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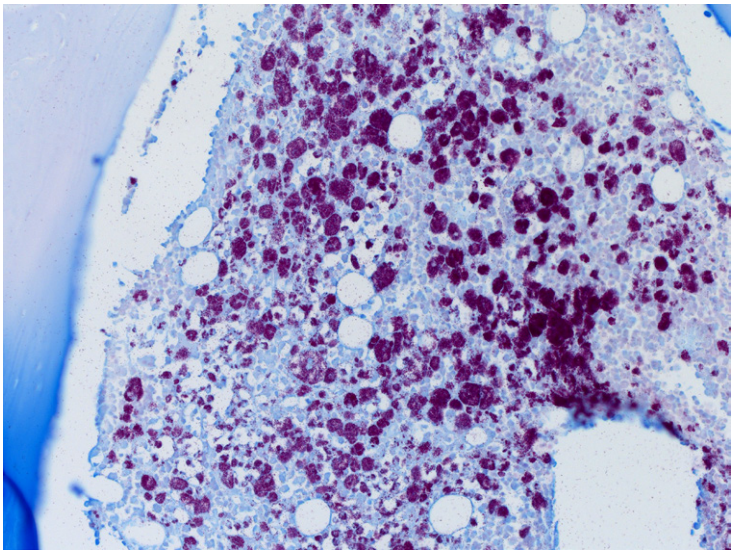

Hyperammonaemia and an abnormal bone marrow biopsy; what is your diagnosis?

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EDITORIAL

High-altitude treatment in severe asthma: Effective and needed in an era of precision medicine

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Asthma is a chronic often inflammatory disorder of the airways characterised by typical symptoms such as wheezing, shortness of breath and cough that vary over time.[1] About 500,000 patients in the Netherlands have asthma, varying from mild to severe. The disease is classified as severe refractory asthma if symptoms cannot be controlled despite treatment with a high dose of inhaled corticosteroids, beta2 agonists, anticholinergica and/or oral steroids. The diagnosis 'severe asthma' is confirmed when a step-wise algorithm is followed.[2] In this algorithm other causes for poor asthma control are excluded, such as treatment adherence and inhalation technique issues. In the Netherlands approximately 3.6% of asthma patients have severe refractory asthma.[3]

Not all asthma is the same: there are different endotypes or underlying inflammatory profiles such as eosinophilic asthma, neutrophilic asthma or allergic asthma, or combinations. Different phenotypes require different treatment strategies.[4,5] In severe asthma, biologicals have been applied since the introduction of anti-IgE for severe allergic asthma. More recently, several anti-IL5 treatments became available for severe eosinophilic asthma.[6] More phase 3 studies with biologicals targeting specific pathways in asthma are currently being performed.

Because of this wide range of biologicals for different phenotypes, one might think that high-altitude treatment has lost its relevance in the treatment of severe asthma. Hashimoto, Rijssenbeek-Nouwens and collegues show the opposite in this issue of the *Netherlands Journal of Medicine*.[7] High-altitude treatment is still very effective in a wide range of severe asthma patients.

A prospective cohort of 136 patients who received high-altitude treatment in the Dutch Asthma Centre in Davos is presented in the article of Hashimoto. The results show, independent of the phenotype, a significant increase in lung function (FEV1), quality of life (measured using AQLQ). Since none of the patient characteristics were correlated with these outcomes, the authors suggest that the non-specific aspects of their treatment, such as improved adherence to medication or removal from stress at home, could be responsible for this effect. However, the reduction in oral corticosteroid use that was observed was related to the severity of symptoms at baseline and younger patients with low blood eosinophil counts, as expected based on the phenotype. Importantly, the reduction in oral steroids was possible with preserved improvement in asthma control. Patients with an allergic phenotype with a high IgE showed less airway inflammation after treatment, probably caused by the minimised exposure to allergens in the high-altitude environment. The authors conclude that high-altitude treatment improves the quality of life as well as the lung function in all patients with severe asthma. Furthermore, patients' characteristics may be used to predict the beneficial effect of the treatment and might optimise selection of patients who are eligible for this treatment.

The results presented by Hashimoto, Rijssenbeek-Nouwens and colleagues are of relevance, first because there remains a group of severe asthma patients who are not eligible for or do not respond to treatment with biologicals. High altitude is often successful in this group of patients as it may act on both inflammatory as well as behavioral aspects of the disease.

Furthermore, high-altitude treatment can play a key role in precision medicine.[8] While entering the era of precision medicine the focus will be on treatable traits rather than phenotypes. Airway inflammation is just one of the treatable traits, medication adherence, infection, allergy and deconditioning are some of the traits that can be relevant in severe asthma. Therefore, high-altitude treatment remains very relevant in the future treatment of severe refractory asthma.

REFERENCES

1. Hering T. [Update of the GINA-Recommendations]. MMW Fortschr Med. 2017;159:63-4.
2. Bel EH, Sousa A, Fleming L, et al. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). Thorax. 2011;66:910-7.
3. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The prevalence of severe refractory asthma. J Allergy Clin Immunol. 2015;135:896-902.
4. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178:218-24.
5. Chung KF. Precision medicine in asthma: linking phenotypes to targeted treatments. Curr Opin Pulm Med. 2018;24:4-10.
6. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti-IL5 therapies for asthma. Cochrane Database Syst Rev. 2017;9:CD010834.
7. Hashimoto S, Rijssenbeek-Nouwens LH, Fieten KB, Weersink EJ, Bel EH. Predictors of benefit from high-altitude climate therapy in adults with severe asthma. Neth J Med. 2018;5:218-25.
8. Agusti A, Bel E, Thomas M, et al. Treatable traits: toward precision medicine of chronic airway diseases. Eur Respir J. 2016;47:410-9.

REVIEW

Methadone use and asymptomatic common bile duct dilation: Re-examining the link

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ABSTRACT

Opiates have long been implicated in causing common bile duct (CBD) dilation but few studies have been done to look at the association between synthetic opiates – methadone – and asymptomatic CBD dilation. The mechanism by which methadone could cause CBD dilation is poorly understood, but it has been postulated that increase in biliary pressures from contraction of the sphincter of Oddi is likely. In the below article, we review all the evidence pertaining towards methadone causing common bile duct dilation.

KEYWORDS

Common bile duct dilation, CBD, methadone, opioid, sphincter of Oddi

INTRODUCTION

Asymptomatic common bile duct (CBD) dilation is one of the frequently faced diagnostic dilemmas in this age of enhanced biliary imaging techniques. Although the aetiologies are quite extensive, clinicians primarily worry about looking at obstruction from malignancy or stones/sludge.[1] Despite the advancements with biliary imaging such as CT cholangiography, magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), and endoscopic ultrasound (EUS), regular transabdominal ultrasound is always the initial test of choice with a sensitivity of 99% for detecting CBD dilation; this can be done twice to confirm the CBD dilation.[2]

A finding of CBD dilation on imaging such as ultrasonography together with symptoms will alert a clinician to perform invasive biliary imaging based on the clinical situation. However, no guidelines have been in place for the management of asymptomatic CBD dilation.[1] According to the systematic review, an overall yield for a malignancy or a treatable condition from an invasive workup for patients with asymptomatic CBD dilation is low.[1] Although never quantified objectively, significant healthcare costs are attached to this workup. The purpose of this review is to examine the various studies that looked at the prevalence and the results of the workup for CBD dilation in patients on methadone.

METHODS

For the purpose of this review we searched PubMed (Medline), Embase and Cochrane until the end of October 2017. We searched combining the MESH terms "opiate", "methadone" and "common bile duct". This yielded 29 articles. These articles were individually reviewed by the authors and five articles were retained after considering the inclusion criteria as mentioned below. We also included the abstract published by our group in this review, making it a total of six studies. The inclusion criteria used for selection of these studies included those that have comparison of methadone as a primary or secondary group.

DISCUSSION

Opiate addiction is a rampant issue which is on the rise. In the United States, as of 2015, there have been at least 12.5 million people who misuse opiates, which is not surprising considering the fourfold increase of opiate prescriptions since 1999.[3] Methadone is one of the opiate substitutions used to treat patients with opiate addiction. According to the estimates from 2015, there are at least 1 million patients on methadone maintenance

therapy (MMT) for opiate addiction.[4] Also, there is a high prevalence of opiate addiction and hence methadone clinics in the inner-city population of major cities.[5] Methadone is a synthetic opiate which has a half-life of 14 hours and is not addictive.[6]

Opiates can cause biliary dilation and specifically CBD dilation, an attribute which is used in biliary scintigraphy to better visualise the biliary tract.[7] To date there have been some studies looking at common bile duct dilation with methadone use.[8-12] Although there is some disparity with the results, most of them support the fact that there could be some degree of CBD dilation in certain sets of patients on methadone. The mechanism by which methadone could cause CBD dilation is poorly understood, but it has been postulated that it could increase biliary pressures by constricting the sphincter of Oddi.[13] Despite the availability of these studies, none of them have a definitive conclusion on factors that could also affect the dilation. Recently, a retrospective single-centre observational study was done comparing the size of the common bile duct among methadone and non-methadone users in an inner city population of a major city. This is the largest study to date with 171 patients on MMT and 273 not on MMT.[14] This study included all patients who underwent abdominal ultrasound after excluding acute biliary pathology. It was found that the CBD diameter in patients on MMT is significantly greater than those not on MMT. It is also interesting to note from this study that the CBD size does not correlate with the dose of methadone, demographics or liver biochemical profile.[14]

An early prospective study on 334 hepatitis C infected patients out of which 36 were on methadone revealed that 3/36 (8.3%) patients on MMT had an asymptomatic dilated CBD, defined as size ≥ 9 mm as opposed to 1/298 (0.03%) in the non-MMT group. This only considered patients with hepatitis C and did not evaluate if there was a mean CBD size difference within the two groups.[8] Later, in 2003, a case series was published on six patients who were referred for ERCP due to CBD dilation revealing no endoscopic abnormalities. All these patients were found to be on methadone and the authors concluded that EUS might be a safer first option in such patients.[9]

A large retrospective cross-sectional study in 2009 included patients with either chronic hepatitis B or C, and compared 215 patients on MMT with 108 patients not on MMT. This study concluded that the patients on MMT have a significantly increased CBD diameter (5.87 mm) as compared with the control group (3.79 mm), $p < 0.001$. On multivariate logistic regression, 26.1% of patients on methadone have a CBD diameter of ≥ 8 mm when compared with 2.8% of patients who are not (OR of 17.5). However, interestingly, it is noted that the group of patients on MMT also have significantly higher aspartate transaminase, alkaline phosphatase and total bilirubin while the control group had more patients with cirrhosis.[10]

A study by Leopold et al., investigated a similar question in chronic hepatitis C patients who underwent hepatic ultrasonography as a part their pretreatment screening.[11] Patients were assessed for factors associated with CBD dilation, defined as a CBD diameter of ≥ 7 mm. Initially a univariate regression model was built and the variables whose p-value was less than 0.25 were included in a multivariate analysis. Increasing age by decades, being on MMT at the time of scan and the dose of methadone (categorised as no dose, low dose, intermediate dose and high dose) are found to be statistically significant for increased odds of having CBD dilation. There were no data on biochemical markers due to non-availability which was mentioned as a major limitation.[11]

A more recent retrospective study performed in Boston looked at all patients on MMT who underwent an abdominal CT or MRI after excluding the ones with acute biliary aetiologies.[12] In the same period, a total of 97 patients were included in the analysis and they were matched with controls imaged at the same date range. This study found that there is a statistically significant difference in the diameter of the CBD, intrahepatic bile duct and pancreatic duct between the two groups with patients on MMT being on the higher side.

CONCLUSION

In conclusion, it is not uncommon to find a dilated asymptomatic common bile duct in patients with chronic opioid use. Despite the search to find aetiology most of the cases rarely point to any structural aetiologies except opiate use. However, evidence is lacking on whether we can safely ignore these findings without performing any further investigations. Prospective control studies need to be performed before ascertaining for a fact that the dilation of the common bile duct in all chronic narcotic users is benign and warrants no further investigations.

DISCLOSURES

All authors declare no conflict of interest. No funding or financial support was received.

REFERENCES

1. Smith I, Monkemuller K, Wilcox CM. Incidentally Identified Common Bile Duct Dilatation: A Systematic Review of Evaluation, Causes, and Outcome. J Clin Gastroenterol. 2015;49:810-5.
2. O'Connor O.J, O'Neill S, Maher M.M. Imaging of biliary tract disease. AJR Am J Roentgenol. 2011;197:W551-8.
3. Vital Signs: Overdoses of Prescription Opioid Pain Relievers -- United States, 1999--2008. Morbidity and Mortality Weekly Report (MMWR). 2011;60:1487-92.
4. 2015 National Survey on Drug Use and Health: Detailed Tables. Center for Behavioral Health Statistics and Quality, 2016. Substance Abuse and Mental Health Services Administration, Rockville, MD.
5. Krantz MJ, Martin J, Stimmel B, Mehta D, Haigney MC. QTc interval screening in methadone treatment. Ann Intern Med. 2009;150:387-95.
6. Kapur BM, Hutson JR, Chibber T, Luk A, Selby P. Methadone: a review of drug-drug and pathophysiological interactions. Crit Rev Clin Lab Sci. 2011;48:171-95.
7. Achong DM, Tenorio LE. Early morphine administration to expedite gallbladder visualization during cholescintigraphy for acute cholecystitis. Clin Nucl Med. 2003;28:723-4.
8. Zylberberg H, Fontaine H, Correas JM, Carnot F, Brechot C, Pol S. Dilated bile duct in patients receiving narcotic substitution: an early report. J Clin Gastroenterol. 2000;31:159-61.
9. Firoozi B, Choung R, Diehl DL. Bile duct dilation with chronic methadone use in asymptomatic patients: ERCP findings in 6 patients. Gastrintest Endosc. 2003;58:127-30.
10. Meyer T, Kapkov D, Lipp MJ, Bodenheimer Jr HC, Siegel JH, Min AD. Bile Duct Dilatation in Patients With Chronic Viral Hepatitis on Maintenance Methadone Therapy. Gastroenterol Hepatol. 2009;5:641-4.
11. Leopold SJ, Grady BP, Lindenburg CE, Prins M, Beuers U, Weegink CJ. Common bile duct dilatation in drug users with chronic hepatitis C is associated with current methadone use. J Addict Med. 2014;8:53-8.
12. Bates DD, Tamayo-Murillo D, Kussman S, et al. Biliary and pancreatic ductal dilation in patients on methadone maintenance therapy. Abdom Radiol (NY). 2017;42:884-9.
13. Wu SD, Zhang ZH, Jin JZ, et al. Effects of narcotic analgesic drugs on human Oddi's sphincter motility. World J Gastroenterol. 2004;10:2901-4.
14. Sunkara T, Idamakanti M, Gupta S, et al. Asymptomatic Common Bile Duct Dilation and Methadone Use: Re-Examining the Link in Minority Inner-City Populations. Gastroenterology. 2017;152:S503.

ORIGINAL ARTICLE

Iodine status during pregnancy and lactation: a pilot study in the Netherlands

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ABSTRACT

Background: Iodine deficiency occurs in West European countries. Iodine is important for brain development of the foetus and infant. The current iodine status of pregnant and lactating Dutch women is unknown.

Methods: In a pilot study we examined the iodine status of 36 women. From 20 gestational weeks (GW) until 4 weeks postpartum, they ingested 150 µg iodine/day in the form of a multivitamin supplement for pregnant and lactating women. Twenty-four hour urine samples were collected at 20 and 36 GW and at 4 weeks postpartum. A breast milk sample was collected at 4 weeks postpartum. Iodine concentrations were analysed by inductively coupled plasma-mass spectrometry. Cut-off values for the urinary iodine concentration (UIC) for pregnant and lactating women are 150 and 100 µg/l, respectively. Adequate intakes (AI) of iodine for infants aged 0-6 months are 1.1 µmol/l (Institute of Medicine recommendations) or 0.5 µmol/l (Nordic Council recommendations).

Results: The median UICs (percentages below cut-off) were 102 µg/l (83%) at 20 GW, 144 µg/l (56%) at 36 GW and 112 µg/l (40%) at 4 weeks postpartum. The median breast milk iodine concentration was 1.2 µmol/l (range 0.5-3.0); 33% and 0% of the infants had estimated iodine intakes below the IOM-AI and Nordic-AI, respectively.

