontinuation of the causative drug improves the prognosis.[9] Determinants of number and severity of organ involvement is unclear; however, genetic factors may be important.[10] This has led to the suggestion of avoiding the same drugs for first-degree family members.[11]

Once the diagnosis is made, corticosteroids are prescribed in a majority of patients. Corticosteroids might inhibit eosinophilic accumulation, which is thought to account for organ involvement, probably by inhibiting the effect of interleukin 5.[1] No randomised controlled trials of corticosteroids in the treatment of DRESS syndrome are available.[12]

It is remarkable that our patient's symptoms started within three days after taking nitrofurantoin. However, eosinophil count reached its maximum after ten days.

Table 2. *DRESS-associated drugs*[13-23]

Anticonvulsants	**Others**
Carbamazepine	Fluoxetine
Phenytoin	Calcium channel blockers
Lamotrigine	Imedeen
Zonisamide	NSAIDs
Phenobarbital	Allopurinol
Antibiotic agents	Mexiletine
Sulphonamides	Efalizumab
Minocycline	Hydroxychloroquine
Cefadroxil	Esomeprazole
Anti-inflammatory agents	Sorbinil
Salazosulfapyridine	Gold salt
Sulfasalazine	Ranitidine
Antiretroviral drugs	Thalidomide
Nevirapine	Dapsone
Abacavir	Zalcitabine

CONCLUSION

DRESS syndrome should always be considered in case of high eosinophil count *and* skin eruption. Multiple organs can be affected causing a wide range of symptoms.

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CASE REPORT

Severe cerebral toxoplasma infection cannot be excluded by a normal CT scan

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ABSTRACT

A fatal case is reported concerning a severely immunocompromised 50-year-old female renal transplant recipient who developed fever and confusion. Cerebral imaging with contrast-enhanced computed tomography (CT) scans showed no abnormalities while subsequently performed magnetic resonance imaging (MRI) showed clear abnormalities in the basal ganglia. By that time serology and polymerase chain reaction had confirmed the diagnosis of cerebral toxoplasmosis. Because of the suboptimal sensitivity of these tests negative results should be handled with care. Once cerebral toxoplasmosis is suspected in at-risk patients, treatment should be started empirically pending the confirmation of the diagnosis. A normal cerebral CT scan does not preclude cerebral toxoplasmosis. In these situations MRI can give important additional information.

What was known on this topic?
Toxoplasmosis is a rare but feared complication after renal transplantation. The varying presentation of this opportunistic infection and the pitfalls in the diagnostic work-up make it difficult to diagnose the condition early.

What does this add?
This case clearly demonstrates the suboptimal sensitivity of CT scan to diagnose cerebral toxoplasmosis after transplantation. MRI can give important additional information and we recommend using it early.

KEYWORDS

Brain imaging, cerebral toxoplasmosis, MRI, renal transplantation, toxoplasma encephalitis

INTRODUCTION

Toxoplasmosis is a rare but feared complication after solid organ transplantation[1] with a high case-fatality rate. Unfortunately the currently used diagnostic tests are sometimes difficult to interpret especially in the heavily immunocompromised patients at risk. Because of the suboptimal sensitivity of these tests negative results should be handled with care. This fatal case shows the limitation of cerebral imaging with contrast-enhanced computed tomography (CT) scanning in this condition. We will briefly review the varying clinical presentations of this opportunistic infection and the pitfalls in the diagnostic work-up.

CASE REPORT

A 50-year-old Ghanese woman with end-stage renal disease due to hypertension received a postmortal renal transplantation in 2001. Immunosuppressive therapy consisted of prednisolone, ciclosporin and mycophenolate mofetil (MMF). Direct post-transplantation a mild acute cellular rejection was successfully treated with 1000

