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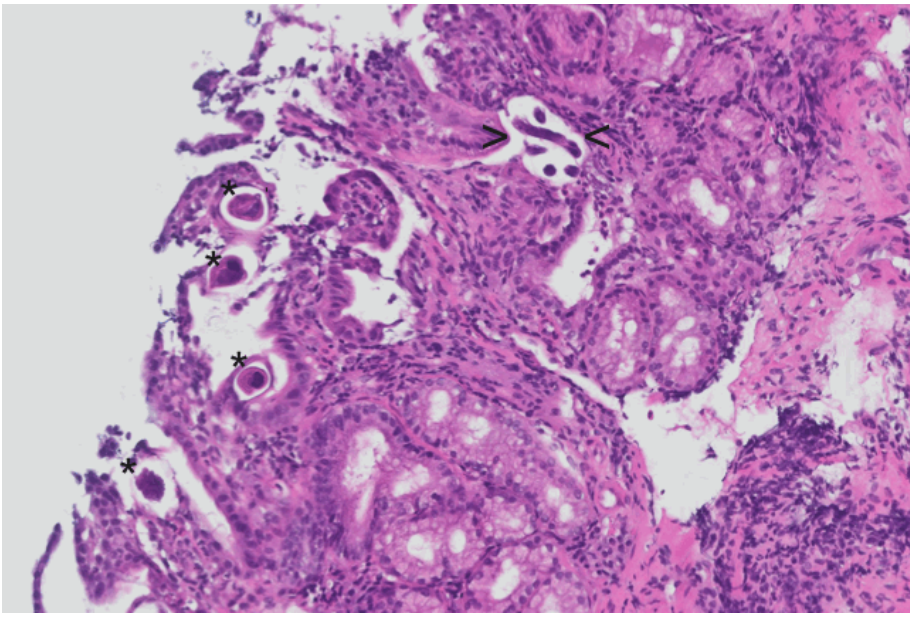

A man from Surinam with intestinal dilatation; what is your diagnosis?

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To serve the need of the physician to practice up-to-date medicine and to keep track of important issues in health care. To promote and to enhance clinical knowledge by publishing editorials, original articles, reviews, papers regarding specialty training, medical education and correspondence.

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Contents

EDITORIAL

The new Dutch antithrombotic management guideline
Treating venous thrombosis with direct oral anticoagulants for a lifetime?

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A new guideline for antithrombotic management has recently been published.[1] This Dutch guideline advises doctors about the prevention and treatment of venous thromboembolism (VTE). Furthermore, the guideline outlines the indication when bridging of anticoagulation is needed, and implicated the HESTIA criteria, a clinical score for outpatient treatment of patients with a newly diagnosed pulmonary embolism (PE).[2] We would like to recommend our readers to read this new guideline, which is easily accessible online[1] as well as a practical Dutch summary published recently.[3]

A main question remains unanswered: what is the length of treatment of a first episode VTE? As compared with the previous guideline,[4] a change of practice has been proposed; namely, to treat a patient with an idiopathic VTE for three months, after which the physician is recommended to evaluate his treatment yearly. The continuation of the antithrombotic treatment should be considered if the risk of a recurrent venous thrombotic event outweighs the bleeding risk.[1]

The recurrent rate after a first idiopathic thrombotic event is 30% in five years.[5-7] Additional treatment of anticoagulant treatment in unprovoked pulmonary embolism did not prevent the recurrence of VTE after antithrombotic treatment was discontinued.[5] The type of VTE is of importance in the recurrence rate as well. Patients presenting with PE are three times more likely to suffer a recurrence PE than patients presenting with a deep venous thrombosis.[6]

It would be a challenge for the physician to annually re-evaluate the continuation of antithrombotic treatment for each individual patient. It is unknown to what extent this patient group will burden the outpatient clinics. Or should the primary physician be left with this yearly consult? The main goal should be the communication with the patient, leading to a shared decision to continue his antithrombotic treatment or not. In addition, we could gain more experience with the usage of direct oral anticoagulants (DOACs) and therefore a yearly consult might be just what is needed to get a better overview of the side effects of DOACs.

The guideline[1] does not elucidate whether patients who are currently using a vitamin K antagonist (VKA) should switch to a DOAC. DOACs have less bleeding complications and are more patient friendly, as no regular laboratory control is needed. In this issue of the Netherlands Journal of Medicine, Brekelmans et al.[8] performed a questionnaire in patients using a VKA, proposing a choice: VKA or DOAC. A majority of the patients were willing to switch to a DOAC. In addition, for VKA and DOACs the 'to switch or not to switch' decision should be based on clinical parameters as well.[9]

Finally, the outcome of antithrombotic treatment versus no-treatment is of great interest, as most probably not many patients will choose to take pills for a lifetime. What would the doctor choose if he were the patient?

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ORIGINAL ARTICLE

Preference for direct oral anticoagulants in patients treated with vitamin K antagonists for venous thromboembolism

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ABSTRACT

Background: Direct oral anticoagulants (DOACs) are an alternative for vitamin K antagonists (VKA) in the treatment and prevention of venous thromboembolism (VTE). Patient preferences for treatment options have not been extensively explored.

Methods: A random sample of 200 patients was obtained from those treated with VKA for deep vein thrombosis, pulmonary embolism or both at the Thrombosis Service Amsterdam. Preference for DOACs relative to VKA was assessed using a treatment trade-off technique administered as a questionnaire sent to all patients. The trade-off consisted of four consecutive scenarios: 1 (no need for laboratory control), 2 (decreased bleeding risk), 3 (less interactions with food and other drugs), 4 (higher efficacy).

Results: The response rate was 68%. In scenario 1, 36% of patients would switch to a DOAC. This proportion rises to 57% (odds ratio [OR] 2.3; 95% confidence interval [CI] 1.6-3.3) for scenario 2. Scenario 3 resulted in 64% of patients preferring a DOAC (OR 3.2; 95%CI 2.2-4.6). The advantage of greater efficacy did not result in a noteworthy change in the preference. Patients who were less satisfied with their current treatment, who were younger and those with higher education were more likely to prefer a DOAC over a VKA. The variables gender, treatment duration, and type of VKA were not significantly associated with DOAC preference.

Conclusion: Almost two-thirds of patients preferred DOACs over VKA. Patients considered the lack of regular laboratory monitoring, the lower risk of serious bleeding and less interactions with food and other drugs the most important arguments to switch to a DOAC.

What was known on this topic?

Treatment of venous thromboembolism with vitamin K antagonists (VKA) has certain disadvantages including frequent INR monitoring and dose adjustments, and interactions with food and other drugs. Direct oral anticoagulants (DOACs) are as effective as VKA, reduce the risk of major bleeding and can be prescribed in a fixed dose regimen. Patient preferences for treatment options have not been explored extensively.

What does this add?

Over the four scenarios, almost two-thirds of patients had a preference for DOACs over VKA. Major reasons for switching to a DOAC were the lack of regular laboratory monitoring, the low risk of bleeding and the absence of interactions with food and other drugs. Patients less satisfied with their current treatment, younger patients and patients with higher education were more likely to prefer a DOAC over a VKA.

KEYWORDS

Direct oral anticoagulants, patient preference, vitamin K antagonists, venous thromboembolism

INTRODUCTION

Oral anticoagulants are indicated for the prevention of stroke and systemic emboli in patients with atrial

fibrillation (AF) or mechanical heart valves, and for the treatment and prevention of venous thromboembolism (VTE). For six decades vitamin K antagonists (VKA) were the only available oral anticoagulants.[1,2] Although highly effective, VKA treatment has certain disadvantages, including the need for frequent INR monitoring and dose adjustments, a risk of bleeding and interactions with food and other drugs.[3,4]

Direct oral anticoagulants (DOACs) have been introduced as an effective and safe alternative for VKA treatment.[5,6] DOACs offer a simplification of anticoagulant treatment due to their stable pharmacokinetic and pharmacodynamic profile, allowing for a fixed dose regimen. Furthermore, they are associated with a significant reduction in major bleeding events.[6] Currently, four DOACs have been registered for the indications AF and VTE: the direct thrombin inhibitor dabigatran etexilate (hereafter dabigatran), and the direct factor Xa inhibitors rivaroxaban, apixaban and edoxaban.

In clinical practice, patients should be informed by their physician about both anticoagulant treatment options and their advantages and disadvantages. However, the final decision of which anticoagulant to prescribe is often made by the physician, following local guidelines and reimbursement restrictions.[1,7] Patient preferences are not always considered or asked for. In a previous study in AF patients,[8] the patient preferences about VKA and DOACs were investigated using a standardised questionnaire. In total 70% of patients would prefer a DOAC over a VKA when they were confronted with different scenarios highlighting the advantages of DOACs. The lack of need for laboratory control and, to a lesser extent, the lower risk of bleeding were considered the most important arguments for preferring DOACs by these patients.[8]

This study aims to investigate the preference of patients with a history of VTE for DOACs versus VKA using the same questionnaire. Furthermore, we explored possible predictors of treatment preference such as age, gender, treatment duration, and treatment satisfaction.

MATERIALS AND METHODS

Study population

A random sample of 200 patients was obtained from patients treated with VKA for deep vein thrombosis (DVT) or pulmonary embolism (PE) at the Thrombosis Service of Amsterdam, the Netherlands. To enrich the sample with respect to experience with VKA at the Thrombosis Service, only patients who were treated for a minimum duration of two years were considered. Hence, the following inclusion criteria were used: 1) Treatment with any VKA, 2) Treatment duration of minimally two years, 3) Treatment indication DVT and/or PE. The survey was sent to the patients by post, together with a return envelope and a recommendation letter from the director of the Thrombosis Service. All patients received a reminder three weeks after the first survey.

Survey

Patient preference for DOACs relative to VKA was assessed using a treatment trade-off technique that was administered as a questionnaire. The treatment trade-off technique allows for a comparison of patients' therapy preference between two different treatment options.[9] The current trade-off consisted of four consecutive scenarios as was the case with the previous investigation in AF patients receiving VKA.[8] In each scenario, one advantage of DOAC treatment was added to the comparison. For each scenario, the patient was asked to express their preference for treatment: stay on the current VKA treatment or switch to DOAC treatment.

In scenario 1, VKA and DOACs have the same efficacy and safety. The only difference between the two options is that there is no need for laboratory control with DOAC treatment. Scenario 1 is the baseline scenario. In scenario 2, a reduced risk of major bleeding with DOAC treatment is added. Scenario 3 adds the advantage of no interactions with food and other drugs. In scenario 4, it is stated that DOACs are more effective than VKA in prevention of recurrent venous thromboembolism (VTE). Patients could indicate for each scenario whether they would definitely or probably stay with their current VKA treatment, were neutral, or would definitely or probably change to a DOAC, on a scale from 1 to 5, respectively.

Statistical analysis

The target response rate of the survey was set at 70%, with a minimum of 50%. The data on treatment preference were analysed using the generalised estimating equations (GEE) method, with a logit link, binomial distribution and an unstructured correlation. GEE enables analysis of repeated measurements or other correlated observations (such as repeatedly assessed preference). It corrects for the fact that patients' answers to each subsequent scenario are related to their answers in previous scenarios. Outcome measures of the GEE were odds ratios and 95% confidence intervals. We calculated 1) whether there was a significant difference in the proportion of patients preferring DOAC over the four scenarios and 2) whether any of the investigated variables were significant predictors for DOAC preference.

To prepare the data for analysis, the 'preference for DOAC' outcome variable was dichotomised. A preference for DOACs was assigned a score of 1, and a neutral preference or preference for VKA was assigned a score of 0. Furthermore, the variable 'educational level' was dichotomised into 'higher education', including university, higher professional education, and preparatory scientific

education, and 'lower education', including all other forms of education. The variable 'patient age' was analysed as a continuous variable and as a dichotomised variable separated by the sample median age. The variable 'treatment duration' was categorised into short VKA treatment duration (< 6 years), intermediate treatment duration (6-10 years) and long treatment duration (> 10 years) groups.

First, we evaluated the change in the percentage of patients that would switch to DOACs over the four scenarios. Second, we investigated whether several variables, such as age, gender, treatment duration, type of VKA, educational level and treatment satisfaction were associated with a preference for DOACs using the GEE method. If a variable turned out to have a significant influence on DOAC preference, the Chi-square test was used to evaluate differences between the variables' categories for each scenario. The significance level was set at $p < 0.05$.

RESULTS

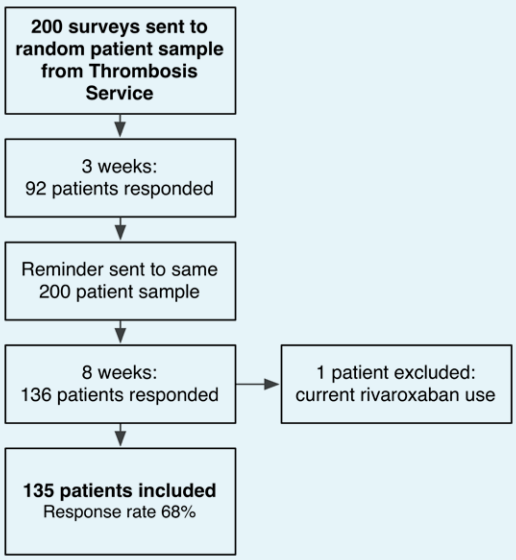
Response and study population

The random sample of 200 patients treated with VKA for the indication of DVT or PE who received the survey by post had a mean age of 71 years, 47% were male, and the mean VKA treatment duration was 18 years. The survey was initially sent on 16 June 2015 and a reminder was sent three weeks later.

In total, 136 of those 200 patients responded with a completed survey, hence a response rate of 68%. One patient was excluded because of current rivaroxaban use, leaving 135 patients treated with VKA for inclusion in the present analysis (*figure 1*). Baseline characteristics of the study population are shown in *table 1*. The average age was 70 years (SD ± 12) and 45% of the respondents were male. The mean treatment duration was 20 years (SD ± 10), and 25% of the respondents were treated for a previous PE, 38% for a past DVT, 22% for both and 13% for previous PE or DVT with an additional indication (i.e. mechanical heart valve, atrial fibrillation). Almost 87% of the patients were (completely) satisfied with their current VKA treatment.

There were missing data for three of the investigated variables. Data on type of VKA were missing in one patient, data on indication for VKA treatment were missing in four patients and data on treatment satisfaction were missing in two patients (*figure 1*).

Figure 1. *Patient inclusion process. Flow diagram of the patient selection and inclusion process*



Preference for DOACs versus VKA

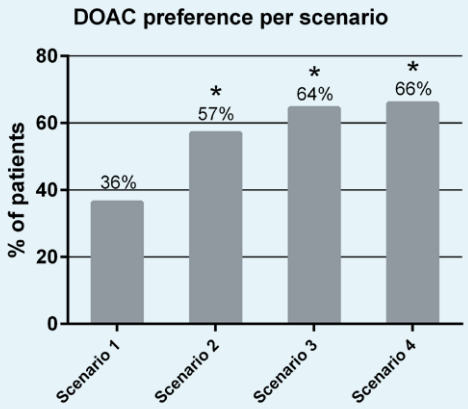
Figure 2 depicts the percentage of patients who would switch from their current VKA treatment to a DOAC per scenario. In scenario 1, where it is explained that DOAC treatment does not require regular laboratory controls, 36% of all patients would switch to a DOAC. In scenario 2, highlighting decreased bleeding risk with DOACs, this

Table 1. *Baseline characteristics. Demographic and clinical characteristics of the 135 respondents*

Patient characteristics		
Age (years)	Mean (± SD)	70 (± 12)
	Median	71
	IQR	62-80
Gender	Male n (%)	61 (45)
Education	Higher* (%)	31 (23)
VKA	Acenocoumarol (%)	105 (78)
	Phenprocoumon (%)	29 (22)
Duration of treatment (years)	Mean (± SD)	20 (± 10)
Indication	PE (%)	34 (25)
	DVT (%)	51 (38)
	PE + DVT (%)	30 (22)
	Other** (%)	16 (13)
Satisfaction VKA	Completely satisfied (%)	59 (44)
	Satisfied (%)	58 (43)
	Neutral/not satisfied (%)	16 (12)

*University, higher professional education, preparatory scientific education. **Deep venous thrombosis or pulmonary embolisation + mechanical heart valve or atrial fibrillation. VKA = vitamin K antagonist; PE = pulmonary embolisation; DVT = deep venous thrombosis.

Figure 2. *DOAC preference per scenario. Percentage of patients preferring a DOAC per scenario. Significance based on GEE, using scenario 1 as reference; p < 0.001 for all scenarios*



percentage rises to 57% (OR 2.3; 95% CI 1.6-3.3; p < 0.01). Scenario 3 added the benefit of no interactions with food or other drugs, resulting in 64% of patients preferring a DOAC (OR 3.2; 95% CI 2.2-4.6; p < 0.01 compared with scenario 1; and OR 1.4; 95% CI 1.1-1.7; p = 0.01 compared with scenario 2). The advantage of greater efficacy did not result in relevant changes in the percentages of patients who would switch to a DOAC (66%, with an OR of 3.4; 95% CI 2.4-4.8; p < 0.01 compared with scenario 1; and OR 1.1; 95% CI 0.89.1-1.3; p = 0.48 compared with scenario 3).

Predictors for DOAC preference

The factors gender, treatment duration, and the type of VKA (acenocoumarol or phenprocoumon) were not significantly associated with DOAC preference. In contrast, the variables treatment satisfaction, patient age, and patient education level did influence patients' preference significantly. Data on age and treatment satisfaction were missing for two patients, whereas patient educational level was known for all patients.

Figures 3 to 5 show DOAC preference percentages for the three variables significantly associated. With regard to treatment satisfaction (*figure 3*), the percentage of patients switching to a DOAC rises in each consecutive scenario for all three categories of treatment satisfaction. The largest increase in DOAC preference is seen in scenario 1 to 3, mainly in the 'neutral/not satisfied' group. This corresponds to the trend seen in the total patient sample. However, 69% of patients who were neutral or not satisfied with their current treatment already switched to a DOAC in scenario 1, compared with 29% of patients who were completely satisfied with VKA treatment. Furthermore, of the neutral/not satisfied patients 94% preferred to switch in scenario 3, versus 53% of the completely satisfied patients.

The distribution of switchers to DOAC according to age is depicted in *figure 4*. Again, in each consecutive scenario the percentage of patients who would switch to a DOAC increases. Patients younger than 71 years were significantly more likely to prefer DOACs in scenario 2 and 3 than their older counterparts.

Patients who had a higher education were more likely to switch to DOACs in each of the scenarios compared with patients who had received a lower education. This difference becomes significant for scenario 3 (*figure 5*).