Conclusion: This pilot study suggested a high prevalence of iodine deficiency during pregnancy. Daily supplementation of 150 µg iodine from 20 GW might be insufficient to reach maternal iodine adequacy. The median breast milk iodine concentration seems adequate. Further studies, using a representative sample of the Dutch population, are needed to establish the current Dutch iodine status of pregnant and lactating women.

KEYWORDS

Infant adequate intake; iodine; iodine status; lactation; pregnancy

INTRODUCTION

Iodine is necessary for the formation of thyroid hormone.[1] Iodine deficiency can cause hypothyroidism. During pregnancy this may lead to pregnancy complications and impaired brain and cognitive development. Iodine is also important for infant brain development during lactation.

Iodine deficiency during pregnancy occurs in West European countries, including the United Kingdom, Sweden, Denmark and Belgium.[2] In the Netherlands, salt has been fortified with iodine since the Second World War. Iodised salt in bread became an important iodine source; other important sources are iodised table salt, seaweed, fish, dairy products and meat.[3] Pregnant and lactating women are vulnerable to iodine deficiency. The Health Council of the Netherlands currently uses the dietary standards of the Nordic Council.[4] Based on iodine requirements to prevent goitre and maintain normal thyroid function, the recommended daily intake for adults is 150 µg/day and the average requirement is 100 µg/day.[5] To cover foetal needs and maintain thyroid gland function, an extra 25 µg/day is required during pregnancy. An extra 50 µg/day is recommended during lactation to provide sufficient iodine in the breast milk.[5] These recommendations for pregnant and lactating women are in agreement with the European Food and Safety Authorisation (EFSA),[6] but are lower than the recommendations of the Institute of Medicine (IOM).[7]

Iodine status is established by measurement of the urinary iodine concentration (UIC). A 100 µg/l cut-off value is

employed for the general population, lactating women included. For pregnant women, the cut-off is set at 150 µg/l.[8] It should be noted that these cut-offs apply for populations, not individuals. The UIC cut-offs as set by the World Health Organisation (WHO) are widely accepted, but there are nevertheless some concerns. These cut-offs are based on an average 24-hour urine volume of 1.5 litres.[9] The Doetinchem study found an average of 2.0 litres in a Dutch population, suggesting an underestimation of iodine status by UIC measurement. The National Institute for Public Health and the Environment (RIVM) in the Netherlands found that, in 2007-2010, the Dutch population aged between 7-69 years 'generally consumed sufficient amounts of iodine'. It was estimated that roughly 10% of pregnant and 50% of lactating women had iodine intakes below the estimated average requirement.[10] In 2008, the maximally permitted iodine content of bakery salt in the Netherlands was reduced from 70-85 to 50-65 mg/kg salt. It was simultaneously allowed to add iodised salt to almost all foods.[11] Since then iodine intake has decreased by 20-25%.[12] A study in Doetinchem (the Netherlands) concluded that from 2006 to 2015, iodine intakes had declined by 37% in men and 33% in women.[13] Until 2008 the iodine status of pregnant Dutch women was not a reason for concern. The Generations R Study, conducted in Rotterdam in 2002-2006, found a median UIC of 230 µg/l (90% range: 55-733 µg/l) at 13 gestational weeks (GW).[14] In 2015, Dutch women of a reproductive age (19-49 years) in Doetinchem had a median (P25-P75) UIC of 76 (45-131) µg/l. Since the urine volume of the study population was higher compared with the WHO study, they might have underestimated the real iodine intake.[13] The 24-hour iodine excretion and estimated iodine intake in the Doetinchem study population were [median (P25-P75)]: 139 (109-190) and 151 (119-207) µg/day, respectively. There are no data on the iodine status of Dutch pregnant women as established after the reduction of iodine in salt in 2008.

We determined the iodine status of a small group of pregnant women at 20 gestational weeks (GW). The study was a secondary aim of our ZOOG-MUM trial. In this trial we provided a multivitamin supplement containing 150 µg iodine/day. We investigated whether this dose is sufficient to reach or maintain an adequate iodine status during pregnancy and lactation. We also investigated whether the breast milk iodine concentrations (BMIC) were in line with the adequate intake (AI) for newborns.

MATERIALS AND METHODS

This was a randomised trial primarily designed to study the dose responses of supplemental fish oil and vitamin D during pregnancy and lactation. It has been named 'ZOOG MUM' and was conducted in Groningen, the Netherlands. A secondary aim was to study iodine status before and after iodine supplementation in an open label observational design. The participants were aware of the composition and the doses of the nutrients in the multivitamin supplement (see below under Supplements). The study was approved by the Ethics Committee of the University Medical Center Groningen (UMCG) (METc number 2014.263) and was registered in the Netherlands National Trial Register (Trial ID NTR4959). All women provided written informed consent. The study was in agreement with the Helsinki Declaration of 1975, as revised in 2013.

Study population

From December 2014 until December 2015, pregnant women in their first trimester were invited to participate. Forty-three apparently healthy women with singleton pregnancies were included. Exclusion criteria were as follows: vegetarian/vegan diet, hyperemesis gravidarum, pregnancy complications or preterm delivery and not having the intention to exclusively breastfeed after delivery. None of the participants used iodine-containing medication at the start or during the study, but most (61%) took iodine supplements prior to the beginning of the study.

Supplements

From 20 GW until 4 weeks postpartum, all participants received a multivitamin supplement (Omega Pharma; Rotterdam, the Netherlands) providing a daily dose of 150 µg iodine and 12-135% of the Dutch Recommended Dietary Allowance RDA/AI for vitamins and minerals for pregnant and lactating women. We did not verify the iodine content of the supplements. Consistent with the primary aim, they received ascending dosages of DHA-rich fish oil (315-1260 mg DHA+EPA) and vitamin D (10-85 µg/day) (both from Bonusan; Numansdorp, the Netherlands). All mothers reported adherence to the supplement protocol. They took > 75% of the supplements, as established by inquiry at appointments, by questionnaire, or both.

Sample collection

The study was started at 20 GW. The participants collected a 24-hour urine sample at the start of the study, at 36 GW and 4 weeks postpartum. At 4 weeks postpartum they also collected a breast milk sample. Urine and breast milk samples were collected on the same day. Participants were instructed to collect their urine for 24 hours, starting after emptying their bladder for the first time in the morning, until at the same time the following day when they again empty their bladder. Participants were asked to store the urine sample in a cold place and to take it along at the

next appointment in the UMCG, preferably on the same day. Participants were instructed to document the time they started and ended the collection. This information was reviewed during the appointment. Completeness of the 24-hour urine collection was established by interview. To ensure that all mothers collected the milk in a similar manner, milk from a completely emptied breast was collected around noon (10.00-14.00). The milk was collected manually or by breast milk pump. The samples were homogenised by careful mixing. They subsequently divided the sample among two sampling tubes. The milk samples were stored in the participant's freezer and taken along to the appointment in the UMCG. The 24-hour urine samples were immediately homogenised and divided into two portions. Urine and breast milk samples were stored at -20 °C until analysis.

Analysis

Iodine in 24-hour urine samples was analysed in the UMCG by inductively coupled plasma-mass spectrometry (ICP-MS; Varian, USA). The intra- and inter-assay coefficients of variation (CVs) were 1.9 and 5.9% at 40 µg/l and 0.9 and 0.5 % at 225 µg/l. BMIC was analysed in the European Laboratory of Nutrients (ELN; Bunnik) by ICP-MS (Agilent, USA). The intra- and inter-assay CVs were 6.3 and 5.5% at 0.7 µmol/l and 5.6 and 5.8% at 2.0 µmol/l.

Cut-off values for iodine insufficiency and newborn adequate intakes

The WHO has issued cut-off values for the evaluation of iodine status based on the UIC.[8] The iodine status in a population is considered sufficient if the median UIC is above the cut-off value. For the general population, including lactating women, cut-off values are set at 100 µg/l. For pregnant women the cut-off value amounts to 150 µg/l.[8] The WHO considers spot urine samples sufficient as day-to-day and within-day variations are averaged in a population.[15] These cut-offs are, however, based on an average 24-hour urine volume of 1.5 litres.[9] As we were dealing with only a small group of pregnant women, we chose to collect 24-hour urine samples and also calculated the 24-hour iodine excretion.

The Health Council of the Netherlands and the European Food and Safety Authority (EFSA) has not established an adequate intake (AI) for infants aged 0-6 months. The iodine-AI established by the IOM,[7] and the Nordic Council[5] are 110 (IOM-AI) and 50 (Nordic-AI) µg/day, respectively. The IOM-AI is based on average milk iodine concentrations in a group of healthy lactating women. The Nordic-AI is based on goitre prevalence and urinary iodine excretion in European children and extrapolated from adults based on energy and growth requirements. Using an average daily milk intake of 780 ml[16] and 126.9 g/mol iodine atomic weight, these AIs translate to milk iodine cut-offs of 1.1 µmol/l (IOM-AI) and 0.5 µmol/l (Nordic-AI), respectively. Since these are adequate intakes, only qualitative comparison is possible. Levels below the AI merely provide indications for a higher risk of iodine deficiency that requires further research.[17]

Data analysis and statistics

We used IBM PASW Statistics 23 software and R studio 1.0.143. Median (ranges) were reported. Between-group differences were analysed with the Mann-Whitney U test for continuous data. Differences between data at the various time points were analysed by the Wilcoxon signed-rank test. A p value < 0.05 was considered significant.

RESULTS

Study population

Forty-three women were included. Seven discontinued the study, of whom three voluntarily and four because of pregnancy complications. At 4 weeks postpartum, three women had discontinued breastfeeding. One woman provided us with a milk sample but did not provide a 24-hour urine sample at 4 weeks postpartum. *Table 1* shows the basic characteristics of the 36 women and their infants who were finally studied.

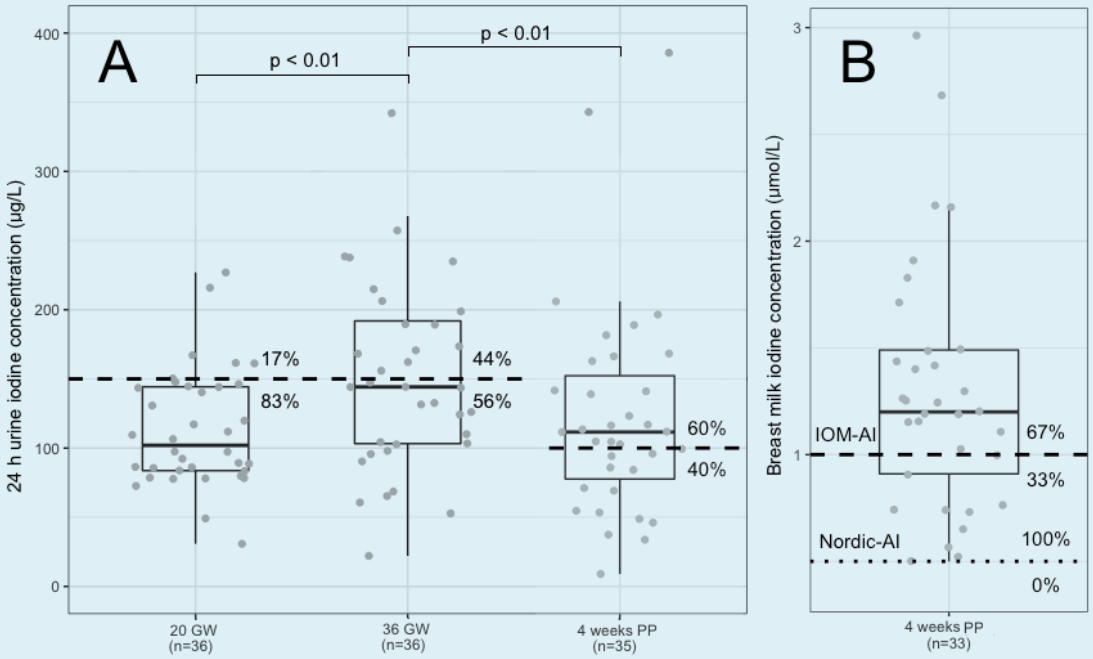
Iodine status during pregnancy and lactation

The median (range) UIC at 20 GW was 102 (31-227) µg/l (*figure 1A*; *table 2*). Median 24-hour urine volume and iodine excretion were 2.0 (1-4.5) l/24h and 210 (94-378) µg/24h, respectively. A total of 83% (30/36) had a UIC below 150 µg/l. The UIC of women who, by their own choice, took iodine-containing multivitamin supplements prior to 20 GW (61% of the women) did not differ from the UIC of counterparts who did not. However, although not significant, there was a trend in the dose response: the median (range) UICs (in µg/l) were: 95 (31-162; n = 14; for those taking 0 µg iodine/day), 87 (n = 1; 50 µg iodine/day), 107 (79-148; n = 11; 75 µg iodine/day) and 141 (78-227; n = 10; 150 µg iodine/day) (*table 2*). The women ingesting the highest doses seemed to have the highest UIC.

At 36 GW the median (range) UIC had increased to 144 (22-342) µg/l (*figure 1A*; p < 0.01; and *table 2*). Median 24-hour urine volume and iodine excretion were 1.9 (0.5-4.5) litres and 304 (95-488) µg/24h, respectively. Of the women, 56% (20/36) had a UIC below 150 µg/l. At 4 weeks postpartum the UIC had decreased to 112 (9-386) µg/l (*figure 1A*; p < 0.01; *table 2*). Median 24-hour urine volume and iodine excretion were 1.7 (0.7-4.0) litres and 157 (35-386) µg/24h, respectively; 40% (14/35) had a UIC below 100 µg/l.

We found a correlation between iodine status at 20 GW and 36 GW (r = 0.459; p < 0.01), and a correlation between

Figure 1. *Panel A. Iodine concentrations (µg/l) in 24-hour urine (UIC) of the investigated women (n = 36) at 20 gestational weeks (GW), following supplementation with 150 µg/day at 36 GW, and at 4 weeks postpartum (PP). Panel B. Iodine concentrations (µmol/l) in breast milk (BMIC) of the investigated women (n = 33) at 4 weeks PP*



The dotted line in panel A indicates the cut-off values for pregnant (150 µg/l) and lactating (100 µg/l) women. It should be noted that these cut-offs apply for populations, not individuals. The dotted lines in panel B represent the IOM-AI (1.1 µmol/l) and Nordic-AI (0.5 µmol/l) for infants aged 0-6 months. Percentages in the figure indicate those below and above the cut-off values. The UICs at 20 GW vs. 36 GW, and 36 GW vs. 4 PP were significantly different (both < 0.01)

iodine status at 36 GW and 4 weeks postpartum (r = 0.622; p < 0.01).

Iodine in milk

Milk samples were available from 33 women. The median (range) BMIC was 1.2 µmol/l (0.5-3.0 µmol/l; *figure 1B*; *table 2*). Of the infants, 33% (11/33) had estimated intakes below the IOM-AI, while none had estimated intakes below the Nordic-AI. We did not find a correlation between UIC at 4 weeks postpartum and BMIC (r = -0.21; p = 0.905).

DISCUSSION

In the present pilot study, 83% of the pregnant women had a UIC < 150 µg/l at 20 GW. The median iodine excretion was 210 µg/24h. After a daily intake of 150 µg iodine the median UIC increased from 102 µg/l at 20 GW to 144 µg/l at 36 GW. The percentage women with a UIC < 150 µg/l decreased to 56% and the median iodine excretion was 304 µg/24h. At 4 weeks postpartum the median UIC had decreased to 112 µg/l, and 40% of the lactating women had a UIC < 100 µg/l. The corresponding median iodine excretion was 157 µg/24h and the median BMIC was 1.2 µmol/l. Of the infants, 33% had an estimated iodine intake below the IOM-AI, whereas none had an estimated intake below the Nordic-AI.

The median UIC of 102 µg/l at 20 GW is lower than the 230 µg/l found in 1525 pregnant women in Rotterdam in 2002-2006,[14] but higher than the median UIC of 76 µg/l, as found in Doetinchem in 2015, in 98 non-pregnant women aged 19-49 years.[13] Although most studies collected spot urines, as opposed to the 24-hour urines in the present study, and information on 24-hour urine volumes is consequently lacking, similarly low median UICs of 87, 88, 98, 101-114, and 124 µg/l were found in pregnant women in Belgium,[18] United Kingdom,[19] Sweden,[20] Denmark,[21] and Austria,[22] respectively. The current 83% of women with UICs below 150 µg/l is much higher than the 10% estimate of the RIVM for pregnant women with iodine intakes below the RDA in 2007-2010.[10]

The UIC cut-offs as set by the WHO are widely accepted, but there are nevertheless some concerns. First, these cut-offs are based on an average 24-hour urine volume of

Table 1.