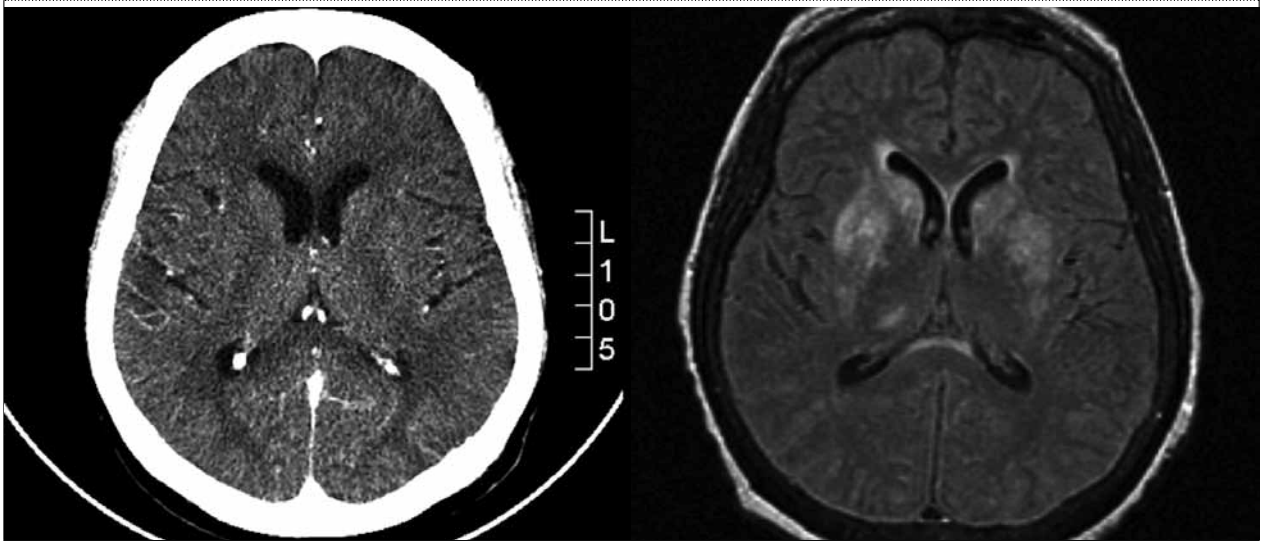
mg of methylprednisolone on three consecutive days. In 2004 it was decided to slowly taper and subsequently stop the MMF. She continued the use of ciclosporin and prednisolone in a daily dosage of 10 mg. After six months a rise in plasma creatinine level from 160 to 375 µmol/l was noted again due to acute cellular rejection. When renal function did not improve after treatment with another 3000 mg of methylprednisolone a course of antithymocyte globulin was given followed by two doses of intravenous immunoglobulins (IVIG). One day after this treatment she developed fever and mental confusion. There were no focal neurological abnormalities and fundoscopy was normal. A CT scan before and after intravenous contrast of the cerebrum did not show any abnormalities. Laboratory analysis showed thrombocytopenia (54 x 10^9/l), leucocytopenia and lymphopenia (2.3 x 10^9/l with 9.4% lymphocytes), elevated lipase (1053 U/l) and rhabdomyolysis (maximum CPK 2570 U/l). Cultures from urine, blood and bone marrow were negative as was a polymerase chain reaction (PCR) on *Mycobacterium tuberculosis*. Analysis of cerebrospinal fluid (CSF) showed a small increase in protein level of 0.87 g/l (normal value <0.5 g/l), a normal Giemsa stain and lymphocyte pleiocytosis. Ciclosporin therapy was stopped. After several days of no improvement a CT scan of the cerebrum was repeated and again showed no abnormalities. However a MRI scan of the brain, performed a day after the second negative CT scan, yielded extensive areas of marked inflammation and oedema around the basal ganglia (*figure 1*) suggestive of *Toxoplasmosis cerebri*. This diagnosis was confirmed by a rise in serum IgG for *Toxoplasma* from 220 IU/ml to 4236 IU/ml, an increase in the titre of the Sabin Feldman test from 1:512 to 1:2048 and a positive CSF PCR on toxoplasmosis. The IgG avidity was high, indicating primary toxoplasma infection had taken place longer before which was confirmed by the 1:32 titre in the Sabin Feldman test, routinely performed before transplantation. This all suggested reactivation instead of an acute infection in this case. Therapy with sulfadiazine, pyrimethamine and folinic acid was started. Despite this, the disease progressed and the patient died four weeks after initiation of therapy. Autopsy was not performed according to the wishes of her family.