When treatment satisfaction, age and educational level were combined in the analysis using the GEE, only patient age (OR 0.94 per year; 95% CI 0.94-0.997) and complete treatment satisfaction (OR 0.24; 95% CI 0.08-0.79)

Figure 3. *DOAC preference and treatment satisfaction. Percentage of patients preferring a DOAC per scenario, per level of satisfaction with current VKA treatment. Significance levels calculated by Chi-square test per scenario. *p < 0.05*

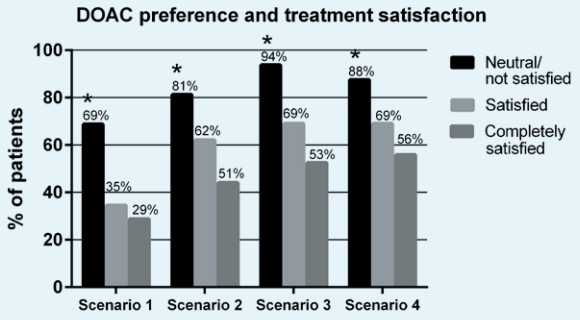


Figure 4. *DOAC preference and patient age. Percentage of patients preferring DOAC per scenario, dichotomised by age younger or older than sample mean. Significance levels calculated by Chi-square tests per scenario. *p < 0.05*

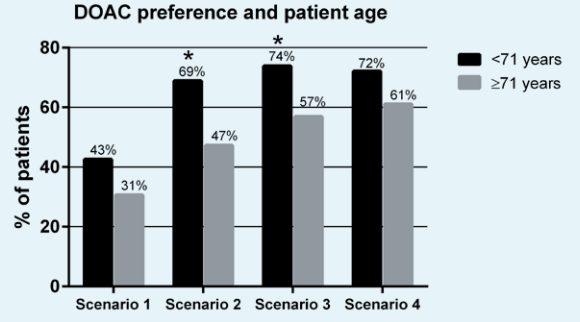
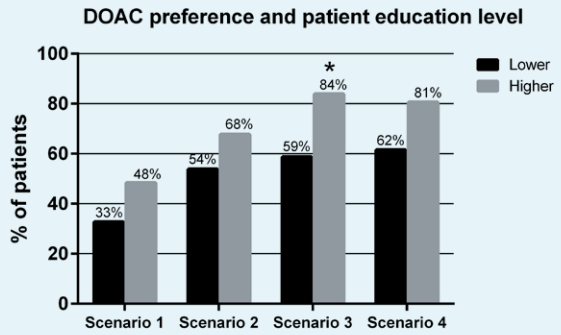


Figure 5. *DOAC preference and patient educational level. Percentage of patients preferring DOAC per scenario, dichotomised by lower or higher education. Higher education = university, higher professional education, preparatory scientific education. Significance levels calculated by Chi-square tests per scenario. *p < 0.05*



remained significant. For higher patient education level, the OR for DOAC preference was 1.76 (95% CI 0.85-3.64).

DISCUSSION

Overall, almost two-thirds of the patients in this study would prefer a DOAC over treatment with a VKA when confronted with different scenarios highlighting the advantages of DOACs. Patients considered the elimination of regular laboratory monitoring, the lower bleeding risk and the reduced interaction with food and other drugs as the most important arguments to switch to a DOAC. Efficacy on the other hand, often considered one of the most important facts by physicians, appeared to be less relevant to patients. Patient gender, treatment duration, and type of VKA were not significantly associated with DOAC preference. However, patient age, educational level, and satisfaction with VKA treatment were. Younger and higher educated patients were more likely to prefer DOACs over VKA, especially in scenarios 2 and 3, where the elements of less bleeding risk and no interaction with food or drugs were evaluated. Patients who were neutral or unsatisfied with their current treatment were significantly more likely to choose DOACs over VKA in all scenarios. Even in patients completely satisfied with VKA treatment, 51% would still like to switch to a DOAC based on the combination of a treatment simplification and a decreased bleeding risk.

As mentioned before, a comparable study using the same questionnaire was performed in patients with AF.[8] There are some differences in baseline characteristics between the present study and the one in AF patients. In the current study, we included relatively more females (55% versus 43%), less patients that were highly educated (23% versus 38%), patients with a longer VKA treatment duration (20 versus 5 years) and more patients who were satisfied with their current VKA treatment (87% versus 76%). In both studies, the total percentage of patients that would switch to a DOAC is consistent at two-thirds. However, in the AF study, the lack of the need for regular laboratory monitoring and the combination hereof with reduced bleeding risk were the main arguments for switching with percentages of 57% and 65% respectively, whereas in our study the diminished interaction with food and drugs turned out to play a role as well. Another difference is that in the AF study only treatment satisfaction was found to be associated with DOAC preference,[8] whereas here we observe that next to treatment satisfaction, also age and educational level played a significant role in switching to DOACs.

A strength of the current study is the high response rate of 68%. This was partly due to the return envelope provided, the accompanying recommendation letter by the director of the Thrombosis Service Amsterdam, and the reminder sent three weeks after the initial survey. Another relevant point is that the patients were randomly sampled. Therefore, our patient group is likely to be representative for VTE patients, albeit treated for a more extensive period of time.

Some aspects of this study require further comment. First, in order to enrich the sample with respect to experience with VKA treatment and the Thrombosis Service and to be able to make a comparison to the previous AF study,[8] we included patients treated for at least two years. This resulted in an average treatment duration of 20 years, with some patients even treated for over 40 years. This is relatively long as most patients with DVT or PE are treated for 3-24 months after a first episode of VTE, and longer if recurrence occurs.[1,10] The consequence of this selection bias is that our study is representative for patients with recurrent VTE rather than for patients with a first event. However, the results may reassure physicians that even if patients are treated for a prolonged period of time and are satisfied with their current treatment, they are open for other treatment options and willing to switch to a DOAC. Because, when presented with the advantages of DOACs, 56% of completely satisfied patients had a preference for a DOAC. The long average treatment duration could be an explanatory factor for the relatively high percentage of patients satisfied with their VKA treatment, as patients would have otherwise stopped or switched to another drug already.

Finally, the questionnaire itself has a few shortcomings. First of all, the lack of a specific antidote for DOACs at the time of study conduction was not mentioned in the questionnaire. It is at present speculation whether the lack

of a reversal agent for DOACs would have led to different results. However, an antidote for dabigatran is currently available,[11] and an antidote for Xa inhibitors is expected within 1-2 years.[12] Second, the order in which DOAC benefits were presented in the questionnaire was the same for all patients and the scenarios were cumulative. The chosen order was the same as in the previous AF study.[8] However, we do not know whether it might have influenced patients' preference patterns. Perhaps efficacy did not add to the preference choice because it was always the last scenario presented and was in addition to the other three scenarios. Third, the trade-off technique might have been too complex for patients with a lower educational level. This might have played a role in the higher likelihood of more educated patients to choose a DOAC. Unfortunately, we cannot correct for this influence. Fourth and last, the argument added in scenario 4 is not proven to be true. Rather than more effective, DOACs are proven to be as effective as VKA. Since the drug efficacy turned out to have the least influence on patients' preference out of all other arguments, we do not expect this has influenced our results.

Extensive trials have been completed for safety and efficacy of DOACs, but limited research has been done on the practical and subjective experiences of its users and factors influencing the treatment decision process. Further research could focus on acquiring more insight into patients' arguments for switching or not switching to DOAC, for instance by changing the order of the scenarios. Furthermore, patients could be included in a follow-up, to retrospectively investigate factors influencing treatment preference in patients who ultimately did or did not switch to DOAC treatment.

In conclusion, almost two-thirds of patients had a preference for DOACs. Patients considered the lack of need for regular laboratory monitoring, the lower risk of serious bleeding and the absence of interactions with food and other drugs the most important arguments for switching to a DOAC. Efficacy was considered less important. Patients who were less satisfied with their current treatment, younger patients and patients with higher education were more likely to prefer a DOAC over a VKA.

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DISCLOSURES

This study did not receive any financial support.

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ORIGINAL ARTICLE

TFR2-related haemochromatosis in the Netherlands: a cause of arthralgia in young adulthood

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ABSTRACT

Background: Type 3 hereditary haemochromatosis (HH) is a rare iron overload disorder caused by variants in the transferrin 2 receptor (*TFR2*) gene. We aim to present characteristics of patients diagnosed with *TFR2*-HH in the Netherlands, in order to increase knowledge and awareness of this disease.

Methods: We collected clinical, biochemical and genetic data from four patients from three families diagnosed with HH type 3 in the Netherlands between 2009 and 2016.

Results: Three women and one man diagnosed with HH type 3 presented with arthralgia and elevated ferritin levels and transferrin saturation (TSAT) at ages 25-41 years. The hepcidin/ferritin ratio as measured in three patients was low. Liver iron content in two patients as assessed by MRI or liver biopsy was highly increased (250 and 362.7 µmol iron/g dry weight, respectively, reference < 35 µmol/g). DNA analysis revealed four different *TFR2* pathogenic variants: one nonsense, one splicing and two missense variants, of which three are novel. Phlebotomy decreased the serum iron parameters but did not relieve the arthralgia.

Conclusion: In patients with a combination of elevated TSAT and ferritin in the absence of anaemia, and after exclusion of *HFE*-related HH, rare forms of HH should be considered. In these cases, presentation with arthralgia in young adulthood, low hepcidin/ferritin ratio and/or liver iron content > 100 µmol/g form an indication for analysis of the *TFR2* gene. Although type 3 HH is extremely rare, awareness of the disease among physicians is important in order to achieve an early diagnosis and prevent complications, such as liver damage.

KEYWORDS

Arthralgia, haemochromatosis, iron, transferrin receptor 2

INTRODUCTION

Hereditary haemochromatosis (HH) is a genetic disease characterised by body iron loading. If not recognised and treated early, the excess iron may lead to complications such as arthropathy, liver disease, diabetes and cardiomyopathy.[1] The most common form, type 1 HH, is caused by mutations in the *HFE* gene.[2] However, mutations in *HJV, HAMP, TFR2* and *SLC40A1* can also cause HH: types 2A, 2B, 3 and 4(A and B), respectively. All these types have an autosomal recessive inheritance, except for type 4A and B, which are autosomal dominant. The HH genes are related to regulation of circulating and tissue iron levels by the hepcidin-ferroportin axis. The hormone hepcidin normally inhibits iron uptake from the gut and iron release from the reticuloendothelial system by promoting degradation of the cellular iron exporter ferroportin.[3]

Five different HH disorders may lead to a classical HH phenotype, i.e. normal haemoglobin (Hb), elevated ferritin and transferrin saturation (TSAT) and iron overload in the parenchymal tissues, such as the liver and pancreas. The pathophysiology of these five conditions is similar: inadequate or ineffective hepcidin-mediated downregulation of ferroportin and subsequent increased iron absorption from the diet, relatively low iron content in the reticuloendothelial system and high parenchymal and circulating iron levels.

HFE-related HH is by far the most common form of HH, and its prevalence is mainly restricted to patients from northern European descent.[4] Most patients with *HFE*-HH do not present until middle age (and women not until after menopause). The other HH subtypes are more rare and less restricted to certain populations.[5,6] Juvenile forms of HH are due to mutations in the genes encoding for haemojuvelin (*HJV*, HH type 2A) and hepcidin (*HAMP*, HH type 2B). These forms of HH are generally characterised by their early onset and a particularly severe phenotype, with patients typically presenting before the age of 30 years with severe systemic iron overload, heart failure and endocrine disorders.[7]

Another form of classical HH is caused by mutations in *SLC40A1* (ferroportin) that interfere with the regulation by hepcidin.[8,9] Since this causes excessive ferroportin-mediated intestinal iron uptake, these mutations are described as gain-of-function mutations. The phenotype of this so-called HH type 4B in affected patients is similar to that in patients with *HFE*-HH.[10,11]

A more atypical form of HH is caused by loss-of-function ferroportin mutations, a condition referred to as 'ferroportin disease',[8] or type 4A HH. Iron overload in this disease is restricted to the reticulo-endothelial system, which leads to the combination of high ferritin concentrations, normal TSAT, and in rare cases iron-restricted erythropoiesis with mild anaemia or impaired tolerance of phlebotomy.[12] Patients with type 4A rarely develop iron-related disease symptoms.

Type 3 HH (*TFR2*-related) is rare and more severe than type 1 HH and presents in young adults.[1,2] Patients typically present in young adulthood, although some children with type 3 HH have been reported as well. Clinical symptoms are similar to those in type 1 and are also treated by phlebotomy. *TFR2* variants have been reported in various populations, most prominently in Italian and Asian families.[13]

Around 50 patients with type 3 HH have been described,[14] comprising around 50 (possibly) pathogenic mutations identified so far.[6,15] The first two type 3 HH families were identified in 2000, when the function of TFR2 was still unknown.[16] A few years later, it was discovered that mutants of this protein cause down-regulation of hepcidin.[17] Current evidence supports a model in which TFR2 senses high levels of circulating iron by its binding to diferric transferrin[18] and subsequently interacts with HFE on the membrane of the hepatocyte, which leads to a SMAD-mediated intracellular signalling resulting in elevated hepcidin expression.[19] Since this upregulation is lost in type 3 HH, hepcidin levels are inappropriately low for circulating iron levels and tissue iron stores, resulting in excessive dietary iron absorption via ferroportin.

We describe three women and one man from three families diagnosed with HH type 3 in our haemochromatosis referral centre in the Netherlands who presented with iron loading and arthralgia in young adulthood. During their diagnostic process we identified in total four different pathogenic *TFR2* variants, of which three are novel. With this report, we aim to facilitate early diagnosis of type 3 HH and related complications by increasing the knowledge and awareness of the disease among physicians.

MATERIALS AND METHODS

Patients

We retrospectively collected and reviewed data on clinical presentation, biochemical tests and DNA analysis of four patients who were diagnosed with type 3 HH at the Radboudumc between 2009 and 2016.

Laboratory methods

Genotyping was performed by DNA sequence analysis of the full coding part of the genes by Sanger sequencing, or whole-exome sequencing (WES), depending on the patient. In case of WES, we used our diagnostic gene panel 'Iron disorders' – consisting of 44 genes with reported roles in iron metabolism disorders – as a filter to analyse the exons of iron-related genes only.[20] The pathogenicity of the variants found was assessed by association of the variant with the phenotype within a family, *in silico* tools and review of the literature and variant databases (ExAC Browser and dbSNP).[21,22] The *in silico* analysis was performed with Alamut Visual (Interactive Biosoftware), which comprises several predictive programmes to assess the consequence of missense and splice site mutations.[23] In case of missense mutations, we also used HOPE (Have (y) Our Protein Explained) to get an *in silico* prediction of the functional consequences of the mutation.[24]

Serum hepcidin-25 measurements were performed with weak cation exchange time of flight mass spectrometry (WCX-TOF MS), according to the method of Kroot et al.[25] The serum hepcidin-25 (hepcidin) and hepcidin/ferritin ratios were interpreted in the context of the reference ranges in the general population.[26]

CASE SUMMARIES

Patient 1

Patient 1 is a woman of British origin, who presented with fatigue at the age of 25. The patient's blood counts (leukocytes and platelets) and serum chemistry profile (glucose, liver enzymes, albumin) were within the reference range. The iron laboratory parameters were as follows: Hb 8.4 mmol/l (= 13.5 g/dl, conversion factor 1.61),

iron 56 µmol/l, TSAT 94% and serum ferritin 831 µg/l. Hepatitis, haematological and inflammatory diseases were excluded. There were no signs of metabolic syndrome. Liver biopsy showed mild architectural disturbance with bridging fibrosis between portal tracts, but no evidence of hepatitis or liver cirrhosis with an amount of 362.7 µmol iron per gram dry weight (= 20.25 mg/g; upper limit of reference in women < 36 µmol/g), which was consistent with haemochromatosis.[27] The patient underwent 16 phlebotomies before she became pregnant at age 26. At 6 months of pregnancy, her ferritin was 440 µg/l and her TSAT was 100%. After her first pregnancy, at age 27, she presented with arthralgia of the pollex of her left hand and back pain. Phlebotomies were restarted after her second pregnancy at age 29, but due to lack of compliance, the patient's serum ferritin and TSAT varied between 50-926 µg/l and 89-100%, respectively, between the ages of 27 and 40 years. At age 37, she also developed arthralgia of the metacarpophalangeal joints of digitus two, three and four of her right hand. X-ray of her hands demonstrated only mild degenerative changes of the carpal joints. Further investigation of the iron overload at age 40 showed a hepcidin/ferritin ratio of 1.6 pmol/µg (reference range premenopausal women 3.0-167.3 pmol/µg) (*table 1*). When the patient was 29 years old, ferrokinetic studies with radioactive labelled iron were regularly used to obtain better insights into the defect of iron metabolism[28-30] and were therefore also performed for our patient. After administration of Fe-59 bound to transferrin, plasma iron clearance, red cell iron incorporation and tissue iron uptake in time were assessed. Plasma T1/2 was prolonged to 186 min (normal 60-120 min).[28] Iron incorporation after 14 days was low: 62.2% (reference 75-85%).[28] Measurement of Fe-59 by a scintillation detector above the sacrum, heart, liver and spleen, 3 times a week for 2 weeks, showed a relatively high liver iron uptake. Finally, whole body counter measurements every 2 to 3 days for 2 weeks showed a body iron retention varying between 95.0% at day 2 to 96.5% at day 14, indicating hardly any body iron losses during this period of time. Taken together, in this patient, we observed a retarded plasma iron disappearance and incomplete iron incorporation in red blood cells, but increased iron uptake by the liver. This is in agreement with dilution of intravenously administrated iron by

Table 1. *Characteristics of our patients with type 3 hereditary haemochromatosis*

		At presentation		At diagnosis HH					At full evaluation (after at least 1 phlebotomy)							DNA analysis	
ID	Sex	Age	Symptoms	Age	Hb (mmol/l)*	MCV (fl)	SF (µg/l)	TSAT (%)	Age	Hb (mmol/l)*	MCV (fl)	SF (µg/l)	TSAT (%)	Hepcidin (nmol/l)	H/F ratio (pmol/µg)	Allele 1	Allele 2
1	F	25	Fatigue	25	8.4	100	831	94	40	7.5	103	493	100	0.8	1.6	c.1870C>T p.Gln624X	c.1606-2A>G (splicing)
2a	F	32	Arthropathy neck, MCP; total hip replacement of both hips	46	na	na	505	94	48	7.4	94	129	78.4	<0.5	<3.9	c.1518C>A p.Ser506Arg	c.1518C>A p.Ser506Arg
2b	M	41	Arthropathy (unknown which joints)	41	na	na	1890	100	54	9.9	92	132	97.2	<0.5	<3.8	c.1518C>A p.Ser506Arg	c.1518C>A p.Ser506Arg
3	F	30	Arthropathy hand and feet, liver damage	31	8.5**	90	5196	100	35	8.2**	84	16	8.5	na	na	c.1300G>A p.Asp343Asn	c.1300G>A p.Asp343Asn

F = female; M = male; na = not available; TSAT = transferrin saturation; SF = serum ferritin; H/F ratio = hepcidin/ferritin ratio, MCP = metacarpophalangeal joints. Patient ID 2a and 2b are siblings.
* In case of Hb, mmol/l is converted to g/dl by multiplying with 1.61.
**Accompanying haematological parameters: mean corpuscular haemoglobin (MCH) 1.95 fmol at diagnosis and 1.84 fmol at full evaluation; erythrocyte count $4.40*10^{12}$/l at diagnosis and $4.46*10^{12}$/l at full evaluation.
Local (Radboudumc) reference ranges: Hb 8.4-10.8 mmol/l (men), 7.4-9.9 mmol/l (women); MCV 80-100 fl; SF 20–300 µg/l (men), 15–200 µg/l (premenopausal women); TSAT 15-45%; MCH 1.75-2.10 fmol; erythrocyte count $4.00-5.10*10^{12}$/l; hepcidin < 0.5-14.7 nmol/l/l (men), < 0.5-12.3 nmol/l (premenopausal women); hepcidin/ferritin ratio 2.9-87.9 pmol/µg (men), 3.0-167.3 pmol/µg (premenopausal women) [www.hepcidinanalysis.com, accessed on 12 September 2016].