Variable	Dimensions	
Maternal characteristics		**(n=36)**
Age at 20 GW	(years)	31 (21-38)
Pre-pregnancy BMI	(kg/m^2)	24 (18-29)
Gravidity	n	2 (1-5)
Parity	n	1 (0-2)
Gestation duration	(weeks)	41 (37-42)
Socio-economic status		
Married/living together	n (%)	36 (100)
Household number	n	3 (2-4)
Education		
High school, intermediate vocational education or less	n (%)	7 (20)
College, university or higher	n (%)	28 (80)
Annual household income		
10,000-30,000 €	n (%)	7 (19)
30,000-50,000 €	n (%)	14 (39)
50,000 € or more	n (%)	15 (42)
Supplement use		
Vitamin supplements at study start:	n (%)	27 (75%)
0 µg iodine	n (%)	5 (14%)
50 µg iodine	n (%)	1 (3%)
75 µg iodine	n (%)	11 (31%)
150 µg iodine	n (%)	10 (28%)
Infant characteristics		**(n=36)**
Gender	(% male)	17 (47%)
Birth weight	(g)	3,790 (2,440-5,020)
Lactation duration	(weeks)	4.4 (3.5-5.3)
Weight at 4 weeks PP	(kg)	4.5 (3.6-5.7)

Data are median (range) or n (%). [1] GW = gestational weeks; PP = postpartum.

1.5 litres.[9] In our study we found median urine volumes of 2.0 and 1.9 l/24h at 20 GW and 36 GW, respectively, and 1.7 l/24h at 4 weeks postpartum, suggesting that the current iodine status may have been underestimated. The WHO UIC of 150 µg/l during pregnancy and 100 µg/l during lactation at an average of 1.5 litres urine/24 hours translate to estimated cut-off values of 225 and 150 µg iodine/24 hours, respectively. When compared with these, our median iodine excretions at 20 GW, 36 GW and 4 weeks postpartum of 210, 304 and 157 µg/24h, respectively, still suggest inadequacy at 20 GW (i.e. prior to supplementation), adequacy at 36 GW and borderline adequacy at 4 weeks postpartum (during supplementation). In addition, Dold et al.[23] recently suggested that maternal UIC alone is not an accurate biomarker of iodine status in lactating women and that additional measurement of

Table 2. *Iodine results*

Variable	Dimensions	
Estimated urine volume		**(n=36)**
20 GW	L	2 (1-4.5)*
36 GW	L	1.9 (0.5-4.5)
4 weeks PP	L	1.7 (0.7-4.0)*
Urine iodine concentration (UIC)		
20 GW	µg/L	102 (31-227)
36 GW	µg/L	144 (22-342)
4 weeks PP	µg/L	112 (9-386)*
WHO cut-off pregnancy	***µg/L***	***150***
WHO cut-off lactation	***µg/L***	***100***
Estimated iodine excretion		
20 GW	µg/24h	210 (94-378)*
36 GW	µg/24h	304 (95-488)
4 weeks PP	µg/24h	157 (35-386)*
Estimated cut-off pregnancy	***µg/24h***	***225***
Estimated cut-off lactation	***µg/24h***	***150***
UIC at 20 GW		
Vitamin supplements at study start:		
0 µg iodine (n=14)	µg/L	95 (31-162)
50 µg iodine (n=1)	µg/L	87 (-)
75 µg iodine (n=11)	µg/L	107 (79-148)
150 µg iodine (n=10)	µg/L	141 (78-227)
Estimated iodine excretion at 20 GW		
Vitamin supplements at study start:		
0 µg iodine (n=14)	µg/24h	202 (94-313)*
50 µg iodine (n=1)	µg/24h	186 (-)
75 µg iodine (n=11)	µg/24h	236 (153-339)
150 µg iodine (n=10)	µg/24h	197 (131-378)
Breast milk iodine concentration		
4 weeks PP	µmol/L	1.2 (0.5-3.0)
IOM-AI	***µmol/L***	***1.1***
Nordic-AI	***µmol/L***	***0.5***

Data are median (range) or n (%). [1] GW = gestational weeks; PP = postpartum. * missing one sample.
AI-IOM and AI-Nordic were calculated from other values based on specific assumptions.

BMIC could be useful in the assessment of the iodine status of lactating women.

Notwithstanding possible confounders, we remain concerned about the current findings, given the observation that 61% of the women already took iodine-containing multivitamins prior to the start of supplementation. Maternal iodine needs are higher during pregnancy and lactation because of iodine losses by transplacental transport, the necessity to maintain increased maternal thyroid hormone production, and the secretion of iodine into the milk.[5] At least three studies showed an association between a mild to medium iodine insufficiency during pregnancy and a lower IQ of the offspring at 3, 8 and 9 years of age.[24-26] For instance,

8-year-old children born to mothers with a urinary iodine/creatinine ratio < 150 µg/g in the first trimester, had higher chances of having a verbal IQ (odds ratio: 1.58), reading accuracy (odds ratio: 1.69) and reading comprehension (odds ratio 1.54) in the lowest quartile,[25] compared with counterparts of mothers with a urinary iodine/creatinine ratio > 150 µg/g in the first trimester.

Most,[14,20,22] but not all,[19] studies showed positive correlations between iodine dosage and the UIC during pregnancy and lactation. We found a trend, probably due to the small number of participants and therefore lack of statistical power. However, the daily supplemental intake of 150 µg iodine did increase the median UIC from 102 µg/l at 20 GW to 144 µg/l at 36 GW. This increase is in line with previous data from pregnant women in two cross-sectional studies conducted in Belgium[18] and Austria,[22] where supplementation with 150 µg/day also increased the median UIC. However, analogous to our study, they found this dosage to be insufficient to reach a median UIC of > 150 µg/l during pregnancy. At 4 weeks postpartum the median UIC was 112 µg/l. This decrease is probably on account of the preferential partitioning of iodine into the breast milk. Taken together, iodine insufficiency seems prevalent and it is unclear whether 150 µg is sufficient to prevent iodine insufficiency during pregnancy and lactation. One suggestion could be to increase the iodine dose, or initiate iodine supplementation prior to conception. Such regimens may optimise thyroid hormone stores with positive effects on both mother and child.[22] Accordingly, both the European Thyroid Association and the American Thyroid Association guidelines currently advocate to start iodine supplementation prior to conception.[27,28]

The median BMIC was 1.2 µmol/l (range: 0.5-3.0). Of the infants, 33% had an estimated iodine intake below the IOM-AI (i.e. 1.1 µmol/l) but none had an estimated intake below the Nordic-AI (0.5 µmol/l). Consequently, inadequate infant iodine intake seems less likely. Based on a dose-response crossover study to determine the minimum daily intake of iodine in early infancy, Dold et al.[29] suggested a 72 µg/day estimated average requirement and an 80 µg/day RDA for iodine for infants aged 2-5 months. These thresholds are in between those of the IOM and the Nordic AIs and would translate to milk iodine cut-offs at 0.72 and 0.8 µmol/l, respectively. Iodine is concentrated in the lactating breast via preferential transport.[30] The milk/plasma ratio amounts to 20-50.[31] We did not find a correlation between maternal iodine status and BMIC. Such a relation has been observed,[32,33] but not consistently.[34,35] In view of the high milk/plasma ratio and the expression of the Na^+/I^- symporter in the lactating breast,[36] it is likely that iodine is transported preferentially to the infant, and that a marginal iodine status notably occurs at the expense of the mother.

Limitations

This open-label study was not primarily designed to study iodine status and the effect of iodine supplementation. Other limitations are: small study numbers, representativeness of the Dutch pregnant population, day-to-day variation of the UIC, lack of a control group, and absence of information on dietary intake. It must, however, be noted that most of the women in the current study were highly educated and had above average incomes. Most of them used an iodine-containing multivitamin supplement prior to the study start. The RIVM found a positive relationship between education level and iodine intake from natural sources.[12] Consequently, the currently studied mothers might have had a better iodine status than the Dutch general population. Information on dietary intake could have improved insights into iodine sources. Other limitations are that we estimated infant iodine intake by using average infant daily milk consumption and that we did not verify the iodine content of the supplements. Conceding the above limitations, but strengthened by similar findings in surrounding countries, we consider the results too worrying to refrain from a report. There is an urgent need to conduct a larger study aiming at the iodine status of a representative group of pregnant and lactating Dutch women.

CONCLUSIONS AND RECOMMENDATIONS

This pilot study suggests a high prevalence (83%) of iodine insufficiency during pregnancy. The insufficiency was not entirely corrected by the use of a daily 150 µg iodine supplement. Due to the potentially severe and easily preventable consequences of iodine insufficiency for both mother and her offspring, a larger and representative study is urgently needed.

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There are no conflicts of interest to report.

REFERENCES

1. Zimmermann MB. Iodine deficiency. Endocr Rev. 2009;30:376-408.
2. Iodine Global Network. Global Scorecard of Iodine Nutrition 2017 in the general population and in pregnant women (PW). 2017; Available at: http://www.ign.org/cm_data/IGN_Global_Scorecard_AllPop_and_PW_May2017.pdf. Accessed 06/08, 2017.
3. Gezondheidsraad, Health Council of the Netherlands. Naar behoud van een optimale jodiuminname. 2008;2008/14.
4. Gezondsheidsraad, Health Council of the Netherlands. Tijdelijke voedingsnormen. 2014.
5. Nordic Council of Ministers 2014. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. 5th edition. Nord. 2014:002.
6. EFSA NDA Panel (EFSA Panel on Panel on Dietetic Products Nutrition and Allergies). Scientific Opinion on Dietary Reference Values for iodine. EFSA Journal 2014;12:3660, 57 pp. doi:10.2903/j.efsa.2014.3660.
7. Panel on Micronutrients, Subcommittees on Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. 2001.
8. World Health Organization. United Nations Children's Fund & International Council for the Control of Iodine Deficiency Disorders. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd ed. Geneva, Switzerland: WHO, 2007.
9. WHO Secretariat, Andersson M, de Benoist B, Delange F, Zupan J. Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation. Public Health Nutr. 2007;10:1606-11.
10. Geurts M, Verkaik-Kloosterman J. De Jodiuminname van de Nederlandse bevolking na verdere zoutverlaging in brood. 11-11-2014;RIVM Rapport 2014-0054.
11. Besluit van 13 juni 2008, houdende wijziging van het Warenwetbesluit Toevoeging micro- voedingsstoffen aan levensmiddelen, inzake het toevoegen van jodium. Staatsblad. 2008;257:1-5.
12. Verkaik-Kloosterman J, Buurma-Rethans EJM, Dekkers ALM. Inzicht in de jodiuminname van kinderen en volwassenen in Nederland. Resultaten uit de Voedselconsumptiepeiling 2007-2010. 2012;RIVM Rapport 350090012/2012.
13. Hendriksen M, Etemad Z, van den Bogaard CHM, van der A DL. Zout-, jodium- en kaliuminname 2015. Voedingsstatusonderzoek bij volwassenen uit Doetinchem. 04-10-2016; RIVM Rapport 2016-0081.
14. Ghassabian A, Steenweg-de Graaff J, et al. Maternal urinary iodine concentration in pregnancy and children's cognition: results from a population-based birth cohort in an iodine-sufficient area. BMJ Open. 2014 Jun 12;4:e005520-2014-005520.
15. World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. 2007 (Third edition).
16. Neville MC, Keller R, Seacat J, et al. Studies in human lactation: milk volumes in lactating women during the onset of lactation and full lactation. Am J Clin Nutr. 1988;48:1375-86.
17. The European Food Information Council. Dietary reference values: A reference for whom? 2013; Available at: http://www.eufic.org/en/understanding-science/article/dietary-reference-values-a-reference-for-whom. Accessed 05/26, 2017.
18. Vandevijvere S, Amsalkhir S, Mourri AB, et al. Iodine deficiency among Belgian pregnant women not fully corrected by iodine-containing multivitamins: a national cross-sectional survey. Br J Nutr. 2013. 28;109:2276-84.
19. Knight BA, Shields BM, He X, et al. Iodine deficiency amongst pregnant women in South-West England. Clin Endocrinol (Oxf). 2017;86:451-5.
20. Granfors M, Andersson M, Stinca S, et al. Iodine deficiency in a study population of pregnant women in Sweden. Acta Obstet Gynecol Scand. 2015;94:1168-74.
21. Kirkegaard-Klitbo DM, Perslev K, Andersen SL, et al. Iodine deficiency in pregnancy is prevalent in vulnerable groups in Denmark. Dan Med J. 2016;63:A5286.
22. Lindorfer H, Krebs M, Kautzky-Willer A. Iodine deficiency in pregnant women in Austria. Eur J Clin Nutr. 2015;69:349-54.
23. Dold S, Zimmermann MB, Aboussad A, et al. Breast Milk Iodine Concentration Is a More Accurate Biomarker of Iodine Status Than Urinary Iodine Concentration in Exclusively Breastfeeding Women. J Nutr. 2017;147:528-37.
24. Abel MH, Caspersen IH, Meltzer HM, et al. Suboptimal Maternal Iodine Intake Is Associated with Impaired Child Neurodevelopment at 3 Years of Age in the Norwegian Mother and Child Cohort Study. J Nutr. 2017 May 17.
25. Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet. 2013;382:331-7.
26. Hynes KL, Otahal P, Hay I, Burgess JR. Mild iodine deficiency during pregnancy is associated with reduced educational outcomes in the offspring: 9-year follow-up of the gestational iodine cohort. J Clin Endocrinol Metab. 2013;98:1954-62.
27. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid. 2017;27:315-89.
28. Lazarus J, Brown RS, Daumerie C, et al. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J. 2014;3:76-94.
29. Dold S, Zimmermann MB, Baumgartner J, et al. A dose-response crossover iodine balance study to determine iodine requirements in early infancy. Am J Clin Nutr. 2016;104:620-8.
30. Harding KB, Pena-Rosas JP, Webster AC, et al. Iodine supplementation for women during the preconception, pregnancy and postpartum period. Cochrane Database Syst Rev. 2017;5;3:CD011761.
31. Leung AM, Pearce EN, Braverman LE. Iodine nutrition in pregnancy and lactation. Endocrinol Metab Clin North Am. 2011;40:765-77.
32. Henjum S, Kjellevold M, Ulak M, et al. Iodine Concentration in Breastmilk and Urine among Lactating Women of Bhaktapur, Nepal. Nutrients. 2016;8:10.3390/nu8050255.
33. Osei J, Andersson M, Reijden OV, et al. Breast-Milk Iodine Concentrations, Iodine Status, and Thyroid Function of Breastfed Infants Aged 2-4 Months and Their Mothers Residing in a South African Township. J Clin Res Pediatr Endocrinol. 2016;8:381-91.
34. Wang Y, Zhang Z, Ge P, et al. Iodine status and thyroid function of pregnant, lactating women and infants (0-1 yr) residing in areas with an effective Universal Salt Iodization program. Asia Pac J Clin Nutr. 2009;18:34-40.
35. Velasco I, Santos C, Limon J, et al. Bioactive Components in Human Milk Along the First Month of Life: Effects of Iodine Supplementation during Pregnancy. Ann Nutr Metab. 2016;68:130-6.
36. Tazebay UH, Wapnir IL, Levy O, et al. The mammary gland iodide transporter is expressed during lactation and in breast cancer. Nat Med. 2000;6:871-8.

ORIGINAL ARTICLE

Predictors of benefit from high-altitude climate therapy in adults with severe asthma

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ABSTRACT

Background: High-altitude climate therapy has been shown to benefit patients with severe asthma but it is not known which patients benefit most from this treatment. In the current study we aimed to identify clinical, functional and inflammatory predictors of favourable outcome of high-altitude climate therapy.

Methods: This is a secondary analysis of a prospective cohort including 136 adult patients with a diagnosis of severe refractory asthma, referred to the Dutch Asthma Centre in Davos (1600 metres above sea level), Switzerland. They had assessments of medication usage, asthma-related quality of life (Asthma-related Quality of Life Questionnaire, AQLQ), asthma control, body mass index (BMI), sino-nasal symptoms, fatigue, lung function (forced expiratory volume in one second, FEV1), exercise tolerance, allergy and inflammation (fraction of exhaled nitric oxide, blood eosinophils) at entry and after 12 weeks of treatment. Five clinically relevant outcomes were considered: AQLQ, oral corticosteroid dose, FEV1, body mass index and blood eosinophils. Independent predictors of beneficial outcome were identified by multiple linear regression analysis.