DISCUSSION

Our patient suffered from a disseminated *Toxoplasmosis gondii* infection due to reactivation after antithymocyte globulin treatment for a late allograft rejection. Neurological symptoms as well as the signs of rhabdomyolysis and pancreatitis fit with this infection. Extracerebral toxoplasmosis can involve any organ system with eye and lung involvement (approximately 50 and 25% of cases, respectively) as the most frequently involved organs.[2] Pancreatic infection in patients with acquired immunodeficiency syndrome (AIDS) with extracerebral toxoplasmosis was found in 26% of cases at autopsy.[3]

In 1996 Renoult *et al.* described six cases and reviewed 25 cases of *T. gondii* infection complicating renal transplantation.[4] The majority of these infections occurred within three months post-transplantation and were assumed to be donor-derived. Late infection was seen in a minority of cases. Twelve of the 31 patients received antilymphocyte therapy. In this series focal neurological

Figure 1. *Contrast-enhanced CT scan of the brain without significant abnormalities (left picture) and MRI in 'FLAIR-setting', showing diffuse abnormal signal around the basal ganglia (right picture) a predilection localisation for toxoplasmic encephalitis in severely immunocompromised patients*



signs were rare. Co-infection with viruses of the herpes virus group occurred in half of the patients. The outcome of symptomatic toxoplasmosis after renal transplantation seems poor. Of these 31 patients reviewed, 20 (64%, all but one left untreated) died. However, ten of the 11 patients given specific treatment survived, indicating that early diagnosis and therapy are essential.

Toxoplasmosis should be considered in the differential diagnosis of fever, sepsis, pneumonia or encephalitis in transplant recipients. Diagnosis can be established by cerebral imaging in combination with serological tests, direct detection of the parasite by staining or quantitative PCR. MRI scanning of the brain seems much more sensitive than CT scanning. In a prospective study of 50 patients with AIDS and neurological symptoms, relevant abnormalities in 40% of the patients were seen on MRI and not on CT.[5] Discrete abnormalities on MRI not seen on contrast-enhanced CT scan represented significant encephalitis at autopsy.[6] Compared with patients with AIDS, patients who develop cerebral toxoplasmosis after transplantation seem less likely to show ring enhancement or oedema on cerebral imaging.[7]

Serological tests usually suffice to establish the diagnosis in immunocompetent patients.[8] IgG antibodies rise one to two weeks after infection and continue to do so until six to eight weeks after primary infection. Tests for avidity of IgG antibodies have become standard to discriminate between recently acquired infection and those obtained in the more distant past. High avidity antibodies are seldom found in the first four months of infection. In the case described, the IgG aviditiy of the serum sample was high indicating an infection in the past, which was confirmed by low positive IgG values on pretransplantation testing. In addition to IgG, IgM can be used to determine if the infection is recent or more distant.[9] In immunodeficient patients antibody titres are much more difficult to interpret. Repeatedly negative IgG to toxoplama virtually excluded toxoplasmosis. Any positive IgG titre, both low and high, can be associated with reactivation of toxoplasmosis in immunocompromised patients. A rise in IgG to toxoplasma, as in the patient described in this study, can indicate clinical reactivation and warrants special attention, especially in case of unknown clinical findings. Rising of IgG antibody titres can also be observed without clinical importance. Proper interpretation of PCR tests is difficult, with different sensitivity and specificity results being reported from different laboratories using the same PCR probes.[9] A positive PCR on CSF, as in the patient described, is a strong indication of cerebral toxoplasmosis. False-negative PCR results on CSF in cases with cerebral toxoplasmosis are, however, not uncommon. A recent letter in this journal concerning a fatal case of disseminated toxoplasmosis after liver transplantation reported that real-time PCR on plasma could be helpful to detect this condition.[10] Direct examination with a Giemsa stain or immunoperoxidase is possible but has a low sensitivity.