TFR2-related haemochromatosis in the Netherlands.

increased circulating iron levels and uptake of excess circulating iron by the hepatocytes.[31] These observations corroborate the pathophysiology of HH, but nowadays do not have added diagnostic value.

DNA analysis revealed no mutations in the *HFE* gene. *HAMP*, *HFE2* and *SLC40A1* also revealed no pathogenic mutations. Analysis of *TFR2* by Sanger sequencing showed heterozygosity for the variants c.1606-2A>G in intron 13 and c.1870C>T (p.Gln624X) in exon 16. The c.1606-2A>G variant is present in the ExAC Browser at a frequency of < 1% (*table 2*) and was previously reported as 'likely pathogenic but not previously reported among patients with HH'.[6] Alamut Visual predicted the loss of a splice site at exon 14, which supports its suspected pathogenicity (*table 2*). Further investigation of the DNA of the patient's mother revealed only the c.1606-2A>G variant on a single allele, confirming that both variants occur on two different alleles and thus that the patient is compound heterozygous for both *TFR2* mutations. This confirmed the diagnosis for HH type 3. As the patient moved back to Britain, she was lost to follow-up.

Patient 2a

Patient 2a is a 56-year-old woman of Dutch descent, who first presented elsewhere at age 32 with arthralgia in her neck and the metacarpophalangeal joints of digitus two and three of both hands. X-radiation of her hands, feet, ankles and knees revealed abnormalities consistent with haemochromatosis arthropathy. At the age of 45 years, she received a total hip replacement because of severe arthrosis, interpreted as a complication of familial hip dysplasia. The other hip was replaced two years later.

At the age of 46, she had a serum ferritin of 505 µg/l and a TSAT of 94%. The blood counts and her serum chemistry profile (glucose, liver enzymes, albumin) were within the reference range. Hepatitis, haematological and inflammatory diseases were excluded and there were no signs of metabolic syndrome. She was diagnosed with HH, yet screening of the *HFE* gene for the common p.Cys282Tyr and p.His63Asp variants was negative. She did not start phlebotomy treatment, as the iron loading was not considered to be severe: the arthrosis was thought to be independent of haemochromatosis. Two years after diagnosis, however, MRI of the liver was performed to further objectify iron overload and revealed an iron content of 250 µmol per gram dry weight (reference range 10-35 µmol/g).[27]

Subsequently, the patient started phlebotomy on an irregular basis and was also referred to our haemochromatosis referral centre for further analysis. A full evaluation revealed a serum hepcidin of < 0.5 nmol/l (reference range for premenopausal women < 0.5-12.3 nmol/l), which means it is below the detection limit for WCX-TOF MS, ferritin of 129 µg/l and a hepcidin/ferritin ratio of < 3.9 pmol/µg (*table 1*). Liver enzymes, α-fetoprotein, thyroid-stimulating hormone and glucose levels were all within the reference range. The rheumatologist diagnosed a nodular inflammatory

Table 2. *Characteristics of TFR2 variants*

TFR2 variant	Allele Frequency	Reference	Family screening	*In silico* analysis
c.1300G>A p.Asp343Asn	new	na	Proband (patient 3): homozygous Mother (healthy): heterozygous Father (healthy): heterozygous	Align GVGD[a]: C15 Polyphen[b]: 1.0 SIFT[c]: 0 HOPE: difference in charge disturbs ionic interaction
c.1518C>A p.Ser506Arg	new	na	Proband (patient 2a): homozygous Brother (patient 2b, affected): homozygous 7 first-degree relatives (healthy): heterozygous	Align GVGD[a]: C65 Polyphen[b]: 1.0 SIFT[c]: 0 HOPE: larger side chain might give steric hindrance
c.1606-2A>G splicing	0.00001648[d]	Wallace et al.[ref 6]	Proband (patient 1): compound heterozygous Mother (healthy): heterozygous	Alamut Visual: loss of splice site at exon 14[e]
c.1870C>T p.Gln624X	new	na	Proband (patient 1): compound heterozygous	na

na = not available.
(a) Align GVGD web-based software scores amino acid substitutions on a 7-scale scoring system, from C0 to C65. Substitutions with a C0 score are considered to be neutral, those with C15 and C25 scores are considered intermediate, as changes to protein structure or function are uncertain, and C35 scores or higher are considered as likely deleterious.[59]
(b) PolyPhen-2 (Polymorphism Phenotyping v2) scores range from 0 ≤ 1 ≤ X. Outcome scores of 0.00 - 0.15 are classified as benign, 0.15 - 1.0 as possibly damaging, 0.85 - 1.0 as more confidently predicted to be damaging.[60]
(c) SIFT algorithm scores range from 0 to 1. The amino acid substitution is predicted damaging if the score is < 0.05 and tolerated if the score is > 0.5.[61]
(d) http://exac.broadinstitute.org/variant/7-100225445-T-C [Accessed on 12 September 2016]
(e) Prediction by Alamut software: http://www.interactive-biosoftware.com/doc/alamut-visual/2.7/splicing.html. Not proven on RNA or protein level.

TFR2-related haemochromatosis in the Netherlands.

polyarthrosis. Phlebotomy treatment was started to normalise ferritin levels to < 50 µg/l; unfortunately, it did not improve her arthralgia.

We found no pathogenic mutations in the *HFE*, *HAMP*, *HFE2* and *SLC40A1* genes. DNA sequencing analysis of *TFR2*, however, revealed a homozygous c.1518C>A transition changing a serine to an arginine at position 506 of the TFR2 protein (p.Ser506Arg). We found no notion of the pathogenicity of this mutation in the literature or variant databases (ExAC Browser, dbSNP).

In silico analysis predicted the variant to be pathogenic (*table 2*). Three-dimensional structure prediction analysis by HOPE showed that the mutation is located in the core of the protein and is not directly involved in ligand interaction or of importance for the dimerisation surface. However, because the arginine side chain is bigger than that of serine, the mutation likely leads to structural changes that prevent proper folding and thus function (*figure 1*).

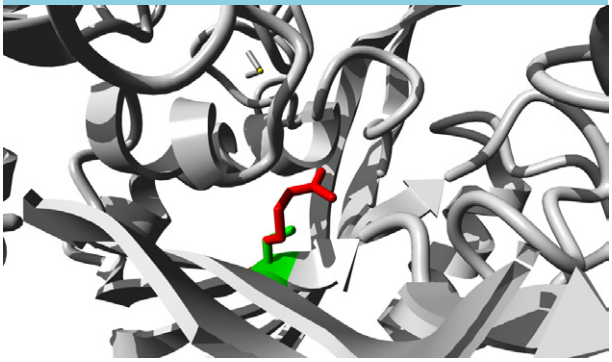
Lastly, we performed restriction analysis for this newly discovered mutation in 100 randomly chosen controls. This revealed no detection of the p.Ser506Arg. In conclusion, the homozygous p.Ser506Arg mutation confirmed the diagnosis of type 3 HH.

Patient 2b

Patient 2b is the brother of patient 2a and presented with severe arthrosis, an elevated serum ferritin level of 1890 µg/l and elevated TSAT at the age of 41 years. He was diagnosed with HH and treated with phlebotomies elsewhere, even though screening for *HFE* showed no mutations. A total of 50 phlebotomies were required to deplete his body iron stores to ferritin levels < 100 µg/l. This did not result in amelioration of his arthralgia. During regular, once every 8 weeks, maintenance phlebotomy, at 54 years of age and BMI 31.8 kg/m², his serum ferritin was 132 µg/l and TSAT was 97.2%. Further investigation showed that the serum hepcidin was < 0.5 nmol/l (reference range for men < 0.5-14.7 nmol/l) and the hepcidin/ferritin ratio was < 3.8 pmol/µg (reference range for men 2.9-87.9). The ALAT level was within the reference range.

After diagnosis of his sister, *TFR2* DNA sequencing analysis was performed and he turned out to be homozygous for the novel p.Ser506Arg mutation as well. Subsequent family screening revealed heterozygosity for the mutation in all other tested family members. None of them showed a HH phenotype, which corroborates the supposed autosomal recessive inheritance.

Figure 1. *HOPE analysis of the p.Ser506Arg mutation. The original amino acid side chain, that of serine, is in green, while the arginine side chain that results from the mutation is in red. The increase in size of the side chain is likely to cause steric hindrance, which prevents proper folding of the TFR2 protein*

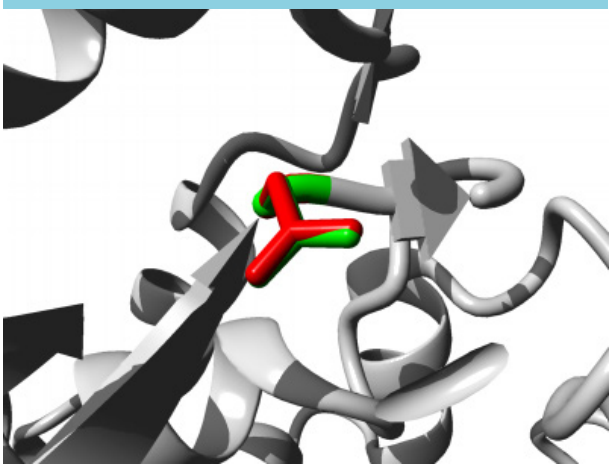


Patient 3

Patient 3 is a woman who presented at age 30 with pre-eclampsia with hepatic dysfunction. After the delivery of a healthy son, one year later, she developed arthralgia in her hands and feet. Persistently mildly elevated liver enzymes (ASAT 51 U/l, reference 0-35 U/l; ALAT 83 U/l, reference 0-40 U/l) at this time warranted further examination, in which a ferritin level of 5196 µg/l and a TSAT of 100% were found, with Hb and other haematological parameters within reference ranges (*table 1*). Based on these values, she was diagnosed with HH. Extensive phlebotomy with a depletion phase of almost two years using a Port-A-Cath was initialised to decrease the ferritin levels to < 50 µg/l. Further analysis at our centre showed that this resulted in an apparent iron deficiency (ferritin 16 µg/l, TSAT 8.5%) without anaemia (Hb 8.2 mmol/l) (*table 1*). Furthermore, blood counts and glucose, but also liver enzymes were all within the reference range (ASAT 26 U/l; ALAT 21 U/l). Other problems in this patient known to be associated with iron overload included impaired fertility and hypothyroidism with subsequent myxoedema, attributed to Hashimoto's disease.

Screening of the *HFE* gene only showed the common heterozygous H63D mutation, which could not clarify the patient's clinical phenotype. Subsequently, WES showed a homozygous c.1300G>A *TFR2* variant, protein coding effect p.Asp434Asn. *In silico* analysis predicted this variant as likely pathogenic (*table 2*). Furthermore, HOPE analysis showed that the wild-type, aspartic acid residue normally forms a hydrogen bond with glycine at position 479 and aspartic acid at position 480 in the protein. It also forms salt bridges with arginine at position 165 and arginine at position 433. While the side chains of the wild-type (aspartic acid) and the mutant (asparagine) form have a similar structure (*figure 2*), the difference in charge (negative and neutral, respectively) disturbs the ionic interaction of the original, wild-type residue. Additionally, the substituted amino acid was highly conserved and part

Figure 2. *HOPE analysis of the p.Asp434Asn mutation. While aspartic acid (green) and asparagine (red) have similar side chains, the difference in charge (negative and neutral, respectively) disturbs the ionic interaction of the original, wild-type residue with other side chains within the protein*



of the M28 peptidase domain, where other pathogenic mutations have also been identified.[32]

This variant has not been reported before in the literature or variant databases (ExAC database, dbSNP). Family screening showed that both parents were heterozygous carriers of the p.Asp434Asn mutation. This verified that this mutation was indeed present on two different alleles in the patient. Based on *in silico* analysis, frequency and family screening, we concluded that the p.Asp343Asn mutant is responsible for the patient's clinical phenotype. Therefore, the diagnosis of HH type 3 was confirmed.

DISCUSSION

We describe four patients who presented with arthralgia at a relatively young age (27-41 years). Laboratory analysis showed elevated ferritin and TSAT levels. When measured, serum hepcidin turned out to be low relative to the ferritin concentration. Genetic testing eventually resulted in the detection of four different variants that are for the first time associated with the *TFR2* defective phenotype. Furthermore, while early arthralgia is commonly found in both type 1 and type 3 HH,[2,33] it is especially pronounced in our case series.

Although our sample number is small, it appears that while function is affected (arthropathy), survival is not. This is an important issue, because iron-related arthropathy can develop even when ferritin concentrations are moderately increased, as in patient 2a. This is in contrast to other symptoms, which are more likely to occur in individuals with serum concentrations of more than 1000 µg/l.[34,35]

A decreased hepcidin/ferritin ratio corroborates the pathophysiology of hereditary haemochromatosis.[36-38] Indeed, in patient 1, the ratio was below the lower limit of the reference range. In patients 2a and 2b, it was < 3.9 pmol/µg and < 3.8 pmol/µg, respectively. The true value may be below the lower limit of the reference range, but this is unsure. In both patients, serum hepcidin was measured after phlebotomy treatment. As this removes iron from the body, it will further decrease the already low hepcidin levels. Moreover, increased erythropoiesis resulting from loss of erythrocytes also inhibits hepcidin production.[39] These processes thus increase the chance that hepcidin levels drop below the detection limit. Therefore, to be able to draw any conclusions, our advice is to measure serum hepcidin levels before phlebotomy, when the hepcidin/ferritin ratio is more likely to be informative.

It is important to realise that while all type 3 HH patients have a low hepcidin/ferritin ratio, making it a sensitive marker, it is by no means a specific marker. For example, low ratios are also observed in some secondary forms of iron overload, such as aceruloplasminaemia and iron-loading anaemias,[40-44] and the metabolic syndrome (Schaap CCM, Janssen MCH, Swinkels DW, unpublished findings). Iron loading anaemias include thalassaemia intermedia and sideroblastic anaemias, which lead to signs of iron overload even without transfusions. However, in contrast to HH patients, this overload is always accompanied by anaemia. A low hepcidin/ferritin ratio combined with a high TSAT and lack of anaemia therefore indicates HH type 1-3 as the most likely diagnosis.

In one of our patients, MRI was performed as recommended in the Dutch guidelines for patients with elevated ferritin and TSAT in the absence of the common *HFE* mutations.[45] The liver iron content in this patient was 250 µmol/g dry weight, more than seven times the upper limit of the reference value (35 µmol/g dry weight).[27] Several other diseases presenting with hyperferritinaemia, including alcoholic liver disease, hepatitis C infection, non-alcoholic fatty liver disease and liver cirrhosis, are also associated with increased hepatic iron deposition. However, in these diseases the liver iron content is typically below 100 µmol/g.[46-48] Therefore, the high liver iron content quantified by MRI in our patient contributed to the diagnosis of HH. Importantly, the iron accumulation in HH is mainly parenchymal and therefore toxic, as indicated by high ferritin combined with high TSAT. For a certain degree of iron accumulation, parenchymal iron will not increase ferritin levels as much as iron accumulation in the reticuloendothelial system (which is for example observed in conditions with low-grade inflammation, such as metabolic syndrome or anaemia of chronic disease), as also demonstrated in transfusion-independent thalassaemia patients.[49,50] Therefore, it should

be kept in mind that in HH patients, liver iron content could reach toxic levels despite a relatively low ferritin.

When HH is suspected, it is useful to first test for the common p.Cys282Tyr and p.His63Asp variants in *HFE*. This is especially the case in patients of Northern European descent, since in these populations the prevalence of the p.Cys282Tyr variant is the highest, whereas in non-Caucasians the common *HFE* mutations are rare. In the absence of homozygosity for the p.Cys282Tyr variant and p.Cys282Tyr/p.His63Asp compound heterozygosity, several causative genes remain. Testing these genes (i.e. *HJV, HAMP, TFR2* and *SLC40A1*) consecutively can be a time-consuming and costly process. With the recent developments in genetics, it may be advantageous to use techniques that screen for multiple genes at once. One suitable technique for this is WES, where the exons of all genes are sequenced. Using a diagnostic filter, genes involved in hereditary haemochromatosis and related diseases can easily be screened for mutations. While more expensive than testing for one gene, WES becomes cost-effective when testing three or more genes, and its costs are rapidly decreasing. We therefore recommend to screen for the common variants in *HFE* first. If the results do not explain the observed phenotype, WES should be considered. Of note, if causative variants are present in non-coding regions of the genome, they will not be detected by WES.

Once type 3 HH is diagnosed, the treatment of choice is phlebotomy, similar to that of type I HH.[51] In the absence of randomised controlled trials, recommendations are based upon the clinical evidence that iron removal before onset of cirrhosis and diabetes in type I HH is associated with reduced morbidity and mortality.[52,53] However, since solid evidence on the optimal endpoint of initial venesection and optimal maintenance therapy is lacking, worldwide guidelines are not identical at this point. According to the European guidelines, the goal is to reduce serum ferritin levels below 50 µg/l and keep them at 50-100 µg/l during the maintenance phase.[54] The Dutch guidelines differ slightly; during the maintenance phase it is sufficient to keep ferritin below the upper limit of the reference range (300 and 200 µg/l for males and females, respectively).[45]

With regard to genetic counselling, it is especially important to screen brothers and sisters of the patient for the presence of *TFR2* mutations. Since mutations are rare, the chance that a patient's partner is a carrier is extremely small, unless there is consanguinity. Therefore, it is normally not necessary to screen any children. The rarity as well as the variety of the mutations also implicate the absence of solid data on the genotype and phenotype relation.[55] For our cases, it is at least striking that the patient with the severest mutations (nonsense and splicing rather than missense) has the earliest age of onset.

Recently, Nai et al. demonstrated the role of TFR2 in the regulation of erythropoiesis using animal studies.[56] They suggest that TFR2 normally decreases the sensitivity of the EPO receptor and balances the amount of erythropoiesis with iron availability to prevent depletion of tissue iron for remaining essential metabolic functions. However, when TFR2 is lost in the erythroid progenitor cell, the balance is lost as well, leading to a constant high level of erythropoietic activity. This is supported by mice without TFR2 in the bone marrow, who fail to develop anaemia in case of iron deficiency. In patient 3, extensive phlebotomy led to iron deficiency (ferritin 16 µg/l, TSAT 8.5%). Yet, the decrease in Hb was limited and she did not develop anaemia (Hb 8.2 mmol/l versus 8.5 mmol/l before phlebotomy), which would be expected considering the iron status. Hb is the product of the mean corpuscular haemoglobin (MCH) and erythrocyte count. MCH will decrease in an iron-deficient state due to haeme-regulated inhibitor kinase,[57,58] a pathway that is most likely unaffected in our patient, as her MCH decreased along with the mean corpuscular volume during phlebotomy. On the other hand, as stated, loss of function of TFR2 leads to uninhibited production of erythrocytes, demonstrated by similar erythrocyte counts in the patient before and after phlebotomy. This limits the decrease in Hb and may explain why the patient did not become anaemic despite very low iron stores. We thus speculate that the loss of TFR2 regulation in the erythroid progenitor cell contributes to the lack of anaemia in this patient. Interestingly, however, both patient 1 and 2a developed mild anaemia, despite adequate iron stores as reflected by their ferritin levels.