Results: Lower blood eosinophil counts ($p < 0.01$), younger age ($p = 0.02$) and poorer asthma control ($p < 0.01$) were independently associated with greater reduction in the dose of oral corticosteroids. Lower fatigue score at baseline ($p = 0.01$) was associated with greater weight loss (reduction in BMI). Higher levels of total IgE at baseline ($p < 0.01$), and higher doses of inhaled corticosteroids ($p = 0.03$) were associated with greater decreases in blood eosinophils. There were no predictors for improvement in AQLQ or FEV1.

Conclusions: The beneficial effect of high-altitude climate therapy in adults with severe asthma can be predicted by patient characteristics, such as age, blood eosinophils and degree of asthma control before admission.

KEYWORDS

Asthma, high-altitude climate therapy, predictors

INTRODUCTION

Severe asthma is a life-threatening disease currently understood to be a complex interaction of clinical, physiological and inflammatory characteristics resulting in different phenotypes. Over the last decade studies have identified at least three different phenotypes for severe asthma, including severe atopic asthma, persistent eosinophilic asthma (formerly called 'intrinsic' asthma) and asthma associated with morbid obesity.[1-4] This phenotypic heterogeneity is mirrored by different responses to asthma treatment. For example, persistent eosinophilic asthma usually responds to high-dose inhaled or oral corticosteroid treatment,[1] whereas the obese phenotype typically does not,[5] which supports the need for targeted treatment.[6,7]

Apart from new biological pharmacological targeted treatments,[8,9] also non-pharmacological treatment strategies such as directly targeting airway smooth muscle with bronchial thermoplasty,[10] optimising the management process with telemonitoring,[11] or treating the patient in a low-trigger environment at high altitude[12] have been shown to be beneficial for patients with severe asthma.

High-altitude climate therapy involves exposure to conditions of altitude ≥ 1500 metres above sea level.[13] It was previously believed to be of benefit specifically for patients with atopic asthma[14] but recent evidence shows that atopy is not the only driver for a successful outcome.[15-18] The determinants of a successful outcome of high-altitude climate therapy are still unclear. From clinical experience it appears that also the type of response to high-altitude climate therapy differs between patients. Some have a more pronounced improvement in lung function,[13] whereas others boost their exercise tolerance or are able to taper their dose of oral corticosteroids.

In the present study, we investigated whether typical clinical, functional and inflammatory characteristics of patients with severe asthma can predict a specific beneficial outcome of high-altitude climate therapy.

MATERIAL AND METHODS

Patients and design of the study

This is a secondary analysis of a prospective cohort study evaluating patients with severe, refractory asthma who were referred to the Dutch Asthma Centre in Davos, Switzerland, in order to optimise their disease. The Dutch Asthma Centre is localised at an altitude of 1600 meters above sea level and offers personalised, multidisciplinary, multifaceted treatment for patients with severe asthma in a low-trigger environment. The characteristics of the cohort have been described previously.[12] In short, participants were adult patients (17-75 years) with a diagnosis of severe refractory asthma using high doses of inhaled corticosteroids ≥ 1000 µg/day of fluticasone or equivalent or chronic oral corticosteroids, combined with long-acting bronchodilators for at least one year. Current smokers and patients with a history of more than 15 pack-years of cigarette smoking were excluded from the study. All patients were symptomatic and had had at least one severe exacerbation during the past year requiring a course of oral corticosteroids.

The study was approved by the Ethics Committee of the Academic Medical Centre of the University of Amsterdam (Amsterdam, the Netherlands) and was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent before taking part in the study. This study was registered at the Netherlands Trial Register, www.trialregister.nl under NTR 1277.

Assessments

Patients participating in this study were assessed and evaluated according to a systematic protocol at entry and after 12 weeks of treatment.

Questionnaires

At baseline, all patients completed standard questionnaires including information on socio-demographics, asthma history, current symptoms, smoking habits and medication usage, including daily doses of inhaled and oral corticosteroids.

The level of asthma control was assessed using the Juniper ACQ-6 score, a 6-item version of the Asthma Control Questionnaire with the FEV1 question omitted.[19] In this questionnaire, patients recall their experiences over the past seven days and respond to each question on a 7-point Likert scale, where 0 represents no impairment and 6 represents maximum impairment.

Asthma-related quality of life was measured using the Juniper Asthma Quality of Life Questionnaire (AQLQ), an asthma-specific questionnaire that measures symptoms, activity limitations, emotional functioning, and environmental stimuli.[20] The mean of the 32 items in the AQLQ between 1 (very poor asthma-related quality of life) and 7 (best asthma-related quality of life) was used.

The 20-question Sino-Nasal Outcome test (SNOT-20) was used to measure rhino-sinusitis-related quality of life. The mean total score ranges from 0 (no symptoms) to 5 (severe symptoms) and is calculated by averaging an individual's responses to all questions.[21]

The severity of fatigue was measured by the subscale Subjective Fatigue from the multidimensional Checklist Individual Strength (CIS).[22] The Subjective Fatigue subscale of the CIS (CIS-Fatigue) consists of eight items, scored on a 7-point Likert-scale, higher scores meaning worse outcomes.

Lung function assessment

Pulmonary function testing was performed using the Masterscreen PTF (Jaeger Viasys, Germany). Forced expiratory volume in one second (FEV1) was assessed before and after inhaled administration of 400 µg salbutamol and expressed as percentage of predicted value.[23]

Exercise tolerance

The six-minute walk test (6MWT) measures the maximum distance an individual is able to walk on a flat, hard surface in a period of 6 minutes.[24] The self-paced 6MWT assesses the submaximal level of functional capacity of the patient and reflects the functional exercise level for daily physical activities. An improvement of 54 meters has been shown to be a clinically important difference.[25]

Assessment of allergy and inflammation

Total IgE and specific IgE to common aero-allergens (house dust mites, mixed grass and birch pollen, cat and dog dander and *Aspergillus fumigatus*) in peripheral blood

was assessed by fluoro-enzyme immunoassay UniCAP® (Pharmacia & Upjohn, Uppsala, Sweden) and expressed in kU.L-1. Eosinophils in peripheral blood were measured by a standard automated cell counter. Fractional exhaled nitric oxide (FeNO) was measured by a chemiluminescence analyser (Niox Aerocrine AB, Solna, Sweden) according to the current guidelines.[26]

Potential predictors of benefit

Nineteen baseline demographic, clinical, functional, and inflammatory parameters were selected as the most clinically relevant and considered as potential predictors of favourable or unfavourable outcome.

Outcomes

Five outcomes considered to be representative of five clinically relevant domains of improvement were defined: change in oral corticosteroid dose (medication requirement domain), change in AQLQ score (patient-reported outcome domain), change in FEV1 (lung function domain), change in blood eosinophils (airway inflammation domain) and change in BMI (lifestyle domain). The choice of these outcomes was based on literature about clinical phenotypes of severe asthma in adults.[1-4]

Statistical analysis

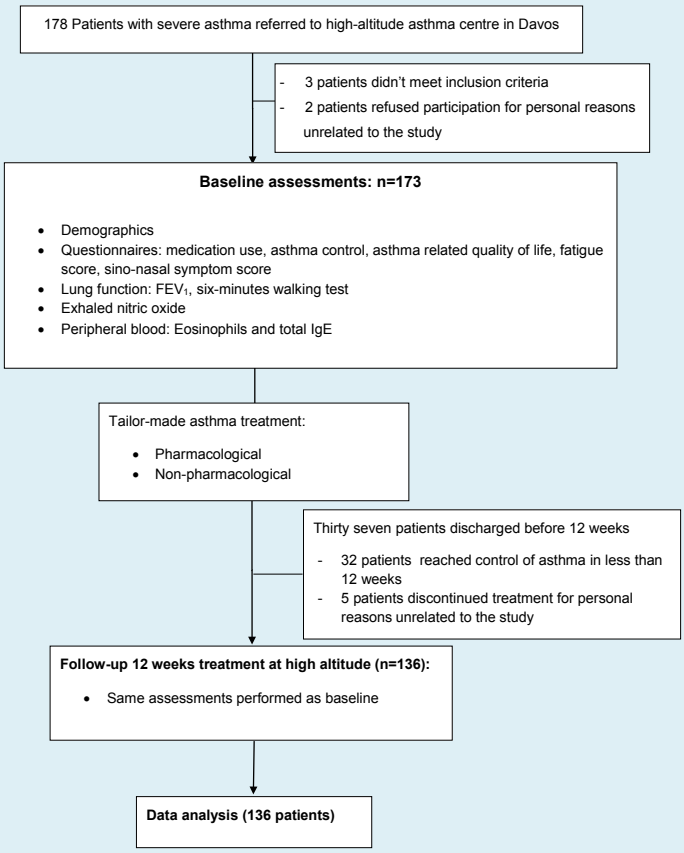
Changes from baseline in BMI, daily oral corticosteroid dose, AQLQ score, FEV1, and peripheral blood eosinophils were analysed using paired t-test or Wilcoxon signed-rank tests, depending on the distribution of the variables.

Spearman rank-order correlation was used to explore the association between the potential predictors of benefit and each of the outcomes. Predictors with a statistical significance (p value < 0.05) were included in the multiple linear regression analysis to identify independent predictors of benefit. Analyses were conducted using PASW Statistics 20.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

A total of 173 patients participated in the study. Of these, 136 patients had assessments performed at baseline and at 12 weeks of treatment, and were included in the analysis (*figure 1*). Thirty-seven patients were only assessed at baseline, but not at 12 weeks for variable reasons: 32 patients achieved control of asthma and were discharged home before 12 weeks and 5 had the high-altitude treatment discontinued for personal reasons.

Figure 1. *Flow diagram of subjects' progress through the study*



Number of patients who were screened, enrolled and completed the study

Table 1. *Baseline characteristics from patients included in the analysis*

Characteristics	n = 136	
Age (years)	45.9	(36.0 to 56.0)
Female gender (n (% n))	92.0	(67.6)
Age asthma onset (years)	8.5	(2.6 to 26.7)
Ever smoker (n (% n))	51.0	(37.0)
Domains		
Medication		
Use of OCS (n (% n))	72.0	(53.0)
OCS dose (mg/day)	20.0	(10.0 to 30.0)
ICS dose (mcg/day)	1000	(500 to 1000)
Patient-reported outcomes		
AQLQ score	3.87	(3.18 to 4.57)
ACQ score	3.16	(2.66 to 3.83)
SNOT-20 score	2.20	(1.67 to 2.75)
CIS-Fatigue score	50.0	(44.0 to 55.0)
Lung function		
FEV1 post β2 (l)	2.87	(1.9 to 3.2)
FEV1 post β2 (%)	89.29	(68.3 to 100.7)
6MWD (m)	460.0	(373.0 to 560.0)
Airway inflammation		
Blood eosinophils (unit/µl)	180.0	(92.5 to 337.5)
FeNO (ppb)	19.0	(11.0 to 40.0)
Atopy (n(% n))	90.0	(66.0)
Total IgE (kU.L-1)	112.0	(32.4 to 384.7)
Sensitisation HDM (n(%n))	65.0	(47.7)
Lifestyle		
BMI (kg/m²)	28.8	(28.0 to 32.9)

Data are presented as median (interquartile range) unless otherwise stated. 6MND = six-minute walking distance; BMI = body mass index; FeNO = fraction of exhaled nitric oxide; HDM = house dust mite; ICS = inhaled corticosteroids; OCS = oral corticosteroids.

There were no differences in baseline characteristics between patients who did and did not finish the study (data not shown).

Baseline characteristics are shown in *table 1*. Changes from baseline in the five main outcomes are shown in *table 2*.

Predictors of benefit from high-altitude treatment

Spearman rank correlations between potential predictors of improvement (baseline characteristics) and each outcome are listed in *table 3*. All patients showed improvements in AQLQ and FEV1 at 12 weeks, but no specific predictors for improvement of these parameters were identified. Poorer asthma control ($p < 0.01$), younger age ($p = 0.02$) and lower blood eosinophil counts at baseline ($p < 0.01$) were independently associated with reduction in daily oral corticosteroid dose at 12 weeks. Higher inhaled corticosteroid dose ($p = 0.03$) and high total IgE at baseline ($p < 0.01$) were independently associated with reduction in blood eosinophils at 12 weeks and a lower fatigue score at baseline was independently associated with reduction in BMI ($p = 0.01$) (*table 4*).

DISCUSSION

In this study we categorised the benefits of high-altitude climate therapy for patients with severe asthma in five different outcome domains represented as reduction in daily oral steroid use, improvements in asthma-related quality of life, FEV1, blood eosinophils and weight loss. Each of these outcomes was independently associated with specific patient characteristics, except for the improvement in asthma-related quality of life and FEV1, which was similar for all patients with severe asthma.

Nearly all patients with severe asthma participating in this study showed a significant improvement in their asthma-related quality of life scores after high-altitude climate therapy. No patient characteristics could predict this benefit, which suggests that non-specific aspects of high-altitude climate therapy played an important role, such as better air quality, improved adherence with the drug regimen or removal from stress at home or at the workplace.[27] Therefore, high-altitude climate therapy might be an option for patients with severe refractory asthma, irrespective of their phenotype.

The majority of the prednisone-dependent patients were able to reduce their dose of oral corticosteroids after 12 weeks of high-altitude climate therapy while maintaining the same level of asthma control. The degree of oral corticosteroid reduction was associated with higher symptom score and younger age, combined with low blood eosinophil count at baseline. Similar characteristics were described by Haldar and colleagues in the highly symptomatic, low eosinophilic non-obese asthma phenotype.[1] This suggests that these patients were symptomatic for other reasons than severe airway inflammation and reinforces previous studies which demonstrated that tapering of oral corticosteroids is easier in patients without eosinophilia.[28]

For the group as a whole there was a significant improvement in post-bronchodilator FEV1, up to 10% of the predicted value in some patients. A previous meta-analysis had also demonstrated benefit of high-altitude climate

Table 2. *Changes from baseline in the five main outcomes*

	Baseline		12 weeks		p value
Domains					
Medication					
Use of OCS (n(% n))	72.0	(53.0)	43.0	(31.6)	< 0.01
Patient-reported outcomes					
AQLQ score	3.87	(3.18 to 4.57)	5.46	(4.87 to 6.29)	< 0.01
Lung function					
FEV1 post β2 (L)	2.87	(1.9 to 3.2)	3.01	(2.23 to 3.53)	< 0.01
FEV1 post β2 (%)	89.2	(68.3 to 100.7)	93.7	(79.3 to 105.6)	< 0.01
Airway inflammation					
Blood eosinophils (unit/µl)	180.0	(92.5 to 337.5)	190.0	(120.0 to 310.0)†	0.29
Lifestyle					
BMI (kg/m²)	28.8	(28.0 to 32.9)	28.3	(23.9 to 31.8)	< 0.01

Data are presented as median (interquartile range) unless otherwise stated; p values from paired t-test or Wilcoxon signed rank tests, depending on the distribution of the variables. †Data available for 100 patients, blood eosinophils measured at 12 or 15 weeks of high altitude climate therapy. BMI = body mass index; OCS = oral corticosteroids.

therapy in the lung function of adult patients with asthma.[13] However, we could not find any clinical or inflammatory predictors of improvement. A reduced post bronchodilator FEV1 indicates persistent airflow limitation, probably linked to inflamed and swollen airway mucosa or airway remodelling. A possible explanation could be that high-altitude climate therapy reduces the thickness of the airway mucosa or might even reverse airway modelling, irrespective of the patient characteristics at admission, but this remains to be investigated in future studies.

Blood eosinophils did not change significantly for the group as a whole, which may be explained by a simultaneous tapering of anti-inflammatory medication. Still, there were patients showing significant reductions of blood eosinophil levels, suggesting an anti-inflammatory effect of high-altitude climate therapy in selected patients. Predictors of such a reduction in blood eosinophils were a high level of total IgE and a high level of inhaled and oral corticosteroid use. This fits with the hypothesis that continuous exposure to allergens (e.g. house dust mites, fungi) at sea level had been responsible for ongoing airway inflammation and elevated blood eosinophil counts despite high dose anti-inflammatory treatment.[27] At high altitude these triggers were likely to be either absent or largely diminished. Thus, in patients with high IgE levels, even if already treated with high doses of anti-inflammatory medications, objective improvement in systemic inflammation can be obtained by high-altitude climate therapy.