CONCLUSION

Once cerebral toxoplasmosis is suspected in at-risk patients, treatment should be started empirically pending the confirmation of the diagnosis.[11] A normal cerebral CT scan does not preclude cerebral toxoplasmosis. In this situation MRI can give important additional information.

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LETTER TO THE EDITOR

Thyroid function in patients with proteinuria

Dear Editor,

As demonstrated by Gilles *et al.*[1] thyroid function is usually not overly affected in proteinuric states. This may be different, however, in patients treated with thyroxine (T4) who develop a nephrotic syndrome.

CASE REPORTS

Patient 1

This 65-year-old woman was treated by her family physician with 275 µg of T4 (Thyrax^{R}) because of primary hypothyroidism. She was referred because of the nephrotic syndrome (s-albumin 13 g/l, 24-hour urinary protein excretion 16 g). She was hypothyroid, the thyroid-stimulating hormone (TSH) being 57 mU/l (normal 0.5 to 4), fT4 6.8 pmol/l (11 to 24). The dose of T4 was increased to 400 µg daily, after which the TSH normalised (1.6 mU/l). The kidney biopsy showed focal glomerulosclerosis. With high-dose steroids, the proteinuria dropped to 11 g/day. TSH decreased to 0.17 mU/l and fT4 rose to hyperthyroid levels (37 pmol/l). Currently, the proteinuria is stable at 2 g/day. The patient is more or less euthyroid (TSH 5 mU/l, fT4 21 pmol/l) on 225 µg of T4.

Patient 2

A 55-year-old woman, euthyroid on 175 µg of T4 (Euthyrox^{R}), became nephrotic (s-albumin 26 g/l; proteinuria 11 g/day) due to minimal lesion nephropathy. The TSH rose to 42 mU/l, fT4 dropped to 11.7 pmol/l. The dose of T4 was increased to 200 µg/day, when the nephrotic syndrome went into remission two weeks after the start of high-dose prednisone. The TSH dropped to 0.016 mU/l and fT4 rose to 35.8 pmol/l. Decreasing the dose of T4 to 175 µg/day rendered the patient euthyroid again. The same sequence was seen two years later when the nephrotic syndrome relapsed and was again brought into remission.

There is a linear relationship between urinary protein and urinary total T4 concentrations in patients with proteinuria.[2] Apparently, patients with hypothyroidism taking thyroxin are unable to compensate for urinary losses of T4, which may result in biochemical and clinical hypothyroidism. This is in contrast to nephrotic patients with a normally functioning thyroid, as described by Gilles *et al.* By increasing the synthesis of T4, these patients make up for the urinary losses of this hormone. I refer to the excellent overview by Chandurkar *et al.*[2] To end with a clinical note: when higher doses are suddenly required in a patient with primary hypothyroidism on a stable dose of T4, do not forget to check the urine for protein.

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PHOTO QUIZ

A productive cough

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CASE REPORT

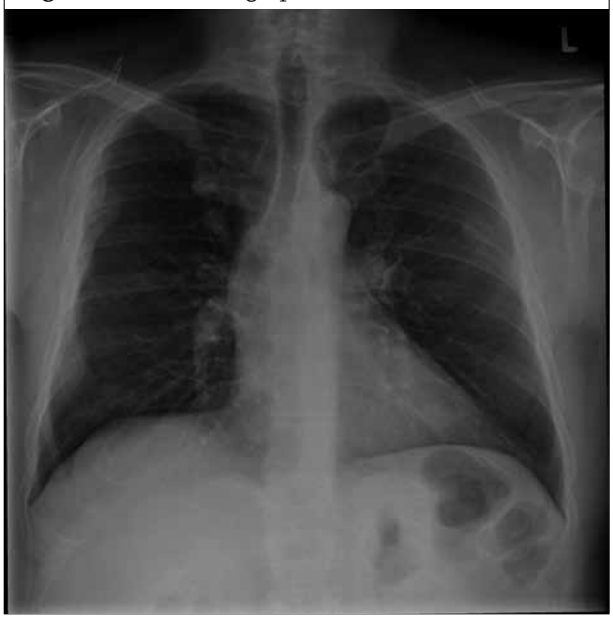
A 48-year-old man with a history of mild asthma presented to the emergency room with intense coughing, progressive over a period of three weeks. In the last week, he has a worsening chest pain while breathing and coughing. He smokes 15 cigarettes and consumes four alcoholic drinks per day. There was no history of a previous trauma.