In conclusion, early recognition of type 3 HH patients is essential to prevent irreversible and incapacitating complications. When a patient with hyperferritinaemia presents, we recommend to rule out secondary iron overload and other causes of hyperferritinaemia using Hb and TSAT. Normal Hb combined with elevated TSAT at presentation is consistent with HH and an indication for screening for the *HFE* p.Cys282Tyr and p.His63Asp mutations. If the common mutations in *HFE* are absent, a hepcidin/ferritin ratio below the lower limit of the reference range may indicate a more severe form of HH. A liver iron content > 100 µmol/g dry weight may further point towards the diagnosis of these rare forms. WES or other massive parallel sequencing techniques can then be used to screen at least *HFE* (whole gene), *HJV, HAMP, TFR2,* and *SLC40A1*. In cases in which causative mutations are not identified, we recommend referring the patient to a centre with expertise in rare iron disorders.

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TFR2-related haemochromatosis in the Netherlands.

DISCLOSURES

We declare that the authors have no conflict of interest.

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ORIGINAL ARTICLE

Ferric carboxymaltose-induced hypophosphataemia after kidney transplantation

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ABSTRACT

Background: Ferric carboxymaltose (FCM) can induce hypophosphataemia in the general population and patients with chronic kidney disease (CKD). Less is known about the effect of FCM in the kidney transplant population. It has been suggested that fibroblast growth factor 23 (FGF-23)-mediated renal phosphate wasting may be the most likely cause of this phenomenon. In the current study, the effects of FCM on phosphate metabolism were studied in a cohort of kidney transplant recipients.

Methods: Two index patients receiving FCM are described. Additionally, data of 23 kidney transplant recipients who received a single dose of FCM intravenously between 1 January 2014 and 1 July 2015 were collected. Changes in the serum phosphate concentration were analysed in all subjects. Change in plasma FGF-23 concentrations was analysed in the index patients.

Results: In the two index patients an increase in FGF-23 and a decrease in phosphate concentrations were observed after FCM administration. In the 23 kidney transplant patients, median estimated glomerular filtration rate was 42 ml/min/1.73 m^2 (range 10-90 ml/min/1.73 m^2). Mean phosphate concentration before and after FCM administration was 1.05 ± 0.35 mmol/l and 0.78 ± 0.41 mmol/l, respectively (average decrease of 0.27 mmol/l; p = 0.003). In the total population, 13 (56.5%) patients showed a transient decline in phosphate concentration after FCM administration. Hypophosphataemia following FCM administration was severe (i.e. < 0.5 mmol/l) in 8 (34.8%) patients.

Conclusion: Administration of a single dose of FCM may induce transient and mostly asymptomatic renal phosphate wasting and hypophosphataemia in kidney transplant recipients. This appears to be explained by an increase in FGF-23 concentration.

KEYWORDS

Ferric carboxymaltose, fibroblast growth factor (FGF)-23, hypophosphataemia, iron, kidney transplantation, phosphate.

INTRODUCTION

Ferric carboxymaltose (FCM) is a non-dextran iron complex that is composed of a ferric hydroxide core stabilised by a carbohydrate shell, carboxymaltose, allowing controlled delivery of iron to the target tissues.[1] FCM is indicated for the treatment of iron-deficiency anaemia when oral iron preparations are either ineffective or not tolerated. The drug allows rapid and convenient repletion of iron stores and is generally well tolerated. Apart from rare anaphylactic reactions, adverse drug reactions are mild and include nausea (reported in approximately 7% of patients), hypertension (4%), flushing (3.5%), dizziness (2%), and vomiting (1.5%).[1-3]

Hypophosphataemia is another side effect of FCM.[1] Transient decreases in serum phosphate occur in 3.7-58.8% of the general population and are mostly asymptomatic.[1,4,7-10] Hypophosphataemia also occurs following intravenous administration of saccharated ferric oxide and iron polymaltose.[4-6,8] The risk of developing hypophosphataemia appears to be greater with FCM as

compared with other intravenous iron preparations.[4,7-9] A decrease in phosphate concentration may be a reflection of cellular uptake of extracellular phosphate associated with a rapid expansion of erythropoiesis.[11] However, fibroblast growth factor (FGF)-23 seems to be a more important factor in the hypophosphataemia induced by intravenous iron.[9,10] Studies in iron-deficient women have demonstrated that parenteral iron reduces kidney phosphate reabsorption, promotes phosphaturia, decreases calcitriol concentration and that this is mediated by an increased concentration of FGF-23. FCM may reduce the cleavage of intact and biologically active FGF-23 (iFGF-23) after it is secreted by osteocytes.[9,10]

In contrast to patients receiving intravenous iron to correct iron deficiency caused by menstrual blood loss, inflammatory bowel disease, or bariatric surgery, chronic kidney disease (CKD) is characterised by unique, chronic, and profound alterations in phosphate and vitamin D metabolism. FGF-23 plays an important role in phosphate homeostasis and vitamin D metabolism. It induces phosphaturia and suppresses calcitriol synthesis.[12-15] In CKD and haemodialysis patients, hyperphosphataemia rather than hypophosphataemia is the rule and relates to a reduced renal capacity to excrete dietary phosphate loads leading to increased concentrations of FGF-23. Blood concentrations of FGF-23 increase as kidney function declines, with haemodialysis patients having the highest concentrations.[12,13,16] In CKD patients (both non-dialysis dependent and haemodialysis-dependent) the reported incidence of FCM-induced hypophosphataemia varies between 3.8% and 75%.[17-19]

Following successful kidney transplantation, some of the abnormalities in phosphate and vitamin D metabolism seen in CKD persist despite normalisation of kidney function. In many kidney transplant recipients, calcitriol concentrations remain low despite good graft function and hypophosphataemia occurs frequently. Several studies have suggested that persistent increases in plasma FGF-23 concentrations rather than tertiary hyperparathyroidism cause post-transplant hypophosphataemia.[15,20]

Only limited information on the safety of FCM in the kidney transplant population is available. At present, two cases of severe FCM-induced hypophosphataemia (necessitating hospital admission) after kidney transplantation have been reported. Only a single FGF-23 concentration was measured in one of these patients and found to be elevated.[21,22] In the current study, we describe a series of kidney transplant recipients developing hypophosphataemia after intravenous FCM administration. The aim of this study was to investigate the incidence and severity of hypophosphataemia following a single dose of intravenously administered FCM after kidney transplantation. Our observations suggest that FGF-23-mediated kidney phosphate wasting is the most likely cause of this phenomenon.

METHODS

Patients

In the Erasmus MC, University Medical Center Rotterdam, the Netherlands, approximately 200 kidney transplantations are performed each year. The number of kidney transplant recipients followed at our outpatient clinic is approximately 1900.

FCM (Ferinject®, Vifor Pharma Ltd., Switzerland) was approved to treat iron-deficiency anaemia in the Netherlands on 6 July 2007 and has been the iron preparation of choice in our unit since the spring of 2011. Two kidney transplant patients received FCM in 2013 and subsequently developed hypophosphataemia. In these index patients more extensive measurements related to phosphate homeostasis (including FGF-23) were performed and these two patients are described first. These observations led us to further analyse the relationship between the administration of a single dose of FCM and changes in the serum phosphate concentration. We retrospectively collected data of all kidney transplant recipients who received FCM intravenously between 1 January 2014 and 1 July 2015. Patients who were treated with FCM were identified by means of the electronic prescription and medication distribution system of the Erasmus MC through the department of hospital pharmacy. The two index patients were not included in this analysis.

In order to be included in this case series patients had to have 1) a functioning kidney transplant and 2) at least one serum phosphate concentration measured within three months after FCM administration. Kidney transplant recipients suffering from delayed graft function or a failed transplant and receiving dialysis treatment were not considered.

For all patients the following data were collected, if available: age, gender, primary kidney disease, date of transplantation, time after transplantation, kidney function and serum phosphate, calcium, calcidiol, calcitriol and parathyroid hormone (PTH) concentration.

Chemical analysis

Plasma creatinine and phosphate concentrations were measured as part of routine clinical care at the department of clinical chemistry of our hospital by the enzymatic creatinine[23] and Molybdenum blue assays,[24] respectively. Estimated GFR (eGFR) was determined by means of the CKD Epidemiology Collaboration (CKD-EPI) study equation.[25] The fractional excretion of phosphate was calculated by multiplying phosphate in urine with creatinine in serum, divided by the multiplication of phosphate in serum with creatinine in urine, which was then multiplied by 100. PTH was measured by enzyme-linked immunoassay (Vitros ECi).[26,27]

Calcidiol and calcitriol were measured by means of a radioimmunoassay (DiaSorin and IDS, respectively).[28,29] C-terminal FGF-23 (cFGF-23) was determined in EDTA plasma using the cFGF-23 immunoassay (Immunotopics), which measures both intact (and biologically active) FGF-23 (iFGF-23), as well as (inactive) C-terminal fragments of FGF-23.[30] FGF-23 was measured in the fasting state. Because no assay to measure FGF-23 was operational in our hospital at the time of writing, it was measured by means of a validated assay in an external laboratory (Department of Clinical Chemistry, VU Medical Center).

Statistical analyses

Data are presented as mean ± standard deviation or median and range, depending on the distribution of the data. For comparison of variables before and after FCM administration, a paired t-test was used. Spearman's rho was performed for rank correlation. The Mann-Whitney test and Chi-square with Yates' correction were used to compare groups. A p value < 0.05 was considered statistically significant.

RESULTS

Index patients

Case 1

A 42-year-old female of Indian descent received a first, pre-emptive, living-related donor, kidney transplant in May 2012 because of end-stage renal disease (ESRD) caused by hypertensive nephropathy. Some seven months after transplantation, iron-deficiency anaemia was diagnosed and considered to be the result of menstrual blood loss in combination with a strict vegetarian diet. She was started on oral iron supplements. Because of persistent iron-deficiency anaemia, she was treated with a single dose of 1000 mg FCM intravenously. At the time of iron infusion, she was treated with tacrolimus and mycophenolate mofetil (MMF). Her other medication consisted of ferrogluconate (695 mg/day), colecalciferol (25,000 IU/month) and amlodipine. Laboratory findings are listed in *table 1*.

Two weeks after an uneventful administration of FCM, her kidney function remained excellent (eGFR 67 ml/min per 1.73 mm^2) and iron stores, haemoglobin and MCV had all normalised. However, considerable hypophosphataemia was noted (0.33 mmol/l; *figure 1A*). This was considered to be the result of renal phosphate wasting as the fractional excretion of phosphate was 34.1% (reference range < 5%) with an absolute urinary phosphate loss of ~1.4 g/day. She had no glucosuria, urinary pH was normal at 6, and there was no evidence for a plasma cell dyscrasia, arguing against a generalised tubular defect (aminoaciduria and plasma bicarbonate were not determined). The calcium concentration was 2.30 mmol/l, calcidiol and calcitriol concentrations were within the normal range (66 nmol/l and 100 pmol/l respectively). PTH had increased slightly (14.5 pmol/l). The cFGF-23 concentration was elevated at 160 RU/ml (reference range < 125).

The patient did not receive any additional treatment. Two months after intravenous FCM administration, the serum phosphate had normalised to 0.80 mmol/l. The PTH concentration had returned to baseline (12 pmol/l), calcidiol remained within the reference range (53 nmol/l) and calcitriol concentration had increased, although it remained within the reference range (168 pmol/l). cFGF-23 decreased to the reference range (106 RU/ml).

Table 1. *Laboratory findings of the two index patients at the time of FCM administration*

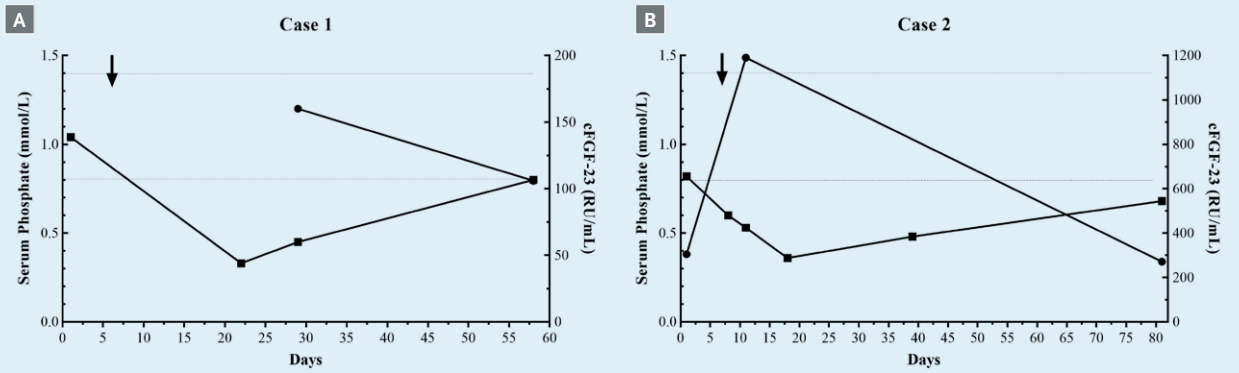
	Reference range	Case 1	Case 2
Creatinine (µmol/l)	55-90	90	153
eGFR (ml/min per 1.73 m^2)	> 90	59	39
Haemoglobin (mmol/l)	7.5-9.5	6.7	6.2
MCV (fl)	80-100	76	79
Iron (µmol/l)	10.0-30.0	4.2	3.9
Ferritin (µg/l)	30-240	26	22
Transferrin (g/l)	2.0-3.5	3.6	2.7
Transferrin saturation (%)	20-45	5	6
Phosphate (mmol/l)	0.8-1.40	1.04	0.82
Calcium (mmol/l)	2.20-2.65	2.41	2.40
Calcitriol (pmol/l)	38.0-183.0	n/a	93.6
Calcidiol (nmol/l)	50-120	68	53
Parathyroid hormone (pmol/l)	1.4-7.3	12.2	16.3
cFGF-23 (RU/ml)	< 125	n/a	305
Urinary protein to creatinine ratio (mg/mmol)		11.4	27.3

eGFR: estimated glomerular filtration rate; MCV = mean corpuscular volume; cFGF-23 = C-terminal fibroblast growth factor 23; n/a = not available.

Case 2

A 70-year-old Caucasian male received his first, pre-emptive, living-unrelated donor, kidney transplant in March 2013 because of ESRD caused by hypertensive nephropathy, possible glomerulonephritis in the 1940s, and nephrolithiasis. In addition, he underwent a right-sided hemi-colectomy three years prior to transplantation because of adenocarcinoma of the

Figure 1. *Serum phosphate and cFGF-23 concentrations over time in the index patients*



Squares represent serum phosphate concentrations and dots represent cFGF-23 concentrations. The dotted line represents the normal range of serum phosphate concentration. The arrow represents the moment of FCM administration. cFGF-23 = C-terminal fragments of FGF-23.

ascending colon. Some five months after transplantation, iron-deficiency anaemia was diagnosed and considered to be caused by chronic blood loss due to a benign ulceration at the site of the neo-ileo-colonic anastomosis. He received a single dose of 1000 mg FCM intravenously followed by oral iron supplementation for the duration of one month.

Some nine months after transplantation and approximately three months after his first FCM administration, recurrent iron-deficiency anaemia was diagnosed. It was decided to treat the patient with a second administration of 1000 mg FCM. At this time, he was treated with tacrolimus, MMF, prednisolone, esomeprazole, trimethoprim/sulfamethoxazole, amlodipine and acenocoumarol. In addition, he received 0.25 µg of alphacalcidol because of persistent hyperparathyroidism (PTH 16 pmol/l). Laboratory results are listed in *table 1*.

Three days after an uneventful FCM infusion, the serum phosphate concentration had dropped to 0.53 mmol/l and decreased further to a nadir of 0.36 mmol/l ten days after FCM infusion (*figure 1B*). There was marked renal phosphate wasting with a fractional excretion of phosphate of 52.7% and 75.1% three and ten days after infusion, respectively. The FGF-23 concentration increased up to 1190 RU/ml three days after the infusion. Kidney function remained stable at 43 ml/min per 1.73 m^2. The calcium concentration was 2.32 mmol/l. The calcidiol concentration was within the normal range (57 nmol/l), but the calcitriol concentration declined significantly (57 pmol/l). PTH concentration had increased to 19 pmol/l. There was no glucosuria or proteinuria on urinalysis.

The patient did not receive additional treatment. Two and half months after the second FMC administration, serum phosphate concentration had increased to 0.68 mmol/l. The PTH and calcitriol concentration had returned to baseline (16 pmol/l and 98 pmol/l), while calcidiol remained normal (52 nmol/l). The fractional excretion of phosphate and cFGF-23 concentration decreased to 19.3% and 271 RU/ml, respectively.

Additional kidney transplant recipients

A total of 30 kidney transplant recipients who received FCM intravenously between 1 January 2014 and 1 July 2015 were identified. Two of these patients were excluded due to the absence of phosphate concentration measurements after FCM administration. Four patients were excluded because they had delayed graft function and were treated with haemodialysis when they received FCM. One patient experienced kidney allograft failure and was started on haemodialysis shortly before receiving FCM. Therefore, a total of 23 kidney transplant recipients were included in this case series. The characteristics of these patients are summarised in *table 2*. The two index patients were not included in this analysis. Only limited laboratory results for PTH, calcitriol and calcidiol were available for these patients.

The median serum creatinine concentration at the time of FCM administration was 200 µmol/l and ranged from 61 to 529 µmol/l with a median eGFR of 42 ml/min/1.73 m^2 (range of 10 to 90 ml/min per 1.73 m^2). One patient was treated with 100 mg FCM intravenously, 3 with 500 mg and 19 with 1000 mg.

As can be seen from *table 2*, serum phosphate concentrations were within the reference range in 11 (47.8%) patients before FCM administration. In nine patients (39.1%) serum phosphate concentrations were decreased and in three patients (13.0%) elevated at the time of FCM treatment. The median time to the first phosphate measurement after a single dose of FCM was 20 days (range 3 to 53 days).