In our study, more pronounced weight reduction was associated with lower fatigue scores at baseline, indicating that patients with more vitality had more possibilities to reduce weight by physical activity. Many studies have shown that weight reduction improves clinical outcomes in patients with asthma.[29,30] but it is not always easy to achieve a significant weight reduction in patients with asthma and obesity with lifestyle modification and physical exercise at sea level. Our study suggests that physical activity might be facilitated in a clean air environment with multidisciplinary support and therefore might help patients with severe asthma to improve their physical condition and subsequently reduction of BMI.

This study has a few potential limitations to be addressed. Firstly, some patient characteristics that could also have influenced treatment outcomes have not been addressed, such as stressful psychosocial conditions, hormonal changes (adrenal gland activation)[31] and unknown exposures to sensitising agents. In particular anxiety and depression might have been important predictors of beneficial response, since it has been shown that these are important contributors to asthma severity.[32] If so, our data suggest that high-altitude climate therapy could have had a beneficial effect on these psychological stressors as well. Secondly, altitude itself could have influenced the partial pressure of gases such as FeNO with potential overestimation of FeNO levels.[33] However, we believe that this did not influence our findings since change in FeNO was not used as an outcome, and baseline FeNO

Table 3. *Associations between patient characteristics at baseline and improvement in the outcomes (Spearman rank correlation)*

Patient characteristic	Outcomes									
	1. Change in OCS		2. Change in AQLQ		3. Change in FEV1		4. Change in blood eosinophils		5. Change in BMI	
	rho	p value	rho	p value	rho	p value	Rho	p value	rho	p value
Gender	0.19	0.09	-0.04	0.64	0.06	0.49	-0.18	0.07	-0.07	0.38
Age	0.30	0.01*	-0.08	0.45	-0.03	0.68	0.06	0.54	0.03	0.68
Asthma onset	0.13	0.25	-0.16	0.07	0.05	0.58	0.16	0.13	0.04	0.57
Asthma duration	0.11	0.33	0.06	0.48	-0.05	0.53	-0.04	0.66	0.01	0.85
Ever smoking	0.02	0.87	0.01	0.97	0.04	0.59	-0.01	0.93	0.02	0.79
BMI	-0.17	0.10	-0.01	0.98	-0.05	0.58	0.06	0.53	--	--
Dose of ICS	-0.16	0.18	0.03	0.76	0.14	0.10	-0.21	0.03*	-0.02	0.80
Dose of OCS	--	--	-0.03	0.74	0.06	0.44	0.22	0.03*	0.01	0.94
ACQ score	-0.46	< 0.01*	0.31	< 0.01*	0.13	0.14	0.02	0.76	0.07	0.37
AQLQ score	0.39	< 0.01*	--	--	-0.09	0.29	-0.11	0.25	-0.12	0.14
SNOT-20 score	-0.23	0.07	0.10	0.22	0.09	0.26	0.10	0.32	0.15	0.07
CIS-fatigue score	-0.10	0.52	0.22	0.01*	0.01	0.90	0.14	0.16	0.18	0.03*
FEV1 %pred	-0.21	0.06	0.01	0.96	--	--	0.14	0.15	-0.18	0.03*
6MWD	0.24	0.05	-0.01	0.89	0.01	0.89	-0.15	0.14	-0.05	0.62
FeNO	-0.02	0.84	-0.13	0.13	0.04	0.67	-0.13	0.20	0.10	0.21
Blood eosinophils	0.35	< 0.01*	-0.07	0.41	0.03	0.71	--	--	-0.10	0.22
Total IgE	0.09	0.43	-0.04	0.63	0.05	0.57	-0.37	< 0.01*	0.03	0.74
IgE-HDM	0.07	0.54	-0.02	0.78	0.01	0.99	-0.25	0.01*	-0.03	0.68
Atopy	0.01	0.97	0.01	0.89	-0.03	0.66	-0.29	< 0.01*	0.01	0.90

Values from Spearman rank correlation between baseline characteristics and degree of improvement in each outcome. rho for Spearman's correlation coefficient. *p value < 0.05. 6MND = six-minutes walking distance; BMI = body mass index; FeNO = fraction of exhaled nitric oxide; HDM = house dust mite ICS = inhaled corticosteroids; OCS = oral corticosteroids

was included as a continuous variable in the regression analyses.

Thirdly, this study was a one-centre intervention and although previous studies on high-altitude climate therapy in children and adults showed similar improvements,[15-17] extrapolation of our results to other high-altitude centres cannot be easily done.

Furthermore, although the current study was not designed to compare high-altitude climate therapy with similar in-house treatment at sea level, it would have been worth evaluating the effect of a similar program at sea level for these patients. A prospective comparative study is currently ongoing in the Netherlands (NTR5182).

Finally, some parameters (total IgE, blood eosinophils, 6-minute walking distance) were not assessed on two occasions in all patients, which might have affected the power of our study. Still, meaningful results could be obtained, despite this potential lack of power.

CONCLUSION

In conclusion, this study shows that high-altitude climate therapy improves quality of life and lung function in all patients with severe asthma. Maintenance oral steroid use, body weight, and blood eosinophils improve to a variable extent depending on patient characteristics, such as age, degree of asthma control, total IgE and fatigue score before admission. For clinicians these results show that the benefits of high altitude are multiple and diverse, and that for each patient with severe asthma a specific treatment target can be formulated. Identifying predictors of improvement of

Table 4. *Independent associations between patient characteristics and outcomes (multiple regression analysis)*

	B*	(95% CI)*	p value
Outcome 1: Change in OCS dose			
ACQ	-8.23	-14.22 to -2.23	< 0.01
Age	0.24	0.04 to 0.49	0.02
Eosinophil	0.02	0.01 to 0.03	< 0.01
AQLQ score	-1.57	-7.67 to 4.51	0.60
Outcome 2: Change in AQLQ			
ACQ score	-0.30	-0.12 to -0.48	< 0.01
CIS fatigue score	0.01	-0.01 to 0.03	0.23
Outcome 3: Change in FEV1	--	--	--
Outcome 4: Change in blood eosinophils			
ICS dose	-0.07	-0.14 to -0.01	0.03
Total IgE	-0.09	-0.13 to -0.06	< 0.01
OCS dose	2.57	-0.26 to 5.41	0.07
Outcome domain 5: Change in BMI			
CIS fatigue score	0.04	0.01 to 0.06	0.01
FEV1 % predicted	-0.01	-0.02 to 0.00	0.05

Values from multiple linear regression analysis between baseline characteristics and improvement in each outcome domain. The model included univariate predictors of improvement with a p value < 0.05. ICS = inhaled corticosteroids; OCS = oral corticosteroids.

high climate therapy is the first step toward understanding mechanisms of the different phenotypes of severe asthma, as well as the beneficial effects of a low-trigger environment.

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DISCLOSURES

The authors report no conflict of interest relevant to the content of this report.

REFERENCES

1. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med. 2008;178:218-24.
2. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181:315-23.
3. Lefaudeux D, De Meulder B, Loza MJ, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. J Allergy Clin Immunol. 2017;139:1797-807.
4. Amelink M, de Nijs SB, de Groot JC, et al. Three phenotypes of adult-onset asthma. Allergy. 2013;68:674-80.
5 Gibson PG. Obesity and asthma. Ann Am Thorac Soc. 2013;10 Suppl:S138-S142.
6 Dixon AE, Pratley RE, Forgione PM, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. J Allergy Clin Immunol. 2011;128:508-15.
7 Freitas PD, Ferreira PG, Silva AG et al. The Role of Exercise in a Weight-Loss Program on Clinical Control in Obese Adults with Asthma. A Randomized Controlled Trial. Am J Respir Crit Care Med. 2017;195:32-42.
8. National Institutes of Health. NationalHeart. Report: Lung and Blood Institute. Global initiative for asthma. Global strategy for asthma management and prevention. NHLBI/WHO workshop, 2006.
9. Bousquet J, Brusselle G, Buhl R, et al. Care pathways for the selection of a biologic in severe asthma. Eur Respir J. 2017;50:1701782.
10. Castro M, Rubin AS, Laviolette M, et al. Effectiveness and safety of bronchial thermoplasty in the treatment of severe asthma: a multicenter, randomized, double-blind, sham-controlled clinical trial. Am J Respir Crit Care Med. 2010;181:116-24.
11. Hashimoto S, ten Brinke A, Roldaan AC, et al. Internet-based tapering of oral corticosteroids in severe asthma: a pragmatic randomised controlled trial. Thorax. 2011;66:514-20.
12. Rijssenbeek-Nouwens LH, Fieten KB, Bron AO, Hashimoto S, Bel EH, Weersink EJ. High-altitude treatment in atopic and nonatopic patients with severe asthma. Eur Respir J. 2012;40:1374-80.
13. Vinnikov D, Khafagy A, Blanc PD, et al. High-altitude alpine therapy and lung function in asthma: systematic review and meta-analysis. ERJ Open Res. 2016; 2:00097-2015.
14. Spieksma FT, Zuidema P, Leupen MJ. High altitude and housedust mites. Br Med J. 1971;1:82-4.

15. Grootendorst DC, Dahlen SE, van Den Bos JW, et al. Benefits of high altitude allergen avoidance in atopic adolescents with moderate to severe asthma, over and above treatment with high dose inhaled steroids. Clin Exp Allergy. 2001;31:400-8.

16. Huss-Marp J, Kramer U, Eberlein B, et al. Reduced exhaled nitric oxide values in children with asthma after inpatient rehabilitation at high altitude. J Allergy Clin Immunol. 2007;120:471-2.

17. Verkleij M, Beelen A, van Ewijk BE, Geenen R. Multidisciplinary treatment in children with problematic severe asthma: A prospective evaluation. Pediatr Pulmonol. 2017;52:588-97.

18. Karagiannidis C, Hense G, Rueckert B, et al. High-altitude climate therapy reduces local airway inflammation and modulates lymphocyte activation. Scand J Immunol. 2006;63:304-10.

19. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med. 2005;99:553-8.

20. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. J Clin Epidemiol. 1994;47:81-7.

21. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the 20-Item Sino-Nasal Outcome Test (SNOT-20). Otolaryngol Head Neck Surg. 2002;126:41-7.

22. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. J Psychosom Res. 1994;38:383-92.

23. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319-38.

24. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166:111-7.

25. Redelmeier DA, Bayoumi AM, Goldstein RS, Guyatt GH. Interpreting small differences in functional status: the Six Minute Walk test in chronic lung disease patients. Am J Respir Crit Care Med. 1997;155:1278-82.

26. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. Am J Respir Crit Care Med. 2005;171:912-30.

27. Rijssenbeek-Nouwens LH, Bel EH. High-altitude treatment: a therapeutic option for patients with severe, refractory asthma? Clin Exp Allergy. 2011;41:775-82.

28. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet. 2002;360:1715-21.

29. Mosen DM, Schatz M, Magid DJ, Camargo CA, Jr. The relationship between obesity and asthma severity and control in adults. J Allergy Clin Immunol. 2008;122:507-11.

30. Peters JI, McKinney JM, Smith B, Wood P, Forkner E, Galbreath AD. Impact of obesity in asthma: evidence from a large prospective disease management study. Ann Allergy Asthma Immunol. 2011;106:30-5.

31. Brimkulov NN, Bakirova AN, Chaltabaev KS. Effect of high-altitude climate therapy on the adrenal cortex function in patients with bronchial asthma. Klin Med (Mosk). 1990;68:82-5.

32. Amelink M, Hashimoto S, Spinhoven P, et al. Anxiety, depression and personality traits in severe, prednisone-dependent asthma. Respir Med. 2014;108:438-44.

33. Brimkulov NN, Bakirova AN, Chaltabaev KS. Effect of high-altitude climate therapy on the adrenal cortex function in patients with bronchial asthma. Klin Med (Mosk). 1990;68:82-5.

ORIGINAL ARTICLE

Clinical predictors of escalating care in hepatic and renal cyst infection in autosomal dominant polycystic kidney and liver disease

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ABSTRACT

Background: Cyst infection may occur in autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD). Antimicrobial agents often fail to control infection, leading to invasive action. We aimed to identify factors predicting escalation of care.

Methods: ADPKD and ADPLD patients were identified from local/national databases (2001-2013). Records were screened for patients meeting criteria for cyst infection (positive cyst aspirate and/or clinical findings). Factors that predict escalated care were identified with multivariate modified Poisson regression.

Results: We screened 1773 patients. A total of 77 patients with cyst infection (4.3%) were included for analysis (hepatic 36%; male 49%; age 54 ± 13 years; ADPKD 95%; dialysis 9%, diabetes 18%, renal transplant 56%, eGFR [IQR 24-78] ml/min/1.73 m^2 (excluding patients with a history of renal transplant or receiving dialysis)). A pathogen was identified in 71% of cases. *Escherichia coli* was the most common pathogen and accounted for 69% of cases. Initial treatment was limited to antibiotics in 87% of patients (n = 67), 40% included a fluoroquinolone. Ultimately, 48% of patients underwent some form of invasive action (escalation of care). Increasing white blood cell count (WBC) (RR 1.04 95%-CI 1.01-1.07, p = 0.008) was associated with escalating care, whereas an increase in time between transplant and infection (RR 0.92 95% CI 0.86-0.97, p = 0.005) and *E. coli* isolation (RR 0.55 95% CI 0.34-0.89, p = 0.02) were protective.

Conclusion: High serum WBC, isolation of atypical pathogens and early infection after transplantation are factors that increase the risk of escalation of care in hepatic and renal cyst infection patients.

KEYWORDS

ADPKD, ADPLD, cyst infection

INTRODUCTION

Cyst infection may occur as a complication in autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD) and results in considerable morbidity and mortality.[1,2] Cyst infections are thought to arise from haematogenous spread of translocated gut bacteria (hepatic cyst infection) or ascending urinary tract infection (renal cyst infection).[3] *Escherichia coli* is the most frequently isolated microorganism in both hepatic and renal cyst infection.[4] Diagnosing cyst infection may be difficult as a definite diagnosis can only be made upon presence of cyst aspirate with inflammatory cells and bacteria.[5] In most cases, a cyst aspirate is not routinely taken. Therefore, physicians are likely to make clinical decisions using a mix of clinical, biochemical and imaging findings,[6] which led to the development of several diagnostic algorithms for patients in whom cyst infection is suspected.[7,8]

The first line of treatment of cyst infection consists of antibiotics. There is no evidence base for the choice of antibiotic therapy. Fluoroquinolones are traditionally selected due to their favourable pharmacokinetic properties as ciprofloxacin readily diffuses in hepatic and renal cysts.[9,10] However, antibiotics often fail to control the infection. This leads to frequent switching between antibiotic classes and ultimately to invasive action in an attempt to control the infection.[11]

We hypothesise that patient-related and clinical factors (age, gender, serum inflammation parameters, comorbidities, history of solid organ transplantation) modify the risk for additional invasive measures that are required to control the infection.[12] The aim of the present study was to investigate factors that predict escalation of care in a nationwide cohort of 77 patients who developed cyst infection.

MATERIALS AND METHODS

Ethical consideration

This study did not require formal review by the Institutional Review Board (IRB) after study protocol evaluation by the local IRB (CMO Radboud University Medical Center Nijmegen, registration number 2013/299).

Study design

We established a multicentre retrospective cohort of patients who were diagnosed with cyst infection. Patients were enrolled at four tertiary referral centres in the Netherlands which participated in the DIPAK-1 Study. This cohort study is reported in accordance with the Strengthening The Reporting of OBservational studies in Epidemiology (STROBE) statement (*supplementary table 1**).[13]

Setting and participants

We identified ADPKD and ADPLD patients through inspection of a number of local and national databases. The local databases are established because of financial reimbursement systems that register the diagnosis of patients using general Diagnosis Treatment Combinations (DTCs). The national database consisted of RENINE (Registration Renal Replacement Therapy the Netherlands), a Dutch foundation that registers all renal transplant recipients in the Netherlands. The DIPAK-1 Study group is an investigator-driven, multicentre, open label, randomised controlled trial, planned to enrol 300 individuals who are aged 18-60 years, with an estimated glomerular filtration rate (eGFR) of 30-60 ml/min/1.73m^2.[14]

We included ADPKD and ADPLD patients diagnosed with either hepatic or renal cyst infection. We diagnosed cyst infection in case of a positive cyst aspirate (white blood cells and bacteria) or when cyst infection was considered by the individual physician to be stated in the medical record in the combined presence of: 1) serum C-reactive protein (CRP) > 50 mg/l, 2) body temperature ≥ 38.5°C and 3) abdominal pain.[6,15] Medical records were screened for patients who met the inclusion criteria for cyst infection. We excluded patients if: a) cyst infection was a complication of aspiration sclerotherapy, b) the site of cyst infection was not documented, c) data on initial treatment were incomplete or d) ADPLD or ADPKD was not reported in the medical chart.