Physical examination revealed no abnormalities except a painful chest on palpitation. Auscultation revealed no abnormal lung sounds. Peripheral oxygen saturation was 99% while breathing ambient air.

Laboratory investigations revealed slightly elevated liver enzymes (gamma-glutamyl transferase 95 U/l and lactate dehydrogenase 269 U/l) and a leucocytosis of 17.1 x 10^9/l.

A chest radiograph was obtained (*figure 1*).

Figure 1. *Chest radiograph on admission*



WHAT IS YOUR DIAGNOSIS?

See page 155 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 154)

A PRODUCTIVE COUGH

DIAGNOSIS

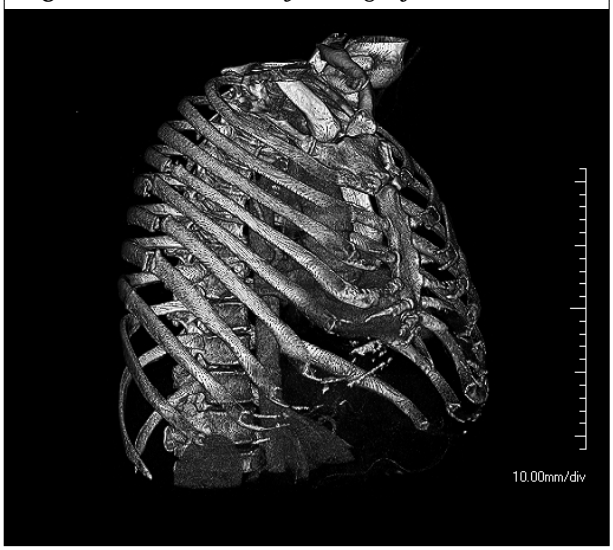
The chest radiograph (*figure 1*) demonstrated multiple fractures of the ribs on both sides, see also the reconstructed 3D-image of the computer tomography scan of the thorax (*figure 2*).

On suspicion of whooping cough, we performed pertussis serology, which was positive.

The rib fractures were considered to be induced by severe coughing secondary to pertussis infection.

Supplementary laboratory investigations revealed a very low level of vitamin D, 25-hydroxy vitamin D 16 nmol/l (30 to 150 nmol/l) and low testosterone, 3.2 nmol/l (6.9 to 28.1 nmol/l). Calcium, phosphate and parathyroid hormone were within normal limits. There were no other hormonal deficiencies. Dual-energy X-ray absorptiometry (DXA) demonstrated severe osteoporosis adjusted for age with a T-score of -3.0. Abdominal ultrasonography showed liver steatosis. The patient had developed severe osteoporosis with hypovitaminosis D and testosterone deficiency due to chronic alcoholic ingestion. His whooping cough was the cause of this revelation.

Figure 2. *Reconstructed 3D image of the CT thorax*



Case reports about rib fractures due to pertussis are extremely rare. Just one was published in 2001 about an 11-year-old boy with a fracture of the first rib due to pertussis.[1] This is the first report of an adult with multiple rib fractures due to pertussis. Further investigations revealed severe osteoporosis due to chronic alcoholism.

Alcohol is known for its impairment of vitamin D metabolism leading to vitamin D deficiency and its depressing effects on bone mineral density. Furthermore chronic alcoholism is related to poor nutrition, malabsorption and alcoholic liver disease. All these factors contribute to the development of osteoporosis and the risk of fractures. The exact pathogenesis is still unclear. There is some evidence for inhibition of bone formation and higher bone resorption rates.[2]

Alcohol also affects the hypothalamic-pituitary-gonadal axis and may have a toxic effect on the testis, resulting in decreased serum testosterone levels and hypogonadism, independently of cirrhosis or nutritional factors. Hypogonadism contributes to the development of osteoporosis too.[3,4]

Referring to this case report, we advise to look for osteoporosis and its underlying causes if patients without a previous trauma present themselves with rib fractures.