Thirteen patients (56.5%) developed hypophosphataemia after FCM administration. The mean serum phosphate concentration decreased by an average of 0.27 mmol/l from

Table 2. *Baseline characteristics and laboratory findings of the 23 kidney transplant recipients receiving FCM*

Case	Gender	Age (years)	Primary kidney disease	Time after Tx to FCM (days)	Time from FCM administration to phosphate measurement (days)	eGFR (mL/min/1.73 m²)	Dose of FCM (mg)	Baseline PTH (pmol/l)	Baseline phosphate (mmol/l)	Phosphate after FCM (mmol/l)	Δ phosphate before and after FCM
1	F	41	Hypertensive and diabetic nephropathy	215	13	54	1000	n/a	1.15	0.33	- 0.82
2	M	40	Membranous glomerulonephritis	1352	12	48	1000	6.3	1.31	0.64	- 0.67
3	M	71	Hypertensive nephropathy, glomerulonephritis	617	21	57	1000	23	0.57	0.44	- 0.13
4	M	32	Unknown	11	7	14	500	n/a	1.55	0.69	- 0.86
5	M	70	Acute glomerulonephritis	5818	15	59	500	5	0.77	0.94	+ 0.17
6	M	79	Hypertensive and diabetic nephropathy	251	22	24	1000	8	1.25	0.77	- 0.48
7	M	62	Unknown; nephrectomy left because of Wilms' tumour	47	17	58	1000	26	0.79	0.56	- 0.23
8	M	83	Granulomatosis with polyangiitis (GPA)	3093	28	23	1000	n/a	1.10	0.87	- 0.23
9	F	57	Unknown	440	3	52	1000	7	0.71	0.38	- 0.33
10	F	65	Hypertensive nephropathy	13	15	55	1000	n/a	1.36	0.34	- 1.02
11	M	34	Alport syndrome	8	6	56	1000	n/a	0.61	0.35	- 0.26
12	M	24	Unknown	8	16	35	100	n/a	0.70	0.33	- 0.37
13	F	36	Membranoproliferative glomerulonephritis (MPGN)	17	14	90	1000	n/a	0.42	0.33	- 0.09
14	M	63	Hypertensive and diabetic nephropathy	1093	20	57	1000	n/a	1.07	0.78	- 0.29
15	F	53	Diabetic nephropathy	4417	17	10	1000	27	1.56	1.08	- 0.48
16	F	69	Hypertensive nephropathy	2766	4	68	1000	n/a	0.77	1.05	+ 0.28
17	M	48	Neurogenic bladder dysfunction due to Spina bifida	312	24	85	1000	n/a	0.73	0.38	- 0.35
18	M	57	Focal segmental glomerulosclerosis (FSGS)	632	6	20	500	28	1.28	0.85	- 0.43
19	F	47	Hypertensive nephropathy	4730	49	16	1000	16	1.21	1.52	+ 0.31
20	M	68	Diabetic nephropathy	1041	53	38	1000	n/a	1.00	0.80	- 0.20
21	M	43	IgA nephropathy	182	50	20	1000	3	1.74	1.40	- 0.34
22	M	43	IgA nephropathy	105	28	19	1000	7	1.18	1.45	+ 0.27
23	M	52	Unknown	10705	25	10	1000	51	1.21	1.59	+ 0.38
Median		54		1647	20	42	896	18	1.05	0.78	
Range		24 - 83		8 - 10705	3 - 25	10 - 90	100 - 1000	3 - 51	0.42 - 1.74	0.33 – 1.59	

Baseline is the moment of FCM administration. F = female; M = male; n/a = not available; eGFR = estimated glomerular filtration rate; FCM = ferric carboxymaltose; PTH = parathyroid hormone; Tx = kidney transplantation.

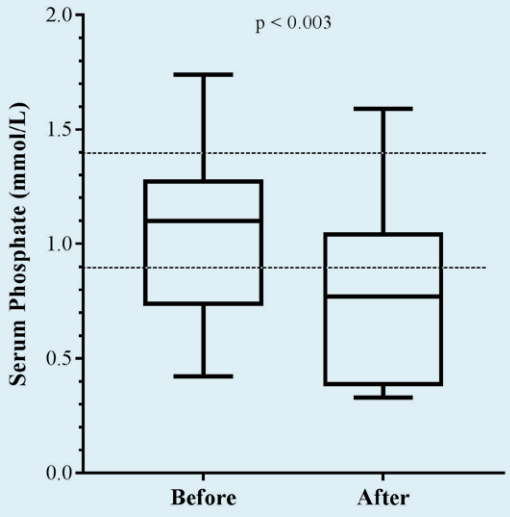
Table 3. *Baseline characteristics of hypophosphataemic and non-hypophosphataemic patients*

	Hypophosphataemia (n = 13)	Non- hypophosphataemia (n = 10)	p value
Baseline phosphate concentration (mmol/l)	0.94 (0.099)	1.18 (0.097)	0.102
Time after Tx to FCM (days)	337.2 (122.2)	3349 (1038)	0.004
eGFR (ml/min/1.73 m²)	52.7 (5.8)	28.2 (6.4)	0.01
Type of transplantation - L(U)R - Deceased donor	 1 12	 5 5	0.06

FCM = ferric carboxymaltose; Tx = kidney transplantation; L(U)R = living (un)related.

1.05 ± 0.35 mmol/l (before FCM) to 0.78 ± 0.41 mmol/l (after FCM administration); p = 0.003 (*figure 2*). In eight (34.8%) patients the hypophosphataemia was severe (defined as < 0.5 mmol/l). The median time to hypophosphataemia was 15 days (range 3 to 24 days). In the three months following the diagnosis of hypophosphataemia, serum phosphate concentrations normalised in eight patients (61.5%), whereas hypophosphataemia persisted in five patients (38.5%). The median time to normalisation of serum phosphate concentrations was 41 days (range 2 to 99 days). From the retrospective patient chart review, none of the patients seemed to have experienced additional side effects of FCM. In one case, phosphate supplementation was started. Of the total population, five patients (21.7%) showed a transient reduction in phosphate concentration without the development of hypophosphataemia. Calcium concentrations were measured in 22 patients. The mean serum calcium concentration did not change significantly: 2.32 ± 0.25 mmol/l (before FCM) vs. 2.30 ± 0.16 mmol/l (after FCM); p = 0.56.

Figure 2. *Change in the serum phosphate concentration before and after FCM administration in 23 kidney transplant recipients*



The dotted line represents the normal range of serum phosphate concentration.

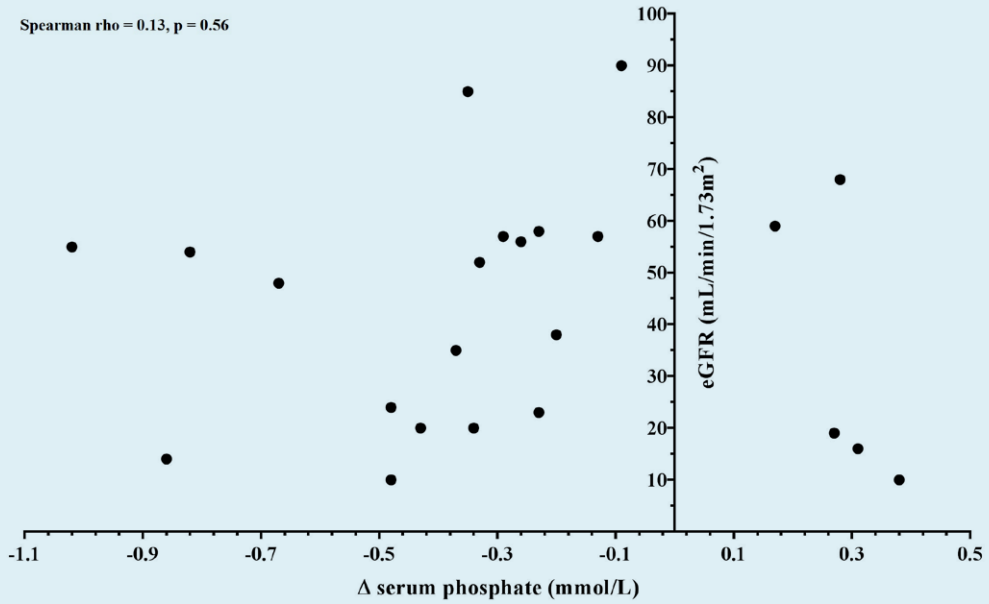
The relationship between renal transplant function and the change of phosphate concentration after FCM administration was analysed (*figure 3*). The delta phosphate concentration (difference before and after FCM) were not correlated with the renal transplant function (Spearman rho = 0.13, p = 0.56).

Next, potential risk factors for the development of hypophosphataemia following FCM infusion were investigated. The change in serum phosphate concentrations (i.e. the difference before and after FCM administration) was analysed in relation to the baseline phosphate level (at the time of FCM administration), renal function, time after transplantation, and type of donor (living vs. deceased donor). These results are described in *table 3*. Taken together, patients who developed hypophosphataemia following FCM treatment had a shorter time after transplantation and had better renal function compared with patients who did not develop hypophosphataemia. Baseline serum phosphate concentration and type of donor were not different between patients with and without hypophosphataemia.

DISCUSSION

Kidney transplantation often only partially reverses the profound alterations in calcium, phosphate, and vitamin D metabolism that characterise CKD. Many kidney transplant recipients will develop a 'tertiary hyperphosphatonism' with persistently elevated FGF-23 concentrations, decreased calcitriol concentrations, and inappropriate kidney phosphate wasting resulting in hypophosphataemia.[15] It is believed that the bones are the source of the excessive FGF-23 production but it is unclear why they continue to release FGF-23 after transplantation.[15,31] Although tertiary hyperparathyroidism may contribute, FGF-23 may

Figure 3. *Relationship between renal transplant function and the change of phosphate concentration after FCM administration*



eGFR = estimated glomerular filtration rate; Δ serum phosphate: difference of phosphate concentration before and after FCM.

be the most important determinant of post-transplant hypophosphataemia.[15,20,31] In case 2 of our series, FGF-23 concentrations remained elevated ten months after kidney transplantation.

Previous studies reported an increase in FGF-23 concentrations after intravenous iron supplementation in non-CKD patients with iron-deficiency anaemia. This was followed by a transient and asymptomatic reduction in serum phosphate.[6,9,11,32] Likewise, in non-dialysis dependent CKD patients and in patients on haemodialysis, a transient and asymptomatic decline in serum phosphate concentration after FCM administration has been reported which may persist for up to three months.[17-19,33] In CKD patients, FGF-23 concentrations are often elevated and FCM administration seems to further increase these concentrations. Our observations are in line with these findings and suggest that FCM also induces kidney phosphate wasting with hypophosphataemia after kidney transplantation and that this is likely mediated by FGF-23. In our population, 56.5% of the patients developed hypophosphataemia after a single dose of FCM. In the two patients in whom FGF-23 was measured, increased concentrations of FGF-23 were observed after FCM administration followed by a decline that was mirrored by normalisation of serum phosphate concentrations. The increase in PTH and decrease of calcitriol concentrations can also be explained by increased FGF-23 activity.

Only few data exist in the literature regarding the risk of kidney transplant recipients to develop hypophosphataemia after FCM therapy. Mani et al.[21] and Blazevic et al.[22] each reported a case of FCM-induced hypophosphataemia after kidney transplantation. The series reported here describes the largest cohort of kidney transplant recipients developing a reduction of serum phosphate concentration after FCM administration and the index cases point towards an important role of FGF-23 in the pathophysiology. Our observation that patients who developed hypophosphataemia after FCM treatment were more recently transplanted and had a better renal function is in line with this hypothesis. Following a successful kidney transplantation, the inability to induce phosphaturia may be reduced and the nephrons may be more sensitive to FGF-23. In a good transplant function it may lead to hypophosphataemia when FGF-23 concentrations are high.

Both iron infusion and iron deficiency appear to stimulate FGF-23 production by osteocytes. Recent studies suggest that iron-deficiency anaemia is associated with normal concentrations of hormonally active, intact FGF-23 (iFGF-23) but markedly elevated inactive cFGF-23 concentrations without influence on the phosphate metabolism.[9,34,35] FCM may increase circulating concentrations of iFGF-23 by inhibiting its cleavage within osteocytes into cFGF-23. Although the mechanism remains unclear, it seems that FCM disrupts the balance between FGF-23 production and cleavage within osteocytes.[9,11,34,35]

The patients described in the current study received a variety of single doses of FCM. Of note, also the one patient who received a single 100 mg FCM dose developed

hypophosphataemia. In one of the three patients receiving 500 mg FCM, the phosphate concentration decreased below the reference value. Observations made in the FIND-CKD study[36] suggest that the risk of reduction in phosphate concentration after FCM is dose-dependent. Apart from being dose-dependent, the risk of developing hypophosphataemia appears to be higher when FCM is prescribed as compared with other intravenous iron preparations.[17-19,33]

It is unknown whether persistent hypophosphataemia after kidney transplantation is merely a laboratory peculiarity or whether it has clinical implications. Hypophosphataemia after kidney transplantation is usually asymptomatic and frequently resolves with time although it may persist for more than a year after transplantation.[20,37] It may be that chronic kidney phosphate wasting, in addition to traditional risk factors such as the use of glucocorticoids, contributes to the increased fracture risk of kidney transplant recipients.[38-40] In our patients, FCM-induced hypophosphataemia did not cause clinical symptoms and resolved spontaneously. However, this was a retrospective analysis and not a prospective study. Therefore mild symptoms and complications following FCM administration may not have been recorded in the patient files. Reports from the literature have indeed suggested that FCM-induced hypophosphataemia may not always be harmless and may necessitate hospital admission and intravenous phosphate supplementation.[21,22,41] In addition, repeated administration of FCM, leading to chronic phosphate wasting, might cause osteomalacia.

It is not clear how to manage post-transplant hypophosphataemia. In analogy to CKD, vitamin D is often prescribed, especially when PTH concentrations are elevated. However, calcitriol stimulates FGF-23 secretion and may thus maintain renal phosphate wasting. Likewise, phosphate supplements may induce FGF-23 and could also increase the risk of kidney calcium-phosphate depositions and kidney stones. Frequent administration of FCM in kidney transplant recipients might further increase this risk. In such cases, close monitoring of phosphate concentrations and prudent use of FCM seems warranted.

Our study has several limitations. First of all, this was a retrospective analysis and the number of cases is relatively small; FGF-23 concentrations were only measured in two index patients. Second, blood sampling following FCM administration was not standardised. As a result, hypophosphataemia (or its nadir) may have been missed in some patients. Finally, both active iFGF-23 and cFGF-23 were measured by the C-terminal FGF-23 assay we used. This precludes a deeper understanding of the pathophysiology of FCM-induced hypophosphataemia and the role of intact FGF-23 and its fragments therein.

CONCLUSION

Correction of iron-deficiency anaemia after kidney transplantation with FCM may induce renal phosphate wasting and hypophosphataemia in as many as 56.5% of patients. This phenomenon may be explained by increased concentrations of FGF-23. The hypophosphataemia following a single dose of FCM administration was transient. Nonetheless, prudent use of FCM seems warranted and close monitoring of such patients seems advisable, especially when chronic therapy with FCM is indicated.

DISCLOSURES

The authors declare that they have no conflict of interest and no relevant financial interests.

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ORIGINAL ARTICLE

Kidney transplantation in patients declined by other centres

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ABSTRACT

Background: Transplant centres show considerable disagreement in the acceptance of transplant candidates with relative contraindications. The aim of this study is to investigate the outcomes of our patients who had been refused at other centres prior to transplantation at our centre.
Methods: We included patients who had been excluded from transplantation or wait-listing at other centres before referral to our centre. We scored the reasons for refusal at other centres, the type of transplantation procedure, postoperative and long-term complications, patient and graft survival and how these patients experienced the transplantation and quality of life at our centre. All regular patients transplanted in 2010 functioned as a control group for outcome parameters.
Results: We identified 23 patients in the period from January 2000 until March 2013. The most frequent reason for the refusal at other centres was obesity. Twenty of the 23 patients (87%) were alive and 19 had a functioning graft (83%) after a median follow-up of 21.0 months after transplantation (range 11.0-48.9). There were significantly more wound-related problems in the study group as compared with the control group (p = 0.029), but their kidney function at one year after transplantation was not significantly different. The patients indicated an improvement of quality of life after transplantation and in general were satisfied with the transplantation.
Conclusions: Patients who had previously had been denied transplantation at other centres generally did well after kidney transplantation with an increased risk of wound complications but a satisfactory graft and patient survival.

KEYWORDS

Dialysis, relative contraindications, survival, transplantation

INTRODUCTION

Current guidelines for the evaluation of renal transplant candidates consist of various recommendations with relative and absolute contraindications for transplantation.[1,2] These guidelines try to balance the possible advantages of transplantation with the risk of the surgical intervention and immunosuppression. Additionally, the selection of transplant candidates has to take into account the shortage of available organs by trying to avoid allocating scarce organs to patients with a short life expectancy or a high risk of early graft failure.
However, even in patients with severe comorbidity, the life expectancy may still be significantly better after transplantation compared with remaining on dialysis.[3-5] Previous studies have reported that kidney transplant recipients have better health-related quality of life than transplant candidates maintained on haemodialysis.[6-8] Transplantation is the treatment of choice for end-stage renal disease; it increases survival and quality of life, while being more cost-effective than dialysis.[9-12]
There is a considerable variation between Dutch transplant centres concerning the acceptance of patients with relative contraindications for transplantation. A general consensus exists concerning the refusal of patients with severe disease limiting the life expectancy to less than five years.[13] However, guidelines and medical practice are less clear with relative contraindications such as obesity, cardiovascular disease and old age. Obesity is one of the major reasons for not putting patients with end-stage renal disease (ESRD) on the renal transplant waiting list.[14]
Over the past years, the kidney transplant program of the Erasmus MC, Rotterdam has adopted a rather liberal policy to accepting transplant candidates with relative contraindications for transplantation. In the course of this approach, numerous candidates who were refused at other

transplant centres were referred to our centre resulting in kidney transplantation in many of them.
The aim of this study is to describe and investigate the outcomes after kidney transplantation in these patients. Additionally, we were interested if these patients judged their quality of life as improved and whether they, in retrospect, are satisfied with the decision to undergo a kidney transplantation despite earlier contrary advice.

MATERIALS AND METHODS

Study design and endpoints

This investigation was a retrospective study that was designed to identify all patients referred from outside our adherence area. First, we identified all patients who had been referred from outside our regular referral area from January 2000 until March 2013. Those patients who had been declined for transplantation or wait-listing at other transplantation centres were included. We excluded patients with: missing information about the reason for declining transplantation, patients with combined liver-kidney transplantation, ABO incompatible transplantation, and patients who chose for our centre after acceptance at another centre. We used our electronic patient information system to identify the reasons for referral. Follow-up data were retrieved from the electronic patient information system and our own transplantation database. We recorded the type of transplantation procedure (living versus deceased donor), postoperative complications (e.g. wound healing problems, infections, cardiovascular complications etc.), long-term complications, renal function (creatinine, estimated glomerular filtration rate (eGFR)) at 3 and 12 months as well as patient and graft survival. Delayed graft function was defined as dialysis treatment performed within seven days after transplantation. As a representative control group we used all patients transplanted in 2010 who were primarily referred to our centre and we noted the duration of initial hospitalisation and additional hospitalisation in the first year after transplantation, wound problems, creatinine and eGFR after 3 and 12 months.
Additionally, the patients were interviewed to evaluate their perceived quality of life after transplantation and asked to compare their post-transplant situation with the situation before transplantation.

All clinical information obtained in the study was considered to be confidential and was used only for research purposes. Patient data were stored in an anonymised fashion. All patients participating in the quality of life questionnaire provided written informed consent. This study did not require institutional ethics committee review.

Statistical analysis

Patient characteristics, such as BMI, number of previous transplantations, as well as outcome measures were presented as means / medians ± standard deviation or interquartile range as appropriate. Hospitalisation duration, differences in creatinine and eGFR were compared with the t-test or the Mann-Whitney U-test as appropriate. Differences in categorical variables between the study and control group were analysed with the chi-square test. All analyses were performed using SPSS 21.0 and statistical significance was defined as $p < 0.05$.