Variables

We extracted patient demographics and clinical variables at the time of cyst infection from medical records into an electronic database. The variables identified included gender, age at cyst infection, type of polycystic disease, site of cyst infection, peak serum CRP, peak serum white blood cell count (WBC), peak estimated glomerular filtration rate (eGFR), microbiological culture results (urine, blood and cyst aspirate), initial treatment, initial antibiotic treatment, treatment duration and details on invasive treatment. We also collected data on comorbidities including history of solid organ transplant, interval between solid organ transplant and cyst infection, dialysis, diabetes mellitus and immunosuppressive therapy. Definitions of variables are discussed in more detail in *supplementary table 2*.

Outcomes

We defined any procedure that aimed to breach the wall of the infected cyst, either through percutaneous access or surgery as escalation of care.

Multiple imputation

We used multiple imputation to account for missing data. We limited imputation of missing variables to patients in whom the outcome was available.[16] Multiple imputation relies on the assumption that data are missing at random, which implicates that the probability that a specific variable is missing depends on the other variables that are available for that patient (i.e. other observed patient characteristics).[17] Literature suggests to impute at least 40 times to allow accurate estimation of missing variables.[16]

Analysis

We performed all statistical analyses using SPSS statistical software package version 22 (SPSS Inc., Chicago, IL). Chi-square test was used for dichotomous data, Student t-test for parametric data and Mann-Whitney U test for non-parametric data. All tests were two-tailed and a p value of < 0.05 was considered statistically significant.

As the expected incidences of the outcomes investigated in this study were high, we calculated adjusted risk ratios

to avoid any possible misinterpretation of odds ratios (OR) as risk ratios (RR).[18] We selected Poisson regression as an alternative for binary logistic regression to obtain adjusted RR instead of OR.[19] To correct for the use of binary outcomes, we used a robust error variance procedure to correct for the incorrect assumption of Poisson distributed outcomes.[20]

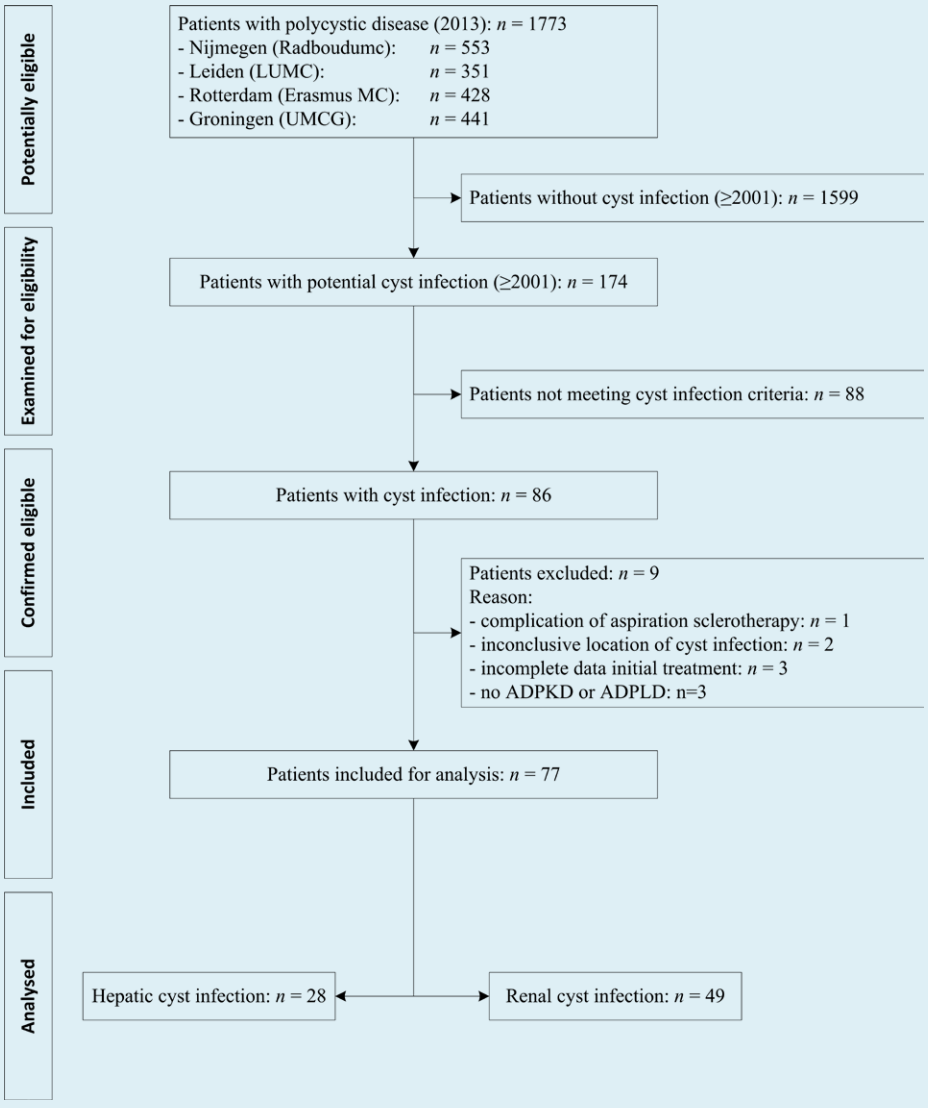
Variables with a p value of < 0.2 in univariate analysis were included in the multivariate modified Poisson regression model with manual backward elimination of non-significant variables. A p value of < 0.05 was considered to be statistically significant. We performed univariate and multivariate analyses on both the original and imputed datasets.

RESULTS

Cyst infection patients

We identified 1773 polycystic disease patients in four Dutch tertiary referral centres (*figure 1*). We examined 174 patients (9.8%) for eligibility and 86 (4.9%) had developed hepatic or renal cyst infection in or after 2001. Patients were excluded if the infection developed following aspiration sclerotherapy (n = 1), infection location was unknown (n = 2), data on initial treatment were missing (n = 3) or polycystic disease was absent (n = 3). Ultimately, we included 77 cyst infection patients (4.3%) for analysis.

Figure 1. *Number of patients at each stage of the study*



Radboudumc = Radboud University Medical Center; UMCG = University Medical Center Groningen; LUMC = Leiden University Medical Center; Erasmus MC = Erasmus Medical Center Rotterdam; ADPKD = autosomal dominant polycystic kidney disease; ADPLD = autosomal dominant polycystic liver disease; *n* = number

Patient demographics

Table 1 shows characteristics of the included patients with cyst infection (n = 77). Almost all patients suffered from ADPKD (95%). Of these patients, 49% were male and the average age at infection was 54 years. Hepatic cyst infection was diagnosed in 28 patients (36%), whereas 49 patients (64%) were diagnosed with a renal cyst infection. Diabetes mellitus was frequent (18%) and 56% of patients with cyst infection had a history of solid organ transplantation (all kidney transplant recipients). The median interval between transplantation and cyst infection was four years.

Table 1. *Characteristics extracted from medical records of cyst infection patients*

Characteristic	Total (n = 77)	Missing (n)
Male, n (%)	38 (49)	
Age at cyst infection, years (± SD)	54 ± 13	
Type of polycystic disease, n (%)		
- ADPKD	73 (95)	
- ADPLD	4 (5)	
Site of cyst infection, n (%)		
- Liver	28 (36)	
- Kidney	49 (64)	
Diabetes mellitus, n (%)	14 (18)	
Dialysis, n (%)	7 (9)	
Solid organ transplant, n (%)[a]	43 (56)	
- Interval solid organ transplant and cyst infection, years [IQR]	4 [1-10]	
Immunosuppressive drug use, n (%)	44 (57)	
Peak serum CRP, mg/l [IQR]	215 [123-283]	1
Peak serum WBC, x10^9/l [IQR]	11.6 [8.3-15.7]	1
Peak eGFR, ml/min/1.73m^2 [IQR][b]		6
- MDRD	50 [24-78]	
- CKD-EPI	57 [26-87]	
Positive microbiological culture, n (%)[c]	55 (71)	
- Hepatic cyst infection patients	23 (82)	
- Renal cyst infection patients	32 (65)	
Atypical pathogen cultured (other than *E. coli*), n (%)	24 (31)	3
Initial treatment		
- Limited to antibiotics, n (%)[d]	67 (87)	
- Containing fluoroquinolone, n (%)	27 (40)	1
- Duration, days [IQR]	8 [4-39]	6
Follow-up, years [IQR]	2 [1-5]	

Parametric variables are expressed as mean ± SD. Non-parametric variables are expressed as median [IQR]. Percentages may not add up to 100 due to rounding.
n = number; SD = standard deviation; IQR = interquartile range; ADPKD = autosomal dominant polycystic kidney disease; ADPLD = autosomal dominant polycystic liver disease; CRP = C-reactive protein; WBC = white blood cell count; MDRD = modification of diet in renal disease; CKD-epi = Chronic Kidney Disease Epidemiology Collaboration; *E.coli* = *Escherichia coli*; N/A = not applicable.
[a] All involved kidney transplantations.
[b] Excluding patients with a history of renal transplant or actively receiving dialysis.
[c] *Supplementary table 4* provides an overview of isolated pathogens.
[d] *Table 2* provides an overview of antibiotic regimens.

Follow-up

Median time of follow-up was two years [IQR 1-5 years]. Follow-up duration was similar between hepatic and renal cyst infection patients (1 year [IQR 0-5 years] vs. 3 years [IQR 1-6 years], p = 0.06) (*supplementary table 3*).

Characteristics at time of diagnosis

Peak serum CRP and white blood cell count were clearly raised, reaching a median level of 215 mg/l (upper limit of normal (ULN): < 10 mg/l) and 11.6 x 10^9/l (ULN: < 11 x 10^9/l), respectively. In most patients renal function was estimated to be mildly impaired (MDRD: 50 ml/min/1.73 m^2, CKD-epi: 57 ml/min/1.73 m^2). *E. coli* was isolated in most urine (57%) and blood (66%) cultures (*supplementary table 4*). A substantial proportion of cultures returned alternative pathogens.

Treatment characteristics

Initial treatment was limited to the use of antibiotics in 87% (n = 67) of cyst infection patients; the remainder of the patients received antibiotics in combination with invasive treatment. Some 40% (n = 67) received a fluoroquinolone antibiotic, either as mono or combination antibiotic treatment (*table 1*). The median duration of initial treatment was eight days. *Table 2* provides an overview of all antibiotics given as initial treatment.

Differences between hepatic and renal cyst infection patients

Characteristics of hepatic and renal cyst infection patients were comparable, except for age (58 years ± 10 vs. 51 years ± 14, p = 0.02) and peak serum WBC (9.4 x 10^9/l [IQR 7.8-12.3] vs. 12.8 x 10^9/l [IQR 9.2-18.5], p = 0.007) (*supplementary table 3*).

Escalation of care during follow-up

In 48% of patients (n = 32) escalation of care occurred (*table 3, supplementary table 5*). The peak serum WBC appeared to be significantly higher in these patients (12.5 x 10^9/l [IQR 9.1-18.5] vs. 10.9 x 10^9/l [IQR 8.3-13.8], p = 0.01). Furthermore, atypical pathogens were more frequent (47% vs. 21%, p = 0.01). In contrast, the interval between transplantation and cyst infection was significantly shorter in patients receiving invasive treatment compared with those who did not (3 years [IQR 0-8] vs. 9 years [IQR 1-14], p = 0.03). Follow-up duration was comparable between patients regardless of escalation of care (2 years [IQR 1-6] vs. 2 years [IQR 1-5], p = 0.61).

Predictors for escalation of care

Selected variables (p value < 0.2 in univariate analysis) were entered into the multivariate model (*table 4*). Multiple imputation had no effect on the selection of predictors (*supplementary table 6*). Exclusive isolation of *E. coli* decreased the risk for escalation of care (RR 0.55, 95% CI 0.34-0.89, p = 0.02) (*figure 2*). In contrast, if peak serum WBC increased or the interval between transplantation and cyst infection decreased, patients were more likely to be exposed to escalation of care (RR 1.04, 95% CI

Table 2. *Antibiotic regimens in patients with initial treatment limited to antibiotics*

Antibiotic regimen	Initial cyst infection treatment limited to antibiotics (n = 67)
Monotherapy, n (%)	**56 (84)**
Fluoroquinolones, n (%)	26 (46)
- Ciprofloxacin, %	100
Cephalosporins, n (%)	16 (29)
2nd generation	
- Cefuroxime, %	56
3rd generation	
- Ceftriaxone, %	19
- Ceftazidime, %	19
- Cefotaxime, %	6
Penicillin, n (%)	9 (16)
- Amoxicillin/clavulanic acid, %	78
- Amoxicillin, %	11
- Piperacillin/tazobactam, %	11
Carbapenems, n (%)	3 (5)
- Meropenem, %	100
Glycopeptides, n (%)	1 (2)
- Vancomycin, %	100
Sulphonamides, n (%)	1 (2)
- Trimethoprim/sulfamethoxazole, %	100
Combination therapy, n (%)	**10 (15)**
- Cefuroxime + gentamicin, %	20
- Cefuroxime + tobramycin, %	20
- Cefuroxime + metronidazole, %	10
- Cefazolin + metronidazole, %	10
- Amoxicillin + teicoplanin, %	10
- Ceftazidime + vancomycin, %	10
- Ciprofloxacin + gentamicin, %	10
- Flucloxacillin + ceftazidime, %	10
Missing, n (%)	**1 (1)**

Percentages may not add up to 100 due to rounding.

Table 3. *Univariate modified Poisson regression to identify predictors for escalation of care during follow-up.*

Variable			Original dataset	Imputed datasets
	Conservative (n = 45)	Escalated care (n = 32)[a]	*P* value	*P* value
Gender, n (%)				
- Male	21 (47)	17 (53)	0.58	0.58
- Female (referent)	24 (53)	15 (47)		
Age at cyst infection, years (± SD)	51 ± 13	57 ± 13	0.06	0.06
Type of polycystic disease, n (%)				
- ADPLD	2 (4)	2 (6)	0.71	0.71
- ADPKD (referent)	43 (96)	30 (94)		
Site of cyst infection, n (%)				
- Liver	18 (40)	10 (31)	0.44	0.44
- Kidney (referent)	27 (60)	22 (69)		
Diabetes mellitus, n (%)				
- No	37 (82)	26 (81)	0.91	0.91
- Yes (referent)	8 (18)	6 (19)		
Dialysis, n (%)				
- No	41 (91)	29 (91)	0.94	0.94
- Yes (referent)	4 (9)	3 (9)		
Solid organ transplant, n (%)[b]				
- No	22 (49)	12 (38)	0.33	0.33
- Yes (referent)	23 (51)	20 (62)		
Interval solid organ transplant and cyst infection, years [IQR]	9 [1-14]	3 [0-8]	0.02	0.03
Immunosuppressive drug use, n (%)				
- No	22 (49)	11 (34)	0.22	0.22
- Yes (referent)	23 (51)	21 (66)		
Peak serum CRP, mg/l [IQR]	194 [126-269]	228 [122-306]	0.22	0.32
Peak serum WBC, x10^9/l [IQR]	10.9 [8.3-13.8]	12.5 [9.1-18.5]	0.002	0.01
Peak eGFR, ml/min/1.73m^2 [IQR][c]				
- MDRD	45 [27-71]	66 [17-88]	0.56	0.55
- CKD-EPI	52 [28-81]	70 [17-95]	0.65	0.67
Atypical pathogen cultured, n (%)				
- No	33 (79)	17 (53)	0.02	0.01
- Yes (referent)	9 (21)	15 (47)		
Initial antibiotic treatment containing fluoroquinolone, n (%)				
- No	26 (58)	16 (53)	0.70	0.68
- Yes (referent)	19 (42)	14 (47)		
Duration initial antibiotic treatment, days [IQR]	9 [5-42]	7 [4-39]	0.55	0.70
Follow-up, years	2 [1-6]	2 [1-5]	0.61	0.61

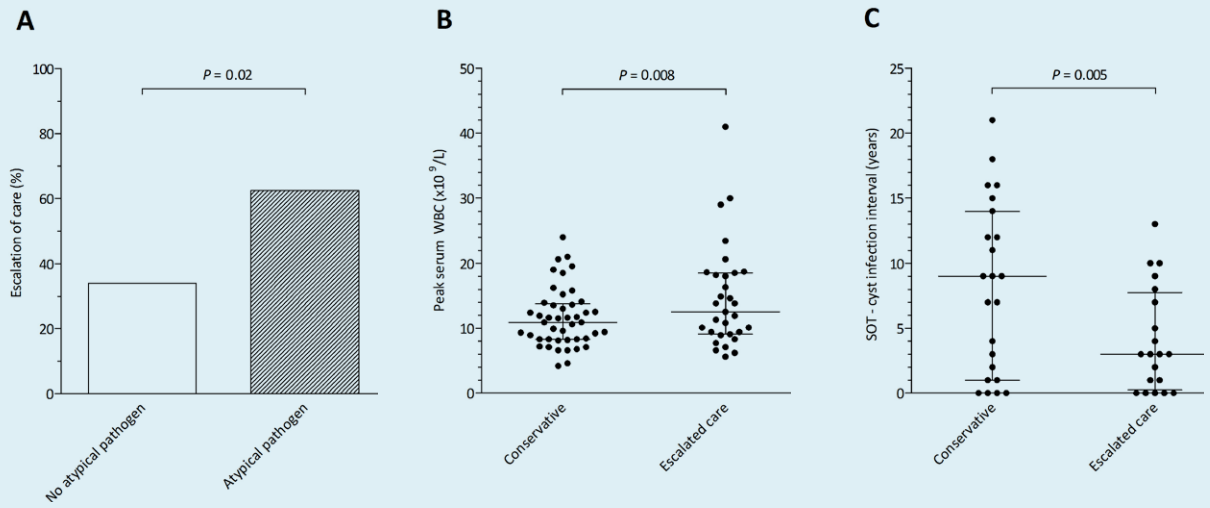
Percentages may not add up to 100 due to rounding.
CI = confidence interval; ADPKD = autosomal dominant polycystic kidney disease = ADPLD = autosomal dominant polycystic liver disease; CRP = C-reactive protein; WBC = white blood cell count; MDRD = modification of diet in renal disease; CKD-epi = Chronic Kidney Disease Epidemiology Collaboration.
[a] *Supplementary table 5* shows details on escalated care.
[b] All involved kidney transplants.
[c] Excluding patients with history of renal transplant or actively receiving dialysis.