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PHOTO QUIZ

Acute ischaemic limb

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CASE REPORT

A 64-year-old man presented to our emergency department with swelling of the right limb and cyanosis of the toes for two days. He had smoked two packs of cigarettes per day for more than 20 years. On physical examination, his vital signs were stable. Painless pulsatile masses over the bilateral groin regions and cyanosis of right toes were noted.

WHAT IS YOUR DIAGNOSIS?

See page 157 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 156)

ACUTE ISCHAEMIC LIMB

DIAGNOSIS AND TREATMENT

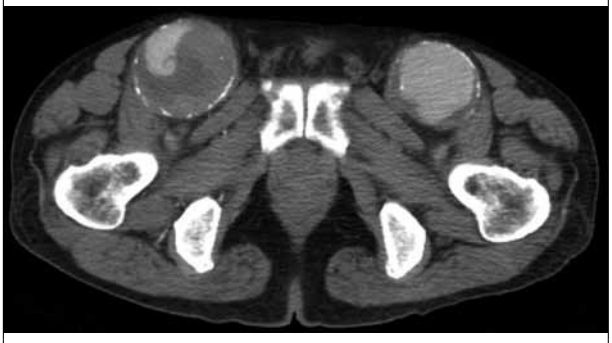
Computed tomography angiography (CTA) showed bilateral thrombosed common femoral artery aneurysms (CFAA) with the one on the right side more severe than the left (*figures 1* and *2*). The right and left CFAAs were about 10 cm and 8.7 cm in length, respectively. Aneurysmectomy of the right CFAA was performed urgently with reconstruction of the right CFAA with an 8 mm polytetrafluoroethylene (PTFE) graft, and reimplantation of the right profunda femoral artery with an 8 mm PTFE graft. The cyanosis of the right toes recovered immediately after reconstructing the artery. Surgery for the left CFAA was performed smoothly in the same way two weeks later. At follow-up six months later, the patient had made an uneventful recovery.

REMARKS

Aneurysms of the femoral artery are rare.[1,2] Cutler and Darling classified femoral artery aneurysms as type 1 and 2 depending on the relationship of the aneurysm to the femoral artery bifurcation in 1973. The aetiology of femoral artery aneurysms may be attributed to arterial degeneration or false aneurysms associated with previous vascular reconstructions or arterial injury. Risk factors include cigarette smoking, diabetes, dyslipidaemia, hypertension, and hyperhomocysteinaemia. Acute thrombosis occurs in 15% of the cases.[1] Studies have suggested that duplex scans or CT should be performed in all patients presenting with femoral artery aneurysms based on the high incidence of associated aneurysms, such as contralateral femoral or abdominal aortic aneurysm.[3] Large or symptomatic aneurysms warrant early operative intervention.