RESULTS

Initially 30 patients, who were referred to our centre in the period from January 2000 until March 2013, were identified for inclusion in the study. Of these, six were excluded from the study because they had been referred to our centre because of live donor-associated problems. One additional recipient was excluded as she indicated that she had actively chosen to be transplanted at our centre and had not been denied transplantation at the initial centre. The 23 remaining patients were included (*table 1*). A total of 21 transplantations were performed from 2007 until 2013. The remaining two were performed in 2001 and in 2004. The group consisted of 16 male (69.6%) and seven female patients (30.4%). The majority were transplanted with a living-unrelated donor (39.1%). Various reasons for refusal at other centres were identified, such as obesity, malignancies, old age, hyperparathyroidism and overall poor condition. In six patients more than one reason for refusal was given in the decision letter. Obesity was the most important reason for refusal in seven patients (30.4%), with a BMI that ranges from 35.0-42.1 kg/m^2. Five patients were denied transplantation because of malignancies in the past: a successfully removed renal cell tumour three years before transplantation, smouldering multiple myeloma, recurrent pheochromocytoma and focal segmental glomerulosclerosis, skin cancer and an ovarian carcinoma which was successfully treated with chemotherapy and surgery 14 years before, and a patient with an oesophageal carcinoma in 1990, which was successfully treated, but still had serious ongoing skin cancer with removal of multiple squamous cell skin cancers in the past.
The median age at the moment of transplantation was 54 years (range 44.0-61.0). At baseline, 14 patients had diabetes mellitus (60.9%) and 8 of the 23 patients (36.4%) had a BMI > 35 kg/m^2. Three patients (13.0%) underwent a pre-emptive transplantation while 12 patients (52.2%) were on dialysis treatment for more than three years at the moment of transplantation. The median follow-up was 21.0 months (range 11.0-48.9) and two patients died in

Table 1. *Baseline characteristics*

Variable	Study population Absolute numbers (%)	Control group Absolute numbers (%)
Participants	23	172
Male	16 (69.6)	115 (66.9)
Reason for the refusal[1]		
BMI	7 (30.4)	-
Age	2 (8.6)	-
High PTH-level	2 (8.6)	-
Overall poor condition	4 (17.3)	-
Cardiac comorbidity	2 (8.6)	-
Medication non-compliance	2 (8.6)	-
MGUS, skin tumours, renal tumour Other reasons[2]	1, 2, 1 (17.3) 4 (17.3)	-
Median age at transplantation	54	54.5
BMI (kg/m²) (n = 22)[3]		
< 18.5	0 (0)	3 (1.7)
18.5 - 25	6 (27.3)	77 (44.8)
30-35	4 (18.2)	32 (18.2)
> 35	8 (36.4)	7 (4.1)
Primary cause of ESRD		
Glomerulonephritis	5 (21.7)	43 (25.0)
Hereditary	2 (8.7)	17 (9.9)
Diabetic nephropathy	11 (47.8)	24 (14.0)
Hypertensive nephropathy	3 (13.0)	34 (19.8)
Others[4]	2 (8.7)	54 (31.4)
Dialysis vintage at transplantation		
Mean (in months)	32	19.6
Preemptive	3 (13.0)	52 (30.2)
Diabetes mellitus	14 (60.9)	35 (20.3)
NODAT[5]	2 (22.2)	n.a.
First transplant	20 (87.0)	142 (82.1)
Type of donor		
DBD	4 (17.4)	35 (20.3)
DCD	4 (17.4)	14 (8.1)
Living – related	6 (26.1)	63 (36.6)
Living – unrelated	9 (39.1)	60 (34.9)

[1]N is 27 as there were multiple reasons for refusal in some patients; [2]Caroli syndrome, risk of recurrence of primary disease (FSGS), non-active hepatitis B, severe hypertension; [3]Height is missing in 1 patient; [4]Haemolytic-uraemic syndrome, chronic pyelonephritis; [5]9 patients at risk ,16 had diabetes mellitus prior to transplantation. n.a. = not available; BMI = body mass index; PTH = parathyroid hormone; MGUS = monoclonal gammopathy of undetermined significance; ESRD = end-stage renal disease; NODAT = new-onset diabetes after transplantation; DBD = donation after brain death; DCD = donation after circulatory death.

the follow-up period. One patient died ten days after the transplantation as result of a major stroke; another patient died 11 weeks after transplantation because of a cardiac arrest.

We compared our study group with a control group consisting of patients who were primarily referred to our centre and transplanted at our centre in 2010 (*table 2*).

Delayed graft function was seen significantly more often in the study population (p = 0.001) while the incidence of rejection treatment was not significantly different (p = 0.200). Immunosuppression consisted of induction with basiliximab plus tacrolimus and a mycophenolate mofetil (MMF) maintenance treatment. Steroids were withdrawn in all patients at three months after transplantation. There was no significant difference between the study and control group regarding mean second ischaemic time: 28.6 vs. 24.2 minutes respectively (p = 0.115).

The group of patients that had been declined at other centres had a significantly higher BMI as compared with the control group, 31.0 vs. 25.5 (p = 0.002), and the study group experienced more wound-related problems (p = 0.029): three patients in our study group had delayed wound healing, all without fascial dehiscence. They were seen frequently for wound inspection and within one year after transplantation all wounds had healed. One out of the three patients with delayed wound healing and one other patient experienced wound infections, which were treated with antibiotics and in one patient surgical exploration was required. After surgery the patient did well and the wound healed promptly. Interestingly, the patients with wound infections all had a normal BMI, but did have diabetes mellitus.

Kidney function one year after transplantation was comparable in both groups. Remarkably, there were no significant differences in rejection rate, patient death and death censored graft loss in the first year after transplantation.

Next to the clinical follow-up, we were interested in how patients rated their quality of life and how they looked back at their transplantation (*figure 1*). Eighteen of 20 living patients were willing to participate in the interview. Before transplantation, patients mainly mentioned limitations in everyday life such as: not being able to work, not having enough energy to walk long distances, to do the daily shopping and housekeeping. Only one patient experienced the preparations for the transplantation as a burden, while 13 patients (72.2%) did not. Fourteen patients (77.8%) reported an improvement in their health since the transplantation, three considered their health to be unchanged after transplantation and one 23-year-old patient thought his health had worsened since transplantation: in the two years following transplantation he experienced multiple ischaemic strokes. Most patients reported feeling less tired and thought that their general physical condition had improved. A number of patients mentioned that the most important change was that dialysis no longer dominated their lives. Sixteen of the 18 patients (88.9%) who completed the questionnaire would choose for kidney transplantation again if they were in the same situation.

Table 2. *Outcomes study and control group*

	Study group			Control group			
	N	Median	IQR	N	Median	IQR	P – value
Age at moment of tx	23	54.00	(44.00 - 61.00)	172	54.50	(42.25 - 65.00)	0.856
BMI (kg/m²)	22	30.95	(26.07 - 35.96)	172	25.49	(22.55 - 29.29)	0.002
eGFR at 3 months after tx	22	42.00	(33.00 - 50.50)	168	45.00	(35.00 - 56.75)	0.478
eGFR at 1 year after tx	16	42.00	(36.25 - 46.75)	157	47.00	(36.50 - 60.00)	0.232
Initial hospitalisation (in days)	23	16.00	(12.00 - 26.00)	172	15.00	(13.00 - 19.00)	0.694
Total hospitalisation (1st year)	16	23.00	(16.50 - 50.25)	164	20.00	(15.00 - 33.75)	0.469
Wound-related problems	23	6 (pts)	26.1%	172	16 (pts)	9.3%	0.029
Delayed graft function	23	11 (pts)	47.8%	172	28 (pts)	16.3%	0.001
Patients treated for rejection	23	6 (pts)	26.1%	172	30 (pts)	17.4%	0.200
Patient death < 1 y	23	2 (pts)	8.7%	172	8 (pts)	4.7%	0.316
Death censored graft loss < 1 y	23	1 (pts)	4.3%	172	4 (pts)	2.3%	0.456

Tx = transplantation; BMI = body mass index; eGFR = estimated glomerular filtration rate; pts = patients.

Only one patient felt that he would not undergo the kidney transplantation again, it was the same patient as mentioned above, mainly because of a negative change in the relationship with his living donor, and other family members. Fourteen patients (77.8%) reported a perceived improvement of their energy in everyday life.

Additionally we performed a Cox proportional hazard analysis for the risk of death and graft failure uncensored for death. Age and study population were included as variables (*figure 2*). Our study population had a significantly higher relative risk for graft failure uncensored for death (RR: 2.3, CI: 1.1-4.5, p = 0.02) compared with the control group. The Cox analysis for patient survival showed that the study population had a significantly higher risk compared with the control population. (RR = 2.9, CI: 1.3-6.8, p = 0.013) (*figure 3*).

DISCUSSION

This study shows the outcomes of patients who were transplanted at our centre after they were denied access to transplantation at other transplant centres in the Netherlands. Of the 23 patients included, 20 are still alive and 19 have a functioning graft at a median follow-up of 21.0 months. Patients in the study group had significantly more wound-related problems when compared with the control group. However, the kidney function was not significantly different between the two groups. The length of the initial hospitalisation and total hospitalisation duration in the first year after transplantation was not significantly higher in the study group.

The ideal control group for this study would be a group with a similar risk profile, but who remained on dialysis. As we do not have a matched group on dialysis, it is difficult to make definite conclusions on the survival and morbidity of our patient population. However, it does seem safe to speculate that a survival rate of 86% after a median follow-up of 22 months is quite favourable in this group of ESRD patients with a large proportion of diabetes. It seems improbable that the mortality would have been as low if these patients had remained on dialysis.

In the study the amount of wound problems was significantly higher than our standard transplant population. This is probably explained by the higher BMI when compared with the control group, which is a well-known problem.[15] However, these wound complications had all resolved within six months after

Figure 1. *Patients were asked to rate four statements concerning their kidney transplantation (18 of the 20 patients alive participated in the interview)*

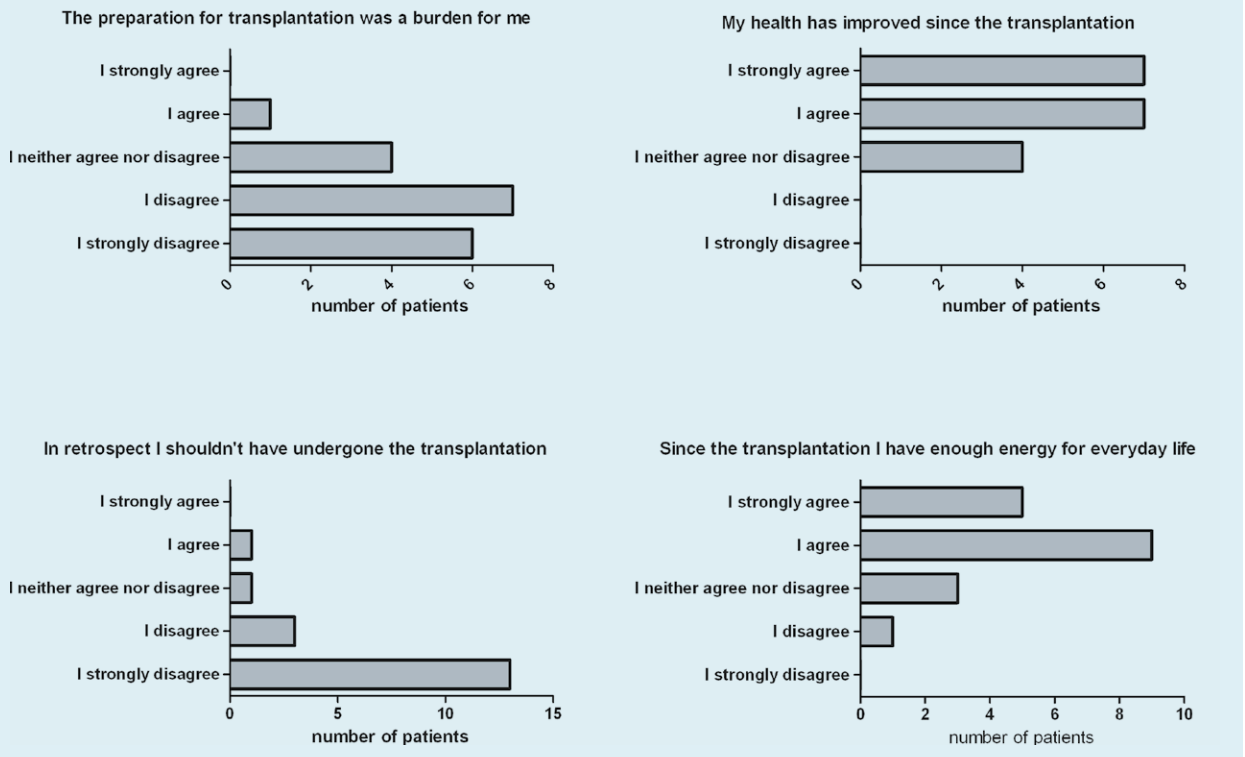


Figure 2. *Cox proportional hazard analysis for graft survival uncensored for death. Age and study population were included as variables (p = 0.02)*

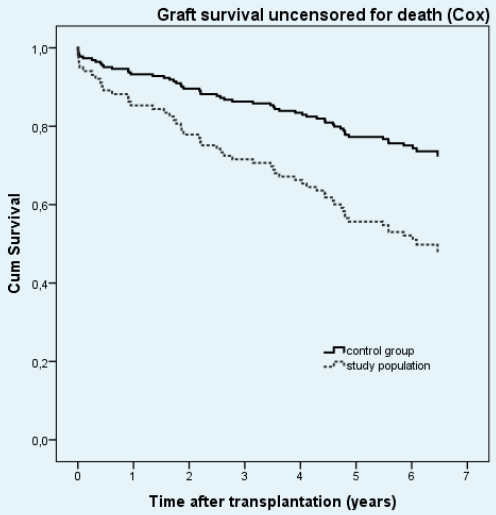
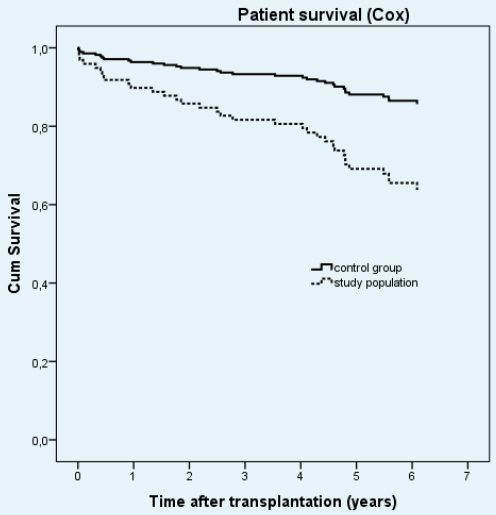


Figure 3. *Cox proportional hazard analysis for patient death. Age and study population were included as variables (p = 0.013)*



transplantation and did not cause long-term morbidity. Transplantation seems to be an appropriate treatment for these patients in the study group and probably resulted in improved survival when compared with the initial decision to deny these patients access to transplantation.

BMI was a frequent reason for other transplant centres to turn these patients down. The number of obese kidney transplant candidates has been growing in the last years.[15-18] Obesity is perceived as a relative contraindication for listing and receipt of renal transplantation and many transplant professionals have been reluctant to offer transplantation to obese candidates because of the risk of surgical complications after transplantation and poorer survival when compared with non-obese transplant recipients.[15,16] However, the impact of obesity on renal transplantation has not been clearly defined.[4] It has been shown that recipient obesity is associated with an increased risk for delayed graft function and local wound complications,[15,19] which is in keeping with the findings in our study group. However, our patients felt that their quality of life was improved by transplantation and they did not report the wound complications as an important burden. Another impact of BMI/ morbid obesity (> 35 kg/m^2) is longer hospitalisation compared with patients with a 'normal' BMI (20-25 kg/m^2).[18,19] We did not see a significant difference in hospitalisation duration when compared with the control group. However, the effect of obesity on hospitalisation may be masked by a relatively long standard length of initial hospitalisation in our program. Older age is another relative contraindication which is an important predictor of kidney transplantation outcomes. The demand for kidney transplantation among the ESRD population of 65 years and older is growing.[20] Elderly recipients, > 65 years, experience more infectious complications, have a lower crude graft and patient survival and a higher risk of mortality, but experience less acute rejection.[11,21] The patients in our cohort who were refused at other centres because of high age are all still alive with a functioning graft after a median follow-up of 21.0 months. We think that the transplantation was a suitable choice for these patients because of the expected high mortality[22] on dialysis and the perceived improvement of their health after transplantation reported by the elderly patients in our cohort. Transplantation in older patients has been shown to be appropriate for a large proportion of elderly patients with renal failure.[3,20]

Malignancies in the recent past (< 5 years) was another reason for refusal. Two of the four patients identified had had a previous transplantation and one of them had recurrent skin tumours after his transplantation within our series. The other three patients did not experience malignant complications within the studied period. All four patients are still alive with a functioning graft.

Figure 2 shows graft survival uncensored for death in the study population of 50% after six years. This was significantly worse than the survival of the unmatched control group. However, as mentioned above, is seems reasonable to speculate that survival would have been poorer when these high-risk patients had remained on dialysis. A recent study of our group confirms that patients with extensive comorbidity enjoy a remarkably good graft and patient survival: 50% of those with the highest comorbidity score survived more than ten years after transplantation, while graft survival was not different from patients with lower comorbidity scores.[23]

Importantly, the interviews demonstrate an improvement of the perceived quality of life after transplantation and almost all patients were still content with the decision to undergo the transplantation despite contrary advice by another transplant centre.

One of the limitations of the study was the small sample size of our identified recipients. Another limitation is the retrospective nature of the analysis. Especially during the interviews, recall bias is a problem as the transplantations had often been performed several years ago. Additionally, the quality of life analysis may be biased due the fact that three patients had died and two patients refused to participate. As mentioned above we were not able to identify a suitable control group on dialysis. Furthermore, the follow-up period was limited. Despite these limitations, we feel that this study provides some insight into an important problem in kidney transplantation.

In conclusion, the acceptance of patients who were declined for kidney transplantation at other centres resulted in successful transplants with high patient satisfaction. Our findings indicate that our current criteria for the acceptance of transplant recipients are far from stringent and that individualisation and shared decision-making are important tools in this process.

DISCLOSURES

The authors declare no conflicts of interest.

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CASE REPORT

Chronic use of metamizole: not so safe after all?

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ABSTRACT

Metamizole can be used in both short- and long-term pain relief therapies and has a relatively favourable safety profile compared with classic NSAIDs. Metamizole is also infamous because of its potential fatal adverse drug reaction, agranulocytosis. Although this risk varies, it is estimated to occur in less than one million metamizole prescriptions. We describe a case of a 68-year-old patient who developed leukopenia after using metamizole.

KEYWORDS

Metamizole, analgesic, agranulocytosis

What was known on this topic?
Metamizole is a non-opioid painkiller, usable for short- and long-term pain relief. The side effect profile of metamizole is favourable, except for the life-threatening side effect agranulocytosis. The occurrence of agranulocytosis is still not precisely known but is estimated to be less than one million metamizole prescriptions.

What does this add?
In spite of the rareness, be aware of agranulocytosis as a side effect of metamizole.