Table 4. *Multivariate modified Poisson regression to identify predictors for escalation of care during follow-up (imputed datasets).*

Variable	Poisson model before backward elimination of non-significant variables		Poisson model after backward elimination of non-significant variables	
	Multivariate adjusted risk ratio (95% CI)	*P* value	Multivariate adjusted risk ratio (95% CI)	*P* value
Age at cyst infection (years)	1.02 (0.99-1.04)	0.14		
Interval solid organ transplant and cyst infection (years)	0.92 (0.87-0.97)	0.003	0.92 (0.86-0.97)	0.005
Peak serum WBC (x10^9/l)	1.04 (1.01-1.07)	0.006	1.04 (1.01-1.07)	0.008
Atypical pathogen cultured				
- No	0.63 (0.39-0.99)	0.05	0.55 (0.34-0.89)	0.02
- Yes (referent)				

CI = confidence interval; WBC = white blood cell count.

Figure 2. *Rate of escalation of care according to (A) cultured pathogen, (B) peak serum white blood cell count and (C) interval between solid organ transplant and cyst infection*



WBC = white blood cell count; SOT = solid organ transplant

1.01-1.07, p = 0.008 and RR 0.92, 95% CI 0.86-0.97, p = 0.005, respectively). Age at cyst infection was discarded in the final model. In contrast to the imputed datasets, the presence or absence of atypical pathogens did not predict escalation of care in the final model using the original dataset (*supplementary table 7*).

DISCUSSION

The key finding of our multicentre retrospective cohort study is that increasing serum WBC, isolation of pathogens other than *E. coli* and a shorter transplant-infection interval increases the risk for escalating care to the point of puncture or operation of the cyst.

We identified several factors in our cohort that increase the risk for treatment escalation. Previous studies on cyst infection involved single-centre cohort studies and focused on the clinical, microbiological and imaging aspects of cyst infection. None specifically investigated factors that influence the need for invasive treatment.[3,4,15,21-23] In this study we found that patients with a longer interval between solid organ transplantation and development of cyst infection were less likely to be exposed to escalation of care. This observation might be explained by a reduced need for immunosuppressive drugs in patients who are

clinically stable.[24,25] This could explain the observation that these patients are more capable of clearing the infection. Alternatively, the early period after solid organ transplantation could warrant a more aggressive (i.e. invasive) approach to cyst infection to benefit the outcome of transplanted allografts.[26,27]

Increasing serum WBC and isolation of atypical pathogens both independently increase the risk for treatment escalation. Serum WBC reflects infection severity. In a study investigating acute pyelonephritis, a severe increase in serum WBC predicted clinical failure.[28] *E. coli* is isolated in most cases of cyst infection.[6] In the presence of an alternative pathogen, treatment limited to antibiotics could be less effective as successful eradication of alternative bacteria might be more challenging. Two systematic reviews on the management of hepatic and renal cyst infection, respectively, show that in case an alternative micro-organism is isolated (e.g. *Enterococcus faecium*), escalation of care is more likely.[11,29]

In patients with high serum WBC, isolation of atypical pathogens or a history of solid organ transplantation, the risk of failing conventional antibiotic treatment increases. Given these results, it is reasonable to consider alternative treatment options such as percutaneous cyst drainage, (partial) resection or even kidney and/or liver transplantation.

The main strength of this study comes with its size as we identified ADPKD and ADPLD patients who developed hepatic or renal cyst infection in four Dutch tertiary referral centres. This approach, combined with the use of broad inclusion criteria, enhances the generalisability of our findings. A potential limitation is the retrospective nature of our study and use of data not primarily intended for study purposes. This resulted in the potential exclusion of a large proportion of patients who did have cyst infection but did not meet the inclusion criteria due to missing data. We used multiple imputation to correct for missing data and, as such, reduced bias and loss of power. Moreover, the modified Poisson regression model is very reliable even in relatively small sample sizes.[20] We did not include total liver volume and total kidney volume as predictors. A recent study showed that infectious complications in ADPKD do not correlate with the total liver volume;[30] it remains unclear whether total kidney volume predicts cyst infection outcome. Finally, we combined patients with hepatic and renal cyst infection. Univariate analysis showed an imbalance for age and peak serum WBC (*supplementary table 5*). We hypothesised that its effect on the outcome would be limited as both covariates have a theoretical effect on the outcomes in both hepatic and renal cyst infection patients. We corrected for this by including these covariates in the multivariate model. Moreover, adding the site of cyst infection to the final multivariate model had no significant effect (*supplementary table 8*).

To conclude, we show that high serum WBC, presence of pathogens other than *E. coli* and early infection after transplantation predict the need for change to invasive procedures.

*The supplementary material is available on request from the editorial office (marina@alphatekst.nl).

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DIPAK Consortium

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DISCLOSURES

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REFERENCES

1. Gevers TJ, Drenth JP. Diagnosis and management of polycystic liver disease. Nat Rev Gastroenterol Hepatol. 2013;10:101-8.
2. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet. 2007;369:1287-301.
3. Suwabe T, Araoka H, Ubara Y, et al. Cyst infection in autosomal dominant polycystic kidney disease: causative microorganisms and susceptibility to lipid-soluble antibiotics. Eur J Clin Microbiol Infect Dis. 2015;34:1369-79.
4. Jouret F, Lhommel R, Beguin C, et al. Positron-emission computed tomography in cyst infection diagnosis in patients with autosomal dominant polycystic kidney disease. Clin J Am Soc Nephrol. 2011;6:1644-50.
5. Jouret F, Lhommel R, Devuyst O, et al. Diagnosis of cyst infection in patients with autosomal dominant polycystic kidney disease: attributes and limitations of the current modalities. Nephrol Dial Transplant. 2012;27:3746-51.
6. Lantinga MA, Drenth JP, Gevers TJ. Diagnostic criteria in renal and hepatic cyst infection. Nephrol Dial Transplant. 2015;30:744-51.
7. Neuville M, Hustinx R, Jacques J, Krzesinski JM, Jouret F. Diagnostic Algorithm in the Management of Acute Febrile Abdomen in Patients with Autosomal Dominant Polycystic Kidney Disease. PLoS One. 2016;11(8):e0161277.
8. Lantinga MA, Darding AJM, de Sevaux RGL, et al. International Multi-Specialty Delphi Survey: Identification of Diagnostic Criteria for Hepatic and Renal Cyst Infection. Nephron. 2016;134:205-14.
9. Hiyama L, Tang A, Miller LG. Levofloxacin penetration into a renal cyst in a patient with autosomal dominant polycystic kidney disease. Am J Kidney Dis. 2006;47(1):e9-13.
10. Telenti A, Torres VE, Gross JB, Jr., et al. Hepatic cyst infection in autosomal dominant polycystic kidney disease. Mayo Clin Proc. 1990;65:933-42.
11. Lantinga MA, Geudens A, Gevers TJ, Drenth JP. Systematic review: the management of hepatic cyst infection. Aliment Pharmacol Ther. 2015;41:253-61.
12. Chapman AB, Devuyst O, Eckardt KU, et al. Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. Kidney Int. 2015;88:17-27.
13. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370:1453-7.
14. Meijer E, Drenth JP, d'Agnolo H, et al. Rationale and design of the DIPAK 1 study: a randomized controlled clinical trial assessing the efficacy of lanreotide to Halt disease progression in autosomal dominant polycystic kidney disease. Am J Kidney Dis. 2014;63:446-55.
15. Sallee M, Rafat C, Zahar JR, et al. Cyst infections in patients with autosomal dominant polycystic kidney disease. Clin J Am Soc Nephrol. 2009;4:1183-9.
16. Graham JW, Olchowski AE, Gilreath TD. How many imputations are really needed? Some practical clarifications of multiple imputation theory. Prev Sci. 2007;8:206-13.
17. Donders AR, van der Heijden GJ, Stijnen T, Moons KG. Review: a gentle introduction to imputation of missing values. J Clin Epidemiol. 2006;59:1087-91.
18. Knol MJ. [Down with odds ratios: risk ratios in cohort studies and randomised clinical trials]. Ned Tijdschr Geneeskd. 2012;156:A4775.
19. Knol MJ, Le Cessie S, Algra A, Vandenbroucke JP, Groenwold RH. Overestimation of risk ratios by odds ratios in trials and cohort studies: alternatives to logistic regression. CMAJ. 2012;184:895-9.
20. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;159:702-6.
21. Bobot M, Ghez C, Gondouin B, et al. Diagnostic performance of [(18)F]fluorodeoxyglucose positron emission tomography-computed tomography in cyst infection in patients with autosomal dominant polycystic kidney disease. Clin Microbiol Infect. 2016;22:71-7.
22. Suwabe T, Ubara Y, Higa Y, et al. Infected hepatic and renal cysts: differential impact on outcome in autosomal dominant polycystic kidney disease. Nephron Clin Pract. 2009;112:c157-63.
23. Suwabe T, Ubara Y, Sumida K, et al. Clinical features of cyst infection and hemorrhage in ADPKD: new diagnostic criteria. Clin Exp Nephrol. 2012;16:892-902.
24. Zand MS. Immunosuppression and immune monitoring after renal transplantation. Semin Dial. 2005;18:511-9.
25. Kirk AD, Mannon RB, Swanson SJ, Hale DA. Strategies for minimizing immunosuppression in kidney transplantation. Transpl Int. 2005;18:2-14.
26. Cainelli F, Vento S. Infections and solid organ transplant rejection: a cause-and-effect relationship? Lancet Infect Dis. 2002;2:539-49.
27. Chong AS, Alegre ML. The impact of infection and tissue damage in solid-organ transplantation. Nat Rev Immunol. 2012;12:459-71.
28. Wie SH, Ki M, Kim J, et al. Clinical characteristics predicting early clinical failure after 72 h of antibiotic treatment in women with community-onset acute pyelonephritis: a prospective multicentre study. Clin Microbiol Infect. 2014;20:O721-9.
29. Lantinga MA, Casteleijn NF, Geudens A, et al. Management of renal cyst infection in patients with autosomal dominant polycystic kidney disease: a systematic review. Nephrol Dial Transplant. 2017;32:144-50.
30. Kim H, Park HC, Ryu H, et al. Clinical Correlates of Mass Effect in Autosomal Dominant Polycystic Kidney Disease. PLoS One. 2015;10:e0144526.

ORIGINAL ARTICLE

Rifampin levels in daily practice: the accuracy of a single measurement

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ABSTRACT

Background: Measurement of rifampin levels is not part of routine practice. However, low levels are associated with failure of tuberculosis treatment. The clinical relevance of serum levels in daily practice is unclear. The objective was to evaluate rifampin serum concentrations and factors associated with insufficient concentrations.

Methods: Patients with at least one rifampin concentration drawn 3 hours after intake (C_3) between 2005 and 2014 were included. Data on demographic and clinical characteristics were collected, including side effects and dose adjustments. Two different criteria were used to define adequate concentrations (criterion 1: C_3 and C_6 ≥ 3 mg/l; criterion 2: C_3 or C_6 ≥ 5 mg/l).

Results: Of 63 patients, 66% and 76% had a sufficient level according to criterion 1 or 2, respectively. C_3 exceeded C_6 in most patients, while a late maximum was significantly associated with diabetes mellitus (p = 0.003). A dose adjustment was made in 19% of cases, more frequently in patients with insufficient levels (p = 0.02) or with ≥ 2 side effects (p = 0.03).

Conclusion: Rifampin levels varied but were mostly adequate and a single measurement at 3 hours after intake provided the required information in most cases, indicating that full $AUC_{0\text{-}24}$ measurements could be limited to specific situations.

KEYWORDS

Absorption, serum levels, therapeutic drug monitoring, tuberculosis

BACKGROUND

Tuberculosis (TB) remains one of the world's most important infectious threats, reflected by 1.8 million deaths in 2015, of which 0.4 million deaths among people living with HIV.[1] Hence, adequate treatment is paramount. Rifampin is a key drug in the first-line treatment of active or latent TB, due to its high activity against *Mycobacterium tuberculosis* with an MIC_{90} of ≤ 0.25 μg/ml.[2-4]

The treatment success rate, especially in new cases, is improving although treatment failure occurs in up to 14% of patients.[5] While multiple factors, including poor treatment adherence, bacterial resistance and even drug quality, may contribute to treatment failure, drug dosage and insufficient concentrations are relevant in this regard. In a previous study, the risk of failure of long-term treatment was almost 9-fold higher in patients with low drug exposure, expressed as 24-hour area under the concentration time curve ($AUC_{0\text{-}24}$) for pyrazinamide, rifampin and/or isoniazid.[6]

That study and other data showed that insufficient serum concentrations may even result in development of drug resistance.[6,7] Apart from the prescribed dose, drug exposure may be influenced by factors such as comorbidities, food intake and inter-individual differences in pharmacokinetics.[7-12]

Therapeutic drug monitoring (TDM) of rifampin is not routinely performed and there is no consensus on adequate levels. In previous studies, rifampin serum concentrations at 2 hours (C_2) and at 6 hours (C_6) after intake have been used to approximate the peak level.[13-15] A recent study found that the rifampin $AUC_{0\text{-}24}$ in TB patients was predicted optimally using sampling at time points 1, 3, and 8 hours,[16]

which would be impractical for most outpatients or require availability of alternative methods such as dry blood spot analysis. During the past decades, a rifampin absorption test at our centre has consisted of measurement of serum concentrations at 0, 3 and 6 hours after intake, and only at the physician's request. The aim of the present study was to retrospectively evaluate the results of these absorption tests of rifampin regarding adequate levels, and factors associated with out of range serum concentrations.

STUDY POPULATION AND METHODS

Study population

The study population consisted of patients in whom one or more rifampin serum concentrations had been measured at Leiden University Medical Centre (LUMC), a tertiary care hospital, between October 2005 and May 2014. Demographic and clinical characteristics were collected from the medical charts, including age, sex, weight, country of origin, clinical diagnosis, comorbidity (HIV infection, present or past malignancy, liver disease, diabetes mellitus, chronic kidney failure, autoimmune disease(s) or other), pregnancy, concomitant medication, rifampin dose at the time of TDM, kidney and liver function, indication for TDM and side effects. Serum concentrations of rifampin at 0, 3 and 6 hours after intake, time of blood sampling, possible dose change and results of possible repeated TDM were collected. Patients were excluded if only a trough level was available or if the clinical data could not be retrieved.