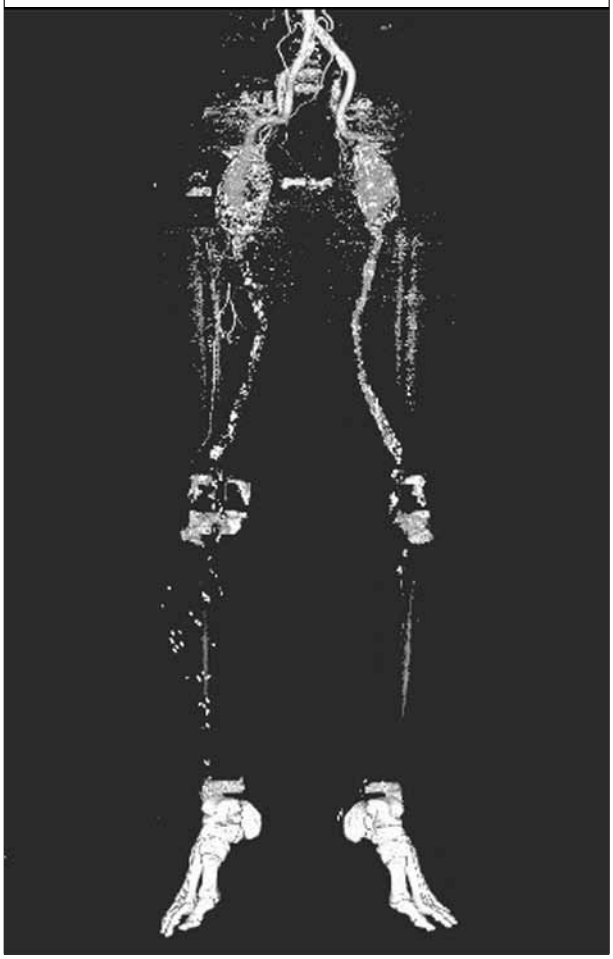
Groin pulsatile mass with distal cyanosis hints at a thrombosed femoral aneurysm. Investigations for other aneurysms should take place simultaneously. Early recognition and immediate surgical management in the clinical setting can avoid a life-threatening catastrophe.

Figure 1. *Contrast CT transverse view demonstrated bilateral thrombosed large common femoral artery aneurysms*



Right and left aneurysms were 7.0 cm and 6.5 cm in diameter, respectively.

Figure 2. *3D CTA revealed bilateral thrombosed CFAAs with the one on the right more severe than left*



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Netherlands
The Journal of Medicine

PHOTO QUIZ

Cystic renal mass

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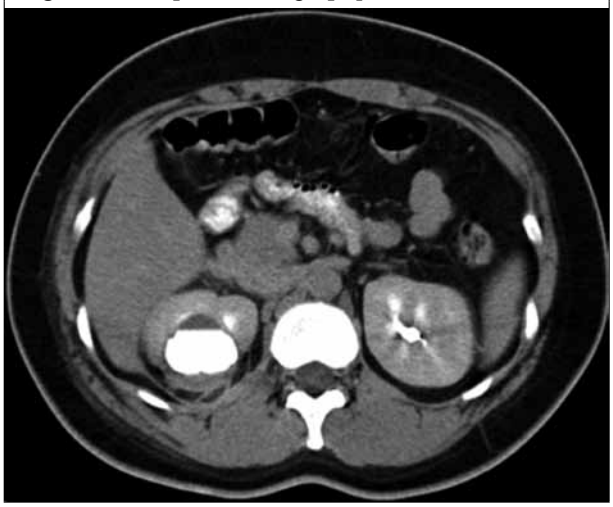
CASE REPORT

A previously well 30-year-old woman presented with two-day history of fever, right loin pain and dysuria. A positive right renal punch was elicited. The white cell count and serum inflammatory markers were raised and urine analysis revealed pyuria. Blood and urine cultures did not yield bacterial growth. A computed tomography was performed (*figure 1*).

Figure 1. *Computed tomography*



WHAT IS YOUR DIAGNOSIS?

See page 159 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 158)

CYSTIC RENAL MASS

DIAGNOSIS

The computed tomography showed stranding of the right perinephric fat and thickening of the right renal fascia consistent with the clinical impression of a right pyelonephritis. In addition, there was a cystic lesion in the upper pole of the right kidney communicating with the pelvicalyceal system (*figure 2*, black arrowhead) and showing retention of contrast medium in the delayed phase of the scan (*figure 2*, arrows), consistent with a calyceal diverticulum. Calculi were seen at the dependent part of the calyceal diverticulum in the precontrast scan (*figure 2*, white arrowhead). The pyelonephritis resolved with antibiotic therapy and the calyceal diverticulum was managed with watchful waiting.