INTRODUCTION

Metamizole (dipyrone) is a compound with analgesic, antipyretic and spasmolytic effects. Metamizole is infamous because of the potential fatal adverse drug reaction, agranulocytosis, with a mortality rate of 23.6%.[1] This adverse event was already discovered in 1922.[2] Several studies indicated a varying risk of agranulocytosis ranging from less than one per million users within one week to one per 1439 metamizole prescriptions.[3] These differences in agranulocytosis risk attributable to metamizole have led to different policies worldwide with regard to the use of metamizole.[4] In some countries metamizole was withdrawn from the market, in other countries it is still available on prescription or as an over-the-counter medication.[1] The Dutch Association of Anaesthesiology propagated the use of metamizole in their revised postoperative pain guideline in 2012.[5] Also the number of side effects has been increasing since 2011.[6] Our case demonstrates that the use of metamizole can cause leukopenia resulting in a severe infection.

CASE DESCRIPTION

A 68-year-old Spanish patient, visiting the Netherlands, was admitted to the hospital because of acute onset of diarrhoea. In his previous history he suffered from diverticulitis, critical ischaemia of both legs due to lower extremity peripheral artery disease, atrial fibrillation and deep venous thrombosis. He used the following medications: acenocoumarol, furosemide, pantoprazole, metamizole and foster.

The patient suffered from dyspnoea and general malaise a few days before admission. The day before admission he had eaten a pizza and since then he had watery stools 14 times daily without blood or mucus. He did not notice any fever at home. His appetite had decreased but he did not complain of abdominal pain or nausea. His travel history was unremarkable other than the Netherlands and Spain.

Physical examination showed a blood pressure of 130/66 mmHg, pulse rate of 80/min, a respiratory rate of 25/min and a temperature of 39.1 °C. Further physical examination showed no abnormalities.

At admission, the following laboratory findings were observed: haemoglobin 10.2 mmol/l; thrombocyte count 196 x 10^9/l; leukocyte count: 0.3 x 10^9/l with 0.1 x 10^9/l neutrophils and no other abnormalities in the white cell differential; urea 5.4 mmol/l; creatinine 75 µmmol/l; eGFR > 60 ml/min and CRP of 264 mg/l. Chest X-ray and abdominal CT revealed infiltrate in the left lower lobe of the lung and some diverticulosis without signs suggestive of inflammation. Intravenous broad-spectrum antibiotics were administered for ten days. Repeated blood, faecal, sputum, and urine cultures showed no abnormalities.

Striking was the neutropenia, while the patient had no history of immunocompromising disease which could explain the neutropenia/leukopenia. We performed a bone marrow biopsy, which showed reduced myelopoiesis, suggestive of a reactive process. Malignancy was excluded. Because of the isolated neutropenia /leukopenia and no thrombocytopenia and/or anaemia, the cause of the leukopenia was probably toxic or due to medication. He had used metamizole for four years because of a painful hip and knee, 1000 mg twice daily. There was no other history of over-the-counter medication. We stopped the metamizole during admission. Three days after stopping the metamizole, the leukocyte count normalised. In retrospect, the symptoms in this immunocompromised patient could be explained by atypical pneumonia with diarrhoea. Microorganisms causing pneumonia with diarrhoea as a symptom are for instance *Legionella pneumophila*, chlamydia pneumonia or *C. pneumoniae*.[7] Unfortunately, in this case we could not find the microorganism.

We concluded that this patient had leukopenia/neutropenia as a result of the use of metamizole with an atypical pneumonia as a complication of the agranulocytosis. In the following seven days his dyspnoea decreased and his stool pattern normalised. His fever resolved with administration of broad-spectrum antibiotics but only after the neutrophil count had normalised. Patient was discharged from the hospital two weeks after admission.

CONSIDERATION

Our case demonstrates a rare adverse event, agranulocytosis, after the use of metamizole. The causality between agranulocytosis and metamizole in this case can be classified as probable. This is because a positive dechallenge reaction was observed on drug withdrawal and the agranulocytosis was unlikely to be attributed to other causes.[4]

Remarkable is that the patient had already been using metamizole for four years, without any episodes of severe infection. However, agranulocytosis caused by metamizole is a hypersensitivity reaction. Exposure over a long period of time with high doses increases the probability for sensitisation and later development of metamizole-related agranulocytosis.[8]

After stopping the metamizole, the patient's leukocyte count normalised within three days. This seems a rather quick recovery, yet not unusual for metamizole-induced agranulocytosis. The white blood cell (WBC) count is able to respond as early as 48 hours after cessation of metamizole, though more commonly the WBC will normalise within one week. Normalisation of the WBC can take as long as one month.[9]

In 2012 the Dutch Association of Anaesthesiology published a revised guideline on postoperative pain. They state metamizole is an effective analgesic with less side effects than classic nonsteroidal anti-inflammatory drugs (NSAIDs). They recommend the use of metamizole as an alternative for patients with a contraindication for the classic NSAID.[5] According to the Drug Information System of National Health Care Institute (GIP database) 18 patients used metamizole in the Netherlands in 2014, four more than in 2013.[10] Since 2011, the Netherlands Pharmacovigilance Centre Lareb has reported 16 side effects of metamizole, of which three patients with neutropenia, two with leukopenia and two with thrombocytopenia. In the period from 1996 to 2010, only one notification was made of a side effect (unclear which side effect) in Lareb.[6]

Metamizole is classified as a drug NSAID, due to the (poor) anti-inflammatory effect.[11] In the analgesic ladder from the World Health Organisation, metamizole is classified in class 1 as monotherapy, as are the classical NSAIDs. In addition, metamizole can also be used as combination therapy in class 2 (weak opiates) or in class 3 (strong opiates).[12]

Metamizole is a prodrug and is hydrolysed and absorbed in the stomach to its main metabolite 4-N-Methylaminoantipyrin (MAA). MAA will be converted to a variety of metabolites.

Although the mechanism of action of metamizole has been examined, as yet it is not completely understood. On one hand, it seems that the peripheral antinociceptive effect of metamizole is caused by an inhibition of cyclooxygenase (COX) whereby COX 2 is more inhibited than COX 1. On the other hand, the analgesic effect of metamizole is associated with a less anti-inflammatory effect in comparison with NSAIDs. This suggests that the antinociceptive effect is in part regulated by central mechanisms.[2] The antipyretic effect of metamizole is purely based on a central COX-dependent effect.[12]

The most important adverse event of metamizole is agranulocytosis. Agranulocytosis is characterised by a neutrophil granulocyte count below 0.5 x 10^9 cells/l and normal counts in other blood cell types.[4]

Regarding the pathophysiology, two types of agranulocytosis are described: an immune reaction and a toxic reaction. In immune-mediated agranulocytosis the drug, or a metabolite of the drug, binds irreversibly to the neutrophil membrane. In doing so, T cells or antibodies are produced which induce lysis of the cell.[9,13] In the toxic reaction, the drug directly damages the myeloid precursor cells.[13]

Metamizole induces agranulocytosis via an immune reaction. The degradation of metamizole eventually results in an antibody reaction.[9,13]

DISCLOSURES

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CASE REPORT

Fatal rupture of a mycotic aneurysm of the right coronary artery post pneumococcal sepsis

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ABSTRACT

Aneurysms of the coronary arteries are rare and mycotic coronary aneurysms are even rarer. We report a unique, yet unfortunately autopsy-proven fatal case of a ruptured atherosclerotic mycotic aneurysm of the right coronary artery with streptococcus pneumoniae in a non-immunocompromised patient resulting in cor tamponade and death.

KEYWORDS

Coronary aneurysm, mycotic aneurysm, streptococcus pneumoniae

What was known on this topic?
Mycotic coronary aneurysms with streptococcus pneumoniae are rare and have only been described in immunocompromised patients.

What does this add?
We describe a fatal case of a ruptured mycotic aneurysm of the right coronary artery with streptococcus pneumoniae in a non-immunocompromised patient. With this case report we wish to emphasise that one cannot safely exclude the possibility of persistent infection, even after 'adequate' antibiotic therapy of a susceptible and even common micro-organism, as there can be occult hiding places – in this case a coronary aneurysm – where antibiotic treatment had not been sufficiently effective.

INTRODUCTION

Coronary aneurysms are a rare finding. The incidence ranges from 1.5% in autopsy studies to 4.9% in angiography studies.[1,2] The most common cause of coronary aneurysm in adults is atherosclerosis and this is found in about half of the cases. Mycotic coronary aneurysms are a rare cause and the exact incidence is unknown. Other causes include congenital, autoimmune arteritis, emboli and in more recent times iatrogenic causes, such as angioplasty.

The first mycotic coronary aneurysm was described by Morgagni in 1761 and was due to syphilis infection.[1] Despite its early historic discovery, up until now only about 150 cases have been published. To our knowledge only two previous studies have reported a coronary mycotic aneurysm with the causative agent being *Streptococcus pneumonia*. However both cases involved immunocompromised patients.[3,4] We report a unique autopsy-proven case of a ruptured mycotic coronary aneurysm due to *S. pneumoniae* in a non-immunocompromised patient.

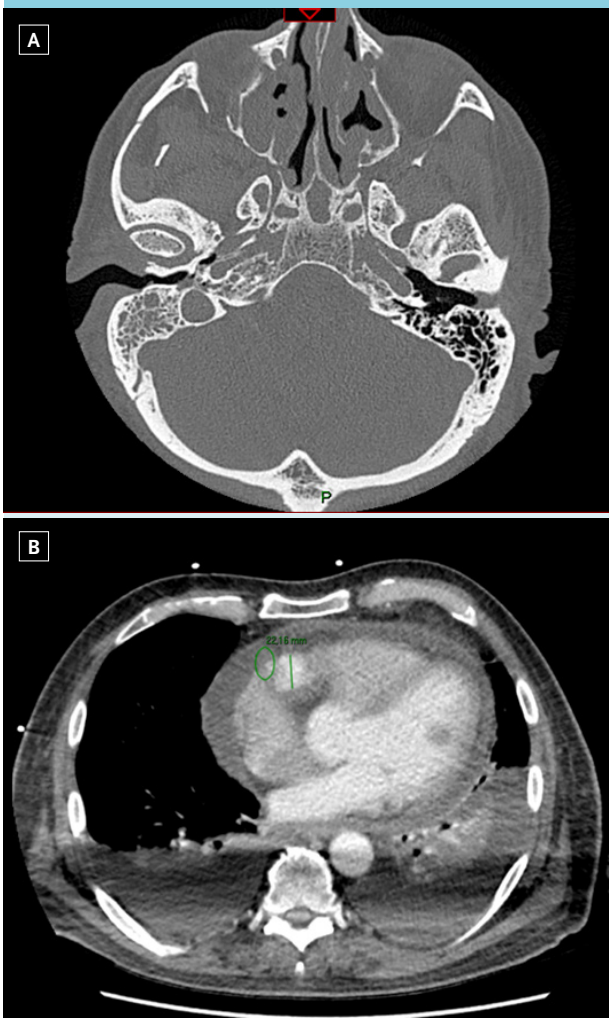
CASE REPORT

A 76-year-old man, with no known cardiovascular history, presented to the emergency ward with a shifting pain of the neck which had started out as chest pain five days previously. In addition fever (39.1 °C), chills, and erythema of the right lower arm and left wrist were noticed. The patient reported a history of an infection of the right ear two weeks before presentation. Physical examination revealed proximal muscle weakness, stiffness and severe neck pain. A neurologist was consulted but considered the symptoms to be unspecific for meningitis and so a lumbar puncture was not performed. Subsequently MRI of the brain and total spine, followed by CT brain revealed right-sided mastoiditis with intact bone structures (*figure 1A*). Because of persistent neck pain and the fear of meningitis a mastoidectomy was performed. The patient was initially treated with intravenous amoxicillin/

clavulanic acid and ciprofloxacin. After admission treatment was switched to clindamycin and ceftriaxone to treat possible meningitis and later to penicillin G when the initial set of blood cultures yielded a penicillin-sensitive strain of *S. pneumoniae*. All subsequent blood cultures collected during antibiotic treatment remained negative. Antibiotics were switched to ciprofloxacin and ceftriaxone after ten days when the patient showed no improvement; despite negative cultures and assumed adequate antibiotic therapy the patient had persistent intermittent fever and elevated CRP, therefore septic emboli were considered. Transthoracic echocardiography (TTE) was performed which showed normal findings. Transoesophageal echocardiogram (TEE) a few days later showed an abnormality in close relationship with the right ventricle at first considered to be an abscess. However, CT of the chest revealed a coronary aneurysm, 2 cm in diameter, of the right coronary artery along with a small amount of pericardial fluid and pleuritic fluid (*figure 1B*). A pleuritic puncture was performed due to fear of empyema but both gram stain and cultures remained negative. The following night the patient was found with no vital signs. Cardiopulmonary resuscitation was unsuccessful.

Post mortem findings revealed the cause of death to be cardiac tamponade due to rupture of an atherosclerotic aneurysm with a diameter of 3 cm in the right coronary artery. There were no signs of pericarditis or endocarditis. Polymerase chain reaction performed on the formalin-fixed paraffin-embedded tissue of the wall of the aneurysm was positive for *S. pneumoniae* and on microscopic examination diplococci were found (*figure 2A-C*).

Figure 1. *A) Computed tomographic (CT) scan of the brain showing right-sided mastoiditis. B) CT scan of the chest showing an aneurysm of the right coronary artery and pericardial fluid*

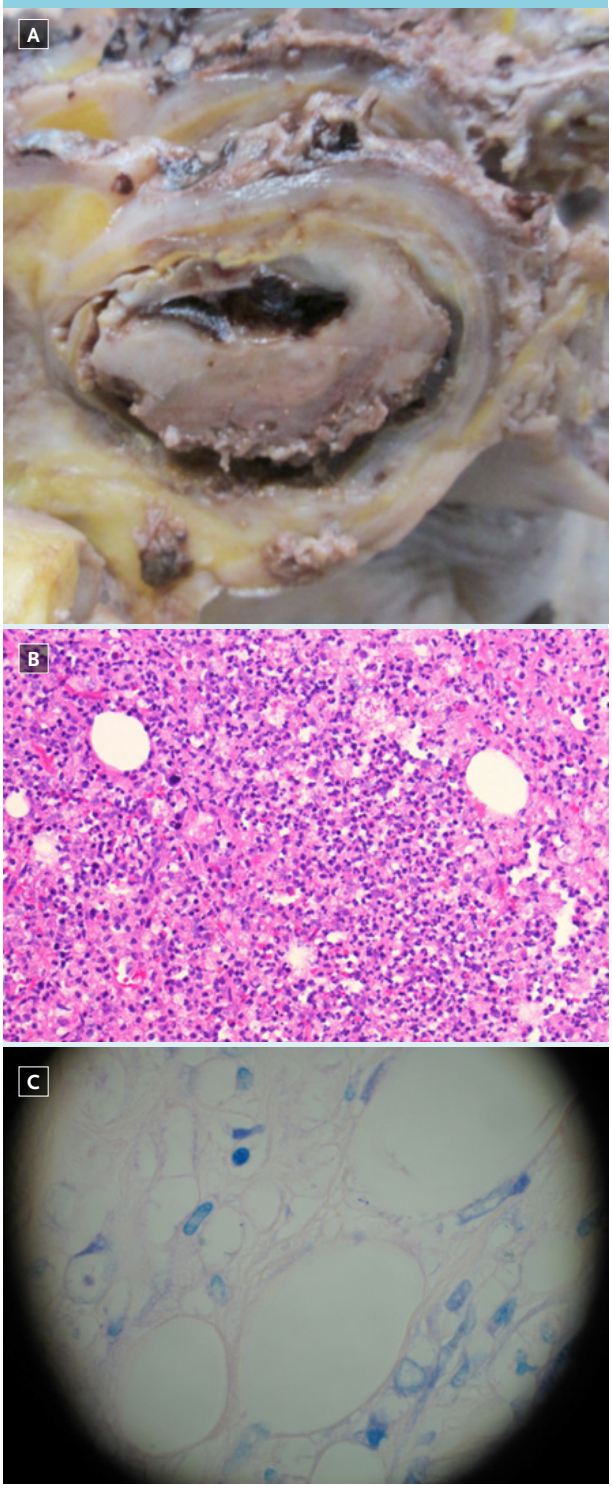


DISCUSSION

Mycotic coronary aneurysms are caused by bacterial colonisation of the arterial wall. This can be due to systemic circulating bacteria, septic emboli, e.g. as complication of endocarditis, direct invasion of the vessel from an extravascular site of infection and traumatic inoculation of contaminated material, e.g. as a complication of stenting procedures. An abnormal intimal surface, in an atherosclerotic plaque or a pre-existent aneurysm, is likely to be prone to infection by any of these mechanisms. In our case infection of a pre-existent atherosclerotic aneurysm due to systemic circulating bacteria is the most likely mechanism. A possible infection of the right coronary by per continuitatem due to pleural empyema seems unlikely since the cultures performed on the pleuritic fluid seen on CT remained negative. We speculate that the antibiotics probably could not reach the bacteria in the atherosclerotic plaque. This would explain why *S. pneumoniae* was detected post mortem by PCR and also why diplococci were seen in the wall of the aneurysm despite antibiotic treatment for 16 days. The infection may have resulted in rapid growth and rupture of the aneurysm. This is supported by the larger diameter of the aneurysm on autopsy compared with that found on chest CT. In addition, pericardial fluid was seen on chest CT but not detected on echocardiography (TTE and TEE). The phenomenon of rapid growth and rupture has also been described in other cases.[3,5,6]

Staphylococcus aureus is the most common culprit in both stent-related and non-stent-related mycotic coronary aneurysms. Several other bacteria have also been described.[2,7] Sporadically mycotic aneurysms may be caused by fungi.[8] In several cases all cultures remained negative. To our best knowledge a mycotic coronary aneurysm due to pneumococcal infection has only been described twice before.[3,4] These cases involved immunocompromised patients, both with a history of lymphoid leukaemia and one with a history of splenectomy

Figure 2. *A) Autopsy: atherosclerotic aneurysm of the right coronary artery. B) HE staining: active inflammation and necrosis of the vessel wall (100 x). C) Giemsa staining: diplococcal (streptococcus pneumoniae) (1000x)*



as well. The causes of infection in these two cases were pneumonia and arthritis of the right knee, respectively. Both cases presented with chest pain and pericardial fluid on presentation. Our patient did mention experiencing chest pain several days before hospital admission. However, because the chest pain did not relapse and the ECG was normal, the initial clinical focus was mainly on the neck pain and mastoiditis. In retrospect this probably caused a diagnostic delay. Unfortunately there is no gold standard for diagnosing a mycotic coronary aneurysm, and this is reflected in the scarce literature. Repeat anatomical imaging techniques seem to be used most frequently,[9,10] since rapid enlargement of the aneurysm seems to be a key characteristic.[6] Also molecular imaging is becoming more sensitive but tailored investigations are mandatory to detect small mycotic coronary aneurysms at an early stage. There is no standardised treatment for mycotic coronary aneurysms either. Several authors suggest that small mycotic aneurysms may resolve with antibiotic treatment. Aneurysms of more than 1 or 2 cm may enlarge and can be complicated by rupture, thrombosis or septic embolisation.[3,5,6,8] Most patients who underwent treatment for a mycotic coronary aneurysm were treated with coronary bypass grafting and/or aneurysmectomy, in combination with antibiotics. There are a few studies reporting successful treatment by stenting.[9,10] However, there is a risk of septic emboli, stent thrombosis and vessel rupture when stenting an infected area and this technique is therefore not recommended by most authors.[3,4]

CONCLUSION

We describe an unique case of a patient without a prior history of cardiovascular disease or an immunocompromised state with a fatal ruptured coronary aneurysm of the right coronary artery due to *S. pneumoniae* bacteraemia. Mycotic coronary aneurysm is rare, even in the age of coronary intervention and stenting, and remains a diagnostic and therapeutic challenge. Mycotic coronary aneurysms can grow rapidly and rupture subsequently, often with fatal consequences. Successful treatment requires timely recognition and intervention involving coronary bypass and resection of the aneurysm, in combination with adequate antibiotic treatment.

DISCLOSURES

The authors declare that there are no funding sources and no conflicts of interest.

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PHOTO QUIZ

Hyperpigmentation of the tongue in a patient receiving chemotherapy

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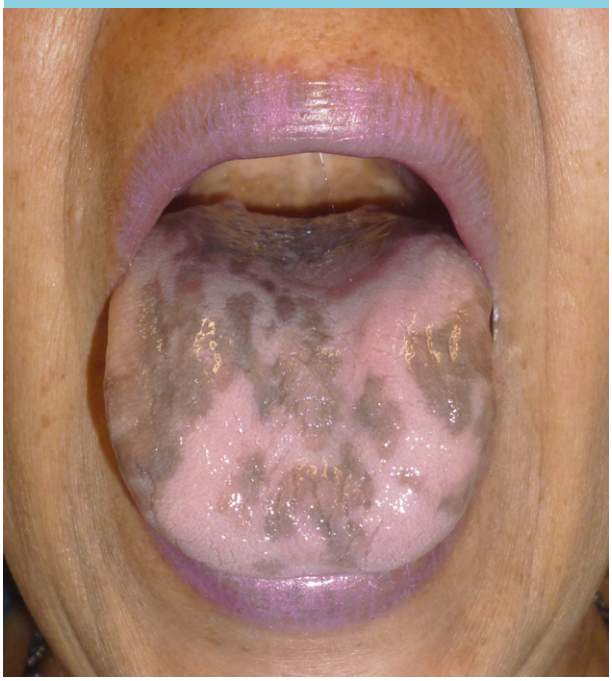
A 54-year-old Indian woman was seen due to hyperpigmented spots on the tongue, which began one month after starting treatment with capecitabine for metastatic breast cancer (*figure 1*). She had a similar episode five years earlier, which coincided with chemotherapy treatment with fluorouracil, doxorubicin and cyclophosphamide, given when the cancer was first diagnosed.

Figure 1. *Hyperpigmented spots on the tongue*



WHAT IS YOUR DIAGNOSIS?

See page 89 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 88)

HYPERPIGMENTATION OF THE TONGUE IN A PATIENT RECEIVING CHEMOTHERAPY

DIAGNOSIS

Given the chronology of events, capecitabine was considered the most likely origin of the clinical picture. Besides, months later, due to new progression of tumour involvement, capecitabine was suspended and the patient started with the next line of therapy. Shortly after, the hyperpigmentation of the tongue disappeared.

Capecitabine is a prodrug of 5-fluorouracil (5-FU), administered orally, which is converted to 5-FU in the tumour. Currently its use is approved for metastatic breast cancer and colorectal cancer. Because capecitabine is a prodrug of 5-fluorouracil, many of the alterations associated with 5-fluorouracil may appear with this drug. Their use is associated with various side effects. The appearance of hyperpigmented spots occurs in a minority of patients, being very rarely reported in the area of the tongue. The cases described appear to be more common in black or oriental race.[1,2]

Many chemotherapeutic agents have been associated with cutaneous and mucocutaneous hyperpigmentation. Mechanisms of action include a direct melanogenic stimulus on melanocytes, post-inflammatory hyperpigmentation secondary to increased photosensitivity, and a combination of the above.[2,3]

It is important to know the side effects of capecitabine given the increasing use of this drug, and thus to avoid performing unnecessary tests.

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Intestinal dilatation: do not forget a tropical origin

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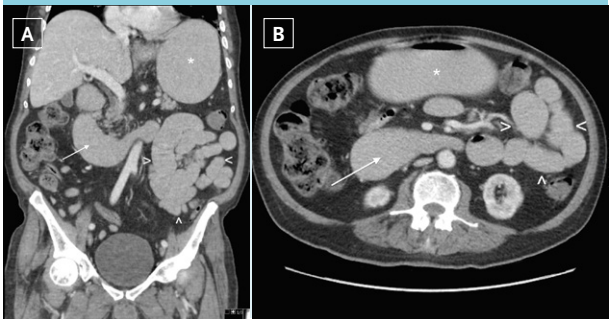
CASE REPORT

A 70-year-old Surinamese man presented to the outpatient clinic with hiccups, burping and bloating after meals, lasting for 1-1.5 hours. His medical history revealed multiple myeloma for which bortezomib (and later lenalidomide) and 40 mg dexamethasone daily had been prescribed for five months. There was no history of travel to a tropical area over the past five years. Physical examination did not reveal any abnormalities. Laboratory findings were within normal ranges except for a pre-existing normocytic anaemia and mild thrombocytopenia. As part of the analysis, a chest X-ray was performed, showing signs of emphysematous lung disease. A gastroscopy revealed an antral gastritis (*Helicobacter pylori* positive) for which he was treated with a combination of amoxicillin, clarithromycin and pantoprazole (no biopsies were taken). After two weeks the hiccups were under control.

The patient returned within five months with progression of the hiccups. Our causal differential diagnosis included goitre, gastrointestinal disorders, toxic-metabolic abnormalities, drugs (such as dexamethasone), central nervous system disorders or an infectious origin. Thyroid function tests and cortisol levels were normal; dexamethasone was stopped. He was treated with ondansetron, haloperidol, baclofen and gabapentin without any relief. The eosinophil count had increased from 0.1 to 1.3 x 10^9/l (normal value ≤ 0.5 x 10^9/l) over 15 months. Due to progressive malaise, anorexia and nausea, a CT scan was performed showing dilatation of the stomach as far as the proximal jejunum (*figure 1*). Another gastroscopy revealed a haemorrhagic antral gastritis and duodenitis, and biopsies were taken (*figure 2*).

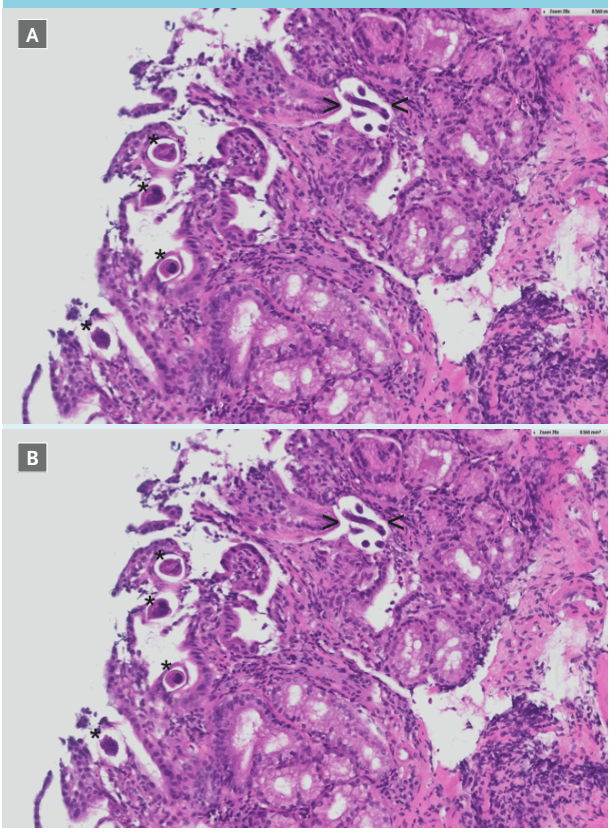
Figure 1. *Abdominal CT in coronal (A) and axial (B) view showing dilatation of the stomach (*), duodenum (arrow) and proximal jejunum (arrowheads) with fluid retention but without obstruction*



WHAT IS YOUR DIAGNOSIS?

See page 91 for the answer to this photo quiz.

Figure 2. *Routine H&E staining of the gastric mucosa*



ANSWER TO PHOTO QUIZ (PAGE 90)

INTESTINAL DILATATION: DO NOT FORGET A TROPICAL ORIGIN

DIAGNOSIS

Routine H&E staining of the gastric mucosa (*figure 2*) showed four *Strongyloides stercoralis* eggs (*) and one larva (arrowheads). In-house ELISA detecting IgG1/IgG4 against *S. stercoralis* showed a titre > 1:2560 (cut-off 1:40; Leiden University Medical Center). A faecal specimen revealed larvae using the Baermann and Ridley tests. In retrospect, the diagnosis of strongyloidiasis should have been considered earlier due to his origin, increased eosinophil count and long-term use of immunosuppressants.

Strongyloidiasis is caused by *S. stercoralis* and is endemic in tropical and subtropical regions. This persistent parasitic infection can lead to intermittent symptoms affecting the gastrointestinal tract, lungs or skin.[1] The severity varies from an asymptomatic infection in immunocompetent patients (eosinophilia) to a life-threatening disease, called hyperinfection syndrome, in immunocompromised patients.[2]

Hyperinfection syndrome is characterised by malabsorption, protein-losing enteropathy and/or colitis or even sepsis. The parasite burden is greatly increased, with high mortality rates if left untreated. Therefore, it is vital to detect and eradicate *S. stercoralis* prior to initiation of immunosuppressive therapy in high-risk patients.[3] However, this is not routine practice and our patient was not screened for this infection prior to the start of immunosuppressants.

In the absence of other causes, we reasoned that the gastric dilatation was the cause of his complaints of malaise, anorexia and hiccups. Gastric ulcers, duodenal obstruction or duodenal dilatation have previously been described in individuals with strongyloidiasis.[4] The exact pathophysiological mechanisms remain unknown. The patient was treated with ivermectin 0.2 mg/kg, a broad-spectrum antiparasitic drug, for three days, after which his symptoms resolved completely and remain absent to date.

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PHOTO QUIZ

Another cause for flagellate dermatitis

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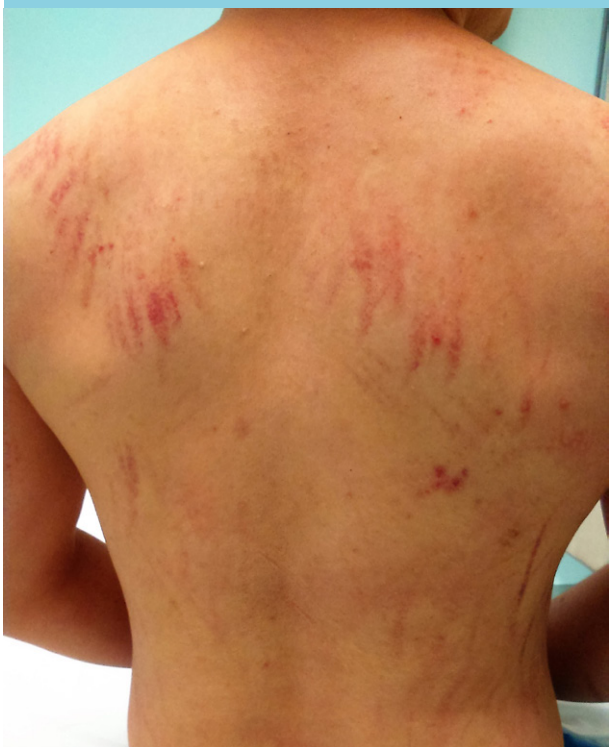
CASE REPORT

A previously healthy 24-year-old Chinese male presented to the emergency department because of a very itchy rash that had evolved in two days. He denied any systemic symptoms or exposure to medication. Physical examination revealed multiple linear erythematous lesions composed of petechiae. The lesions were located primarily on the back (*figure 1*), chest, and proximal areas of the lower limbs. There was no dermographism. The mucosal surfaces were normal.

WHAT IS YOUR DIAGNOSIS?

See page 93 for the answer to this photo quiz.

Figure 1. *Linear erythematous lesions composed of petechiae on the back of the patient*



ANSWER TO PHOTO QUIZ (PAGE 92)

ANOTHER CAUSE FOR FLAGELLATE DERMATITIS

DIAGNOSIS

Shiitake dermatitis or flagellate mushroom dermatitis

When the patient was directly asked, he acknowledged that he had eaten fish with Shiitake mushrooms five hours before the appearance of the rash. The diagnosis of Shiitake dermatitis was finally made, based on the morphological features of the clinical lesions and the clinical history. Antihistamines were prescribed and the rash resolved in one week without post-inflammatory hyperpigmentation.

The shiitake mushroom (*Lentinus edodes*), primarily used in Asian cooking, is now the second most widely consumed mushroom species worldwide. In addition to their culinary uses, it is known for having antihypertensive, lipid-lowering, and also anti-carcinogenic properties.

Shiitake dermatitis was first reported by Nakamura in 1977 as a toxicoderma developing after the consumption of raw or half-cooked mushrooms.[1] It usually presents as a linear erythematous eruption with papules, papulovesicles or plaques, and severe pruritus.

Onset can occur within hours to 4 or 5 days after ingestion of the mushrooms, but the time delay between ingestion and eruption is usually 24 to 48 hours.

The exact pathogenesis remains unknown. Shiitake dermatitis is considered a toxic reaction to lentinan, a thermolabile polysaccharide that increases interleukin-1, causing vasodilation and haemorrhage. Patch testing and skin prick testing have not been proven useful for diagnosing this condition, suggesting a non-allergic aetiology.[2] Histological examination is not mandatory but if performed it usually reveals non-specific histopathological features, including hyperkeratosis, spongiosis, dermal oedema and perivascular lymphocytic infiltrate with eosinophils.[3]

Differential diagnosis includes other causes of flagellate dermatitis induced by drugs such as bleomycin or bendamustine and autoimmune diseases as dermatomyositis or adult-onset Still's disease.[4] Symptomatic dermographism (also called urticaria factitia) and dermatitis artefacta should also be considered in the differential diagnosis.

Diagnosis is usually made based on clinical history and physical examination. Only symptomatic care with topical steroids and emollients, and in cases of associated pruritus, oral antihistamines, is necessary. Most authors consider that re-ingestion of shitake mushrooms is safe if they are thoroughly cooked as this allows denaturation of the toxin.

The growing popularity of Asian cuisine has led to an increasing number of cases of Shiitake dermatitis in Europe. Physicians must be aware of this condition, to provide a prompt diagnosis, and management.

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PHOTO QUIZ

A rare but lethal yeast...

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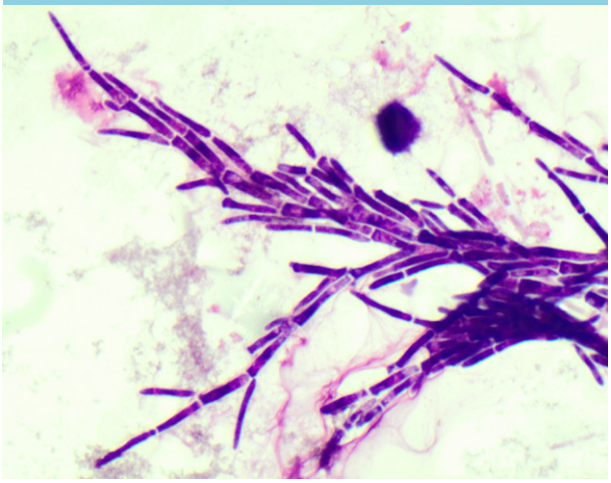
CASE REPORT

A 76-year-old patient was admitted to the intensive care unit (ICU) with respiratory distress. He was of Moroccan descent and had recently been diagnosed with a diffuse large cell B-cell non-Hodgkin lymphoma. He received his second chemotherapy (R-CHOP) 20 days prior to this admission, both times developing profound neutropenia (maximum decline of neutrophilic granulocytes to 0.15 x 10^9/l). Physical examination showed a tachypnoeic patient using his accessory respiratory muscles to breathe, his respiratory rate was 25/minute, $SatO_2$ 95% with 12 litres/minute oxygen, blood pressure of 140/60 mmHg, heart rate of 95/minute and a tympanic temperature of 35.4 °C. On auscultation of the lungs we noticed some crackles and mild bronchospasm. Blood gas analysis showed metabolic compensated respiratory acidosis (pH 7.36, pCO_2 9.2 kPa, HCO^{3-} 39.1 mmol/l, base excess 12.7 mmol/l) and hypoxia (pO_2 6.9 kPa, $SatO_2$ 83%). CT imaging of the chest showed diffuse bilateral lung consolidation and ground glass opacity.

A bronchoscopy with bronchoalveolar lavage (BAL) was done prior to ICU admission. Due to the immunocompromised status of this patient, broad-spectrum empirical therapy had been commenced, with amoxicillin, ceftazidime, voriconazole and co-trimoxazole. Cultures of the BAL liquid showed high numbers of yeast strings, as shown in *figure 1*.

Figure 1. *GIEMSA colouring, showing multiple yeast strings*



WHAT IS YOUR DIAGNOSIS?

See page 95 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 94)

A RARE BUT LETHAL YEAST…

DIAGNOSIS

Our patient was diagnosed with a severe pulmonary infection with *Saprochaete capitata* (teleomorph: *Magnusiomyces capitatus*, previously called *Geotrichum capitatum*, *Trichosporon capitatum* or *Blastoschizomyces capitatus*), most probably due to his immunocompromised state (determination method: MALDITOF). We continued therapy with voriconazole. On the ICU he was initially supported with non-invasive ventilation, but eventually he had to be intubated and mechanically ventilated. Despite full ICU treatment his situation worsened, and he died in cardio-respiratory arrest.

Saprochaete capitata is a non-fermentative, non-encapsulated, urease-negative ascomycetous yeast. It is part of the normal microbiota of human skin and is frequently isolated from the sputum and the digestive tract of healthy people.[1] It is a rare, but emerging yeast responsible for severe infections in patients with profound neutropenia in the haemato-oncology setting.[1-3] The prognosis is poor with a mortality rate exceeding 50%.[2]

Most cases of *Saprochaete capitata* infections have been diagnosed by means of blood cultures or cultures on BAL liquid. Galactomannan antigen enzyme-linked immunosorbent assay (GM-ELISA) is now widely used in the serological diagnosis of invasive *Aspergillosis* as an essential diagnostic method. As the serological G test (1,3-β-D-glucan), which detects 1,3-β-D-glucan as a component of the fungal wall and which is also applicable for early diagnosis of all fungal infections (especially *Candida* and *Aspergillus* and except for *Cryptococcus* and *Zygomycetes*), it can also be used for the diagnosis of other fungal infections such as *Saprochaete capitata*. However, neither of these tests can determine the specific infectious species.[3]

Saprochaete capitata is considered intrinsically resistant to echinocandins,[1] several breakthrough infections in neutropenic patients have been reported.[4] Voriconazole exhibits a promising activity in vitro, and voriconazole and amphotericin B combination therapy has been suggested.[1]

CONCLUSION

In case of severe illness in neutropenic patients, fungal infection should always be considered. *Saprochaete capitata* can play a role as an opportunist. Treatment with echinocandins is probably ineffective; therefore treatment with voriconazole (perhaps in combination with amphotericin B) is advised.

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