Urinary tract infection can be secondary to underlying structural abnormalities of the urinary system such as calyceal diverticulum. Calyceal diverticulum is a cystic cavity lined by transition cell epithelium within the renal parenchyma that communicates with the collecting system.[1] It is important to distinguish calyceal diverticulum from renal cyst because the management is different. Communication with the pelvicalyceal system differentiates calyceal diverticulum from renal cyst and this may be seen on computed tomography and is confirmed by contrast filling of the calyceal diverticulum in the delayed phase of computed tomography or intravenous urography.[2] The mobility of the calculi within the calyceal diverticulum, which can be demonstrated on ultrasound or computed tomography, differentiates the calculi from the mural calcification of renal cyst.[3]

Although rare and usually asymptomatic, calyceal diverticulum can result in a variety of urological problems including renal colic, haematuria, stone formation, urinary tract infection and hypertension.[2] It is therefore important to be aware of the complications associated with calyceal diverticulum and the need for definitive treatment if there are recurrent symptoms or complications.[4]

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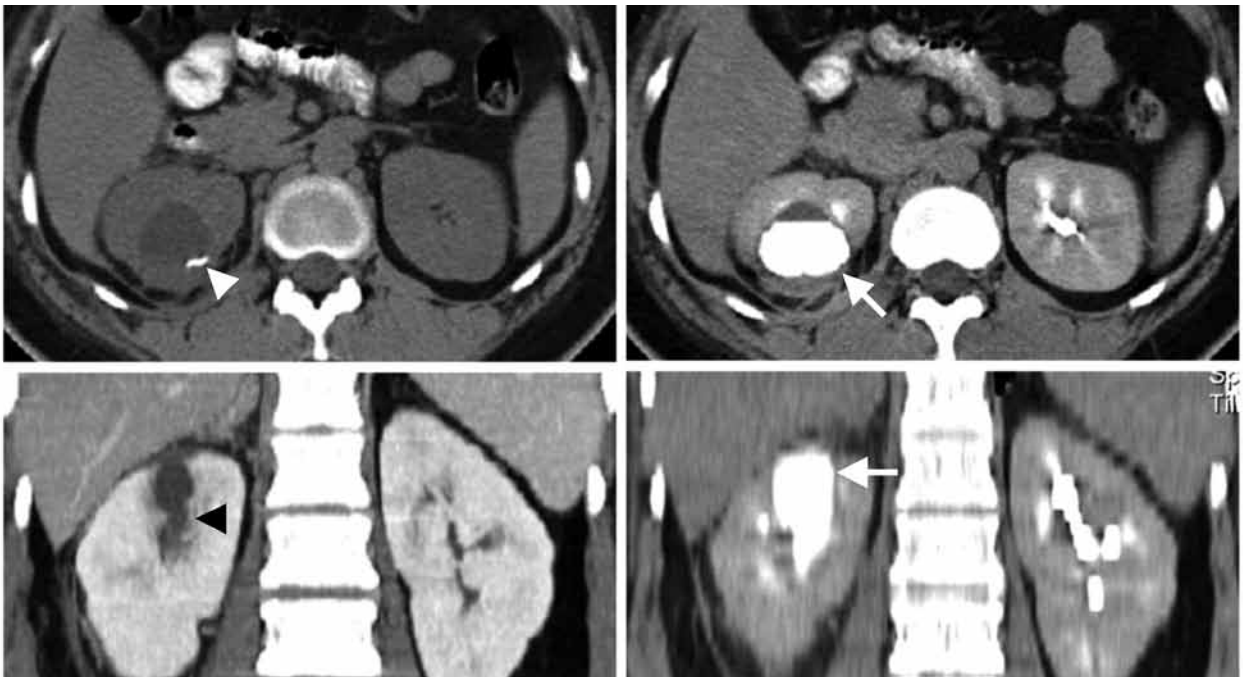
Figure 2. *Computed tomography*



A cystic lesion was seen in the upper pole of the right kidney communicating with the pelvicalyceal system (black arrowhead) and showing retention of contrast medium in the delayed phase (arrows). Calculi were seen at the dependent part of the calyceal diverticulum in the pre-contrast scan (white arrowhead).

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.

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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.

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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).

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

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