The protocol of this retrospective study with anonymised data collection was evaluated by the Medical Ethics Committee of the LUMC and waived from the requirement of informed consent (protocol G16.017).

Criteria for interpretation of serum concentrations

As there are no uniform criteria for adequate rifampin levels, we used two different criteria. According to the original protocol used at our institution for several decades, the source of which could not be retrieved, serum levels of the sum of rifampin and desacetyl-rifampin ≥ 3 mg/l at 3 hours (C_3) and 6 hours (C_6) after intake were defined as adequate (criterion 1: C_3 and C_6 ≥ 3) and clinical decisions therefore were only based on this criterion. As an alternative criterion, adequate absorption was defined as a single measurement of the sum of rifampin and desacetyl-rifampin ≥ 5 mg/l (criterion 2: C_3 or C_6 ≥ 5) as is nowadays implemented in several institutions. The data were analysed according to both criteria.

Method of measurement of rifampin concentrations

Serum concentrations of rifampin and desacetyl-rifampin were measured by high performance liquid chromatography according to the method published by Chandi et al.[17] The method was linear in a concentration range of 0.5 mg/l up to at least 15 mg/l rifampin and/or desacetyl-rifampin. Accuracy was > 98.8% and imprecision < 5.7%.

Statistics

Descriptive statistical parameters were used. To compare proportions or continuous values between two groups, two-way chi square tests (or Fisher's exact probability test in case of comparison of proportions including numbers < 5), and ANOVA tests were used, respectively. Differences using two-sided testing were considered significant at $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics version 23.

RESULTS

Study population

Of 90 patients in whom rifampin levels had been determined, 63 met the inclusion criteria (15 were excluded because only a trough level had been measured and 12 because clinical data were unavailable). Patient characteristics are shown in *table 1*. The majority (42/63, 67%) were immigrants from TB endemic regions. Most patients had one or more comorbidity, with autoimmune disease, chronic liver disease and malignancy being most frequent.

The most frequent reason for TDM was control of compliance (52%), followed by suspected high (29%) or low concentration (6%). More than half of the patients had received rifampin for active TB and one-third for latent TB.

Serum rifampin concentrations

In 63 patients, a total of 138 rifampin concentrations (at 0, 3 and/or 6 hours) were available. Rifampin levels were not always available for all three time points (*table 2*). C_3 was available for all 63 patients, C_0 was available for 34/63 patients (54%) and C_6 for 41/63 patients (61%). According to the guidelines for TB treatment the standard dose of rifampin is 10 mg/kg, with a maximum of 600 mg. Most patients (45/63, 71.4%) were treated with a dose of 600 mg (*table 2*). The dose was 600 mg in 42/46 (91.3%) patients with a body weight ≥ 55 kg. The mean ± SD dose per weight was 11.2 ± 3.9 mg/kg. Maximal rifampin levels did not differ according to dose per weight (data not shown). Maximal levels did not vary by any demographic or clinical parameter (*table 1*).

Trough levels were < 2 mg/l in 31/34 patients (91.2%) and were 3.2 mg/l, 5.6 mg/l and 9.9 mg/l respectively in the remaining three patients. In the last of these three patients (patient 41 in *figure 1*), C_0 exceeded C_3 and C_6 and thus had most likely been measured after intake of

Table 1. *Clinical characteristic and rifampin levels in 63 patients*

Characteristic	Categories	No. (%)	Maximal rifampin level (average ± SD) in mg/l	P value
Sex	Men Women	37 (58.7) 26 (41.3)	8.6 ± 4.9 9.5 ± 6.0	0.5
Age (range in years)	0-15 16-30 31-45 46-60 61-75 > 75	11 (17.5) 13 (20.6) 12 (19.0) 14 (22.2) 11 (17.5) 2 (3.2)	9.2 ± 5.0 9.5 ± 4.9 9.0 ± 5.9 7.8 ± 4.3 10.4 ± 6.9 3.5 ± 4.9	0.6
Immigration	No Yes	19 (30.2) 44 (69.8)	7.65 ± 6.4 9.5 ± 4.8	0.2
Region of origin	Western Europe Eastern Europe/Russia Africa Middle East Asia (other than Middle East) North and Central America South America	19 (30.2) 4 (6.3) 19 (30.2) 7 (11.1) 11 (17.5) 2 (4.5) 1 (2.3)	7.6 ± 6.4 5.6 ± 2.7 9.8 ± 5.6 10.9 ± 2.2 10.5 ± 4.7 4.4 ± 2.8 9.9	0.4
Comorbidities	None ≥ 1 HIV Malignancy Chronic liver disease Diabetes mellitus Pregnancy Chronic kidney failure Autoimmune disease Other	8 (12.7) 55 (87.3) 4 (6.3)[a] 13 (20.6) 10 (15.9) 6 (9.5) 4 (6.3) 3 (4.8) 20 (31.7) 29 (46.0)	7.4 ± 3.5 9.2 ± 5.5 4.5 ± 1.8 11.4 ± 6.4 9.4 ± 5.4 7.5 ± 2.2 9.6 ± 7.0 9.3 ± 2.3 8.5 ± 4.8 9.6 ± 6.4	0.4
No. of comorbidities[b]	0 1 2 3	16 (25.4) 35 (55.6) 11 (17.5) 1 (1.6)	8.6 ± 5.2 9.1 ± 5.8 9.2 ± 4.5 6.9	1.0
Indication for rifampin	Active tuberculosis Latent tuberculosis IV catheter-related infection Other	35 (55.6) 20 (31.7) 6 (9.5) 2 (3.2)	9.3 ±6.0 8.3 ± 4.5 8.8 ± 5.6 9.1 ± 0.6	0.9

HIV = human immunodeficiency virus; iv = intravascular. [a]The sum of the comorbidities exceeds 63 (100%) as patients could have more than one comorbidity; [b]based on the reported seven specific comorbidities as listed in this table, thus excluding the category of other comorbidities.

rifampin. The average individual maximal concentration, which could be either at 3 or at 6 hours, was 8.9 mg/l (range 0.0 mg/l to 26.7 mg/l). With regard to criterion 1: C_3 and $C_6 \geq 3$, 41 patients could be evaluated. Criterion 1 was met in 27/41 (65.9%). Criterion 2: C_3 or $C_6 \geq 5$ was met in 48/63 patients (76.2%). There was no significant relation between age, sex, comorbidities, co-medication or indication for rifampin comorbidities and meeting the criteria or not. Levels in immigrant patients more frequently met criterion 2 than did those from native Dutch patients (86.4% vs 52.6%, p = 0.004).

Figure 1 shows all individual rifampin concentrations, ranked by the value of C_3 which was available for all 63 patients. C_3 exceeded C_6 in all but 8 patients (case 2, 9, 12, 17, 18, 24, 46 and 53 in *figure 1*). C_6 was ≥ 5 mg/l and often even much higher in all of these eight patients with late maximal concentrations. In 7/8 patients criterion 1: C_3 and $C_6 \geq 3$ was also met. Of the eight patients with late maximal levels, four (50%) had diabetes mellitus and one additional patient suffered from systemic sclerosis. In the remaining three patients no factors associated with delayed absorption could be identified. The proportion of patients with diabetes in those with late maximal levels (4/8 patients with $C_6 > C_3$) was significantly different from that in patients with early maximal levels (1/33 patients with $C_3 > C_6$; Fisher's exact probability test p = 0.003).

In 12 patients (19%) rifampin measurements including at least C_3 were later repeated after a median interval of 11 days (range 1-50 days, and one outlier at 248 days) because of out of range first levels, newly experienced side

Table 2. *Dose, side effect, available concentrations, interpretation and dose adjustments*

Parameter	Category	No. (%)[a]
Dose (mg)	600	44 (70.1)
	450	6 (9.7)
	300	3 (4.8)
	Other	9 (14.5
Side effects	≥ 1 side effect	27 (42.8)
	≥ 2 side effects	13 (20.6)
	General symptoms	19 (30.2)[b]
	Gastrointestinal complaints	7 (11.1)
	Drug induced hepatitis	6 (9.5)
	Skin involvement	5 (7.9)
	Headache	2 (3.2)
	Neurological symptoms	1 (1.6)
	Other	6 (9.5)
Available rifampin levels	Only C_3	18 (28.6)
	Only C_3 and C_6	11 (17.5)
	Only C_0 and C_3	4 (6.3)
	C_0, C_3 and C_6	30 (47.6)
Criterion C_3 and C_6 ≥3 mg/l[c]	Yes	27/41 (65.9)
	→ dose change	2/27 (7.4)
	No	14/41 (34.1)
	→ dose change	3/14 (21.4) p = n.s.
Criterion C_3 or C_6 ≥5 mg/l	Yes	48 (76.2)
	→ dose change	6/48 (12.5)
	No	15 (23.8)
	→ dose change	6/15 (40.0) p = 0.02

[a]Denominator was 63 unless otherwise specified; [b]the sum of the side effects exceeds 27 as patients could have more than one side effect; [c]this criterion could only be tested for 41 patients for whom at least C_3 and C_6 were available.

effects and/or after adjustment of the dose based on initial levels. The results of paired individual maximal serum concentrations are shown in *figure 2*.

Side effects

At least one side effect was reported in 27/63 patients (42.8%). Side effects varied from mild to very severe, ranging from minor nausea to drug-induced hepatitis (*table 2*). The maximal rifampin level in patients experiencing side effects was not significantly different from that in patients without side effects. In the six patients with serum transaminases > 100 IU/l, the maximal level was not different from that in patients without liver function disturbances.

Dose adjustments

Twelve out of 63 patients (19.0%) had a dose adjustment. Six of 15 patients (40%) who did not meet criterion 2 had a dose increase. Six of 48 patients (12.5%) meeting criterion 2 had a dose reduction. This difference in proportion with a dose adjustment was significant (p = 0.02).

A dose adjustment was made in 5/13 patients who experienced ≥ 2 side effects, in 3/14 patients with one side effect and in 4/36 patients without side effects (p = 0.03 for comparison of patients with ≥ 2 to those without side effects).

Of 12 patients who had a second measurement of the rifampin level, dose changes were reported in five (*figure 2*). In four of these, the maximal levels were adequate after a dose increase (n = 3) or reduction (n = 1).

Follow-up

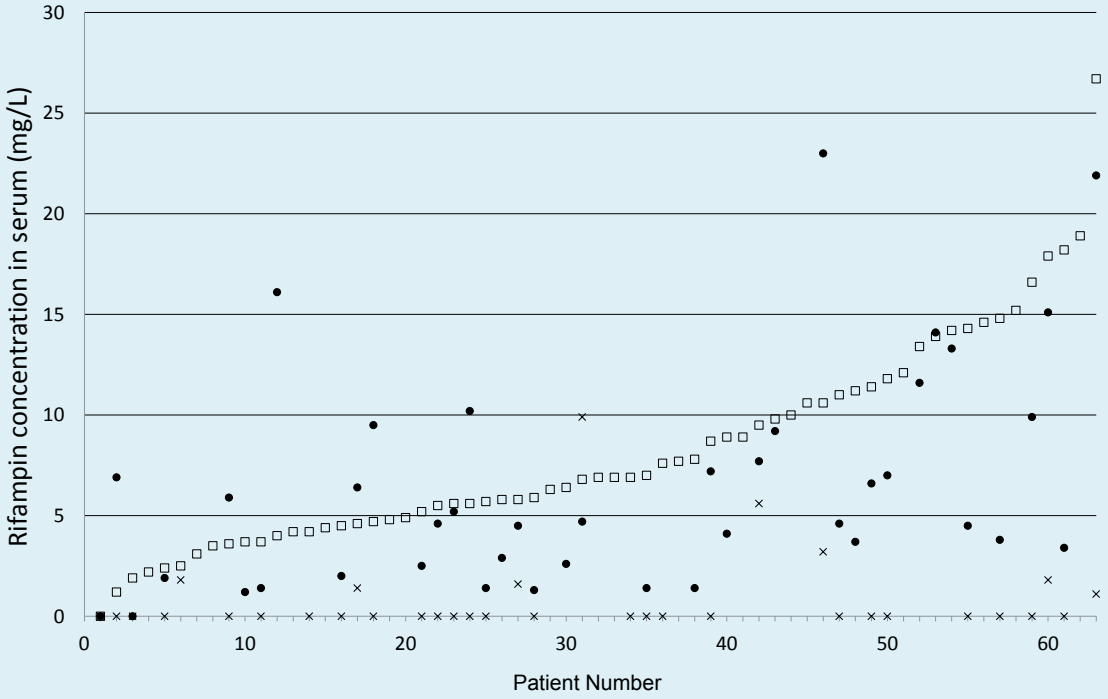
None of the patients with active TB had treatment failure and none of the patients treated for latent TB infection and who later received immunosuppressive drugs had a TB reactivation during a follow-up time between two and ten years.

DISCUSSION

In the present study we retrospectively evaluated rifampin levels which had been determined in routine practice in a mixed population consisting mainly of patients treated for active or latent TB. The data showed considerable inter-individual variation but in the majority of patients serum levels were adequate as based on two different criteria, one of which had been in use for decades at our institution and an alternative criterion based on a single peak level of at least 5 mg/l, which is nowadays implemented in several Dutch institutions. Nevertheless, the dose was adjusted in 20% of patients because of either too low or very high levels. In most patients in whom both C_3 and C_6 were available, C_3 was highest and therefore most informative. Maximal serum levels were not affected by demographic parameters, the presence of comorbidities or use of co-medication.

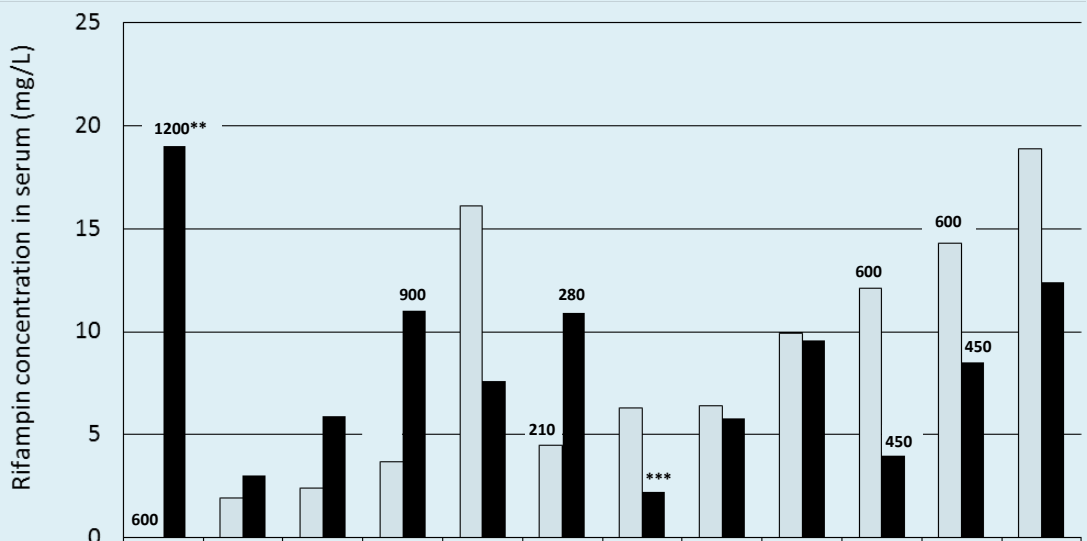
Despite the recognition that adequate rifampin concentrations are crucial for treatment success, TDM is not common practice. In addition, there are no clear criteria for the interpretation of concentrations. Studies in animals showed that the $AUC_{0\text{-}24}$ in steady state divided by the MIC was the best predictive parameter for efficacy of rifampin.[18,19] In humans, treatment failure has been associated with low $AUC_{0\text{-}24}$,[6] and with development of bacterial resistance.[6,7] In a population pharmacokinetic model in patients with active TB, the rifampin $AUC_{0\text{-}24}$ could be predicted with high precision using sampling at 0, 1, 3, and 8 hours after intake.[16] However, such timing is not practical for most outpatients and the investment of the patient's time and the costs must be weighed against the value of the information thus obtained. In a previous study a single measurement of rifampin at four hours after intake gave the best estimate for $AUC_{0\text{-}24}$.[20] While

Figure 1. *Distribution of rifampin levels in 63 patients, ranked by the concentration at 3 hours after intake*



Trough value is indicated by ×; C_3 (concentration 3 hours after intake) is indicated by □; C_6 is indicated by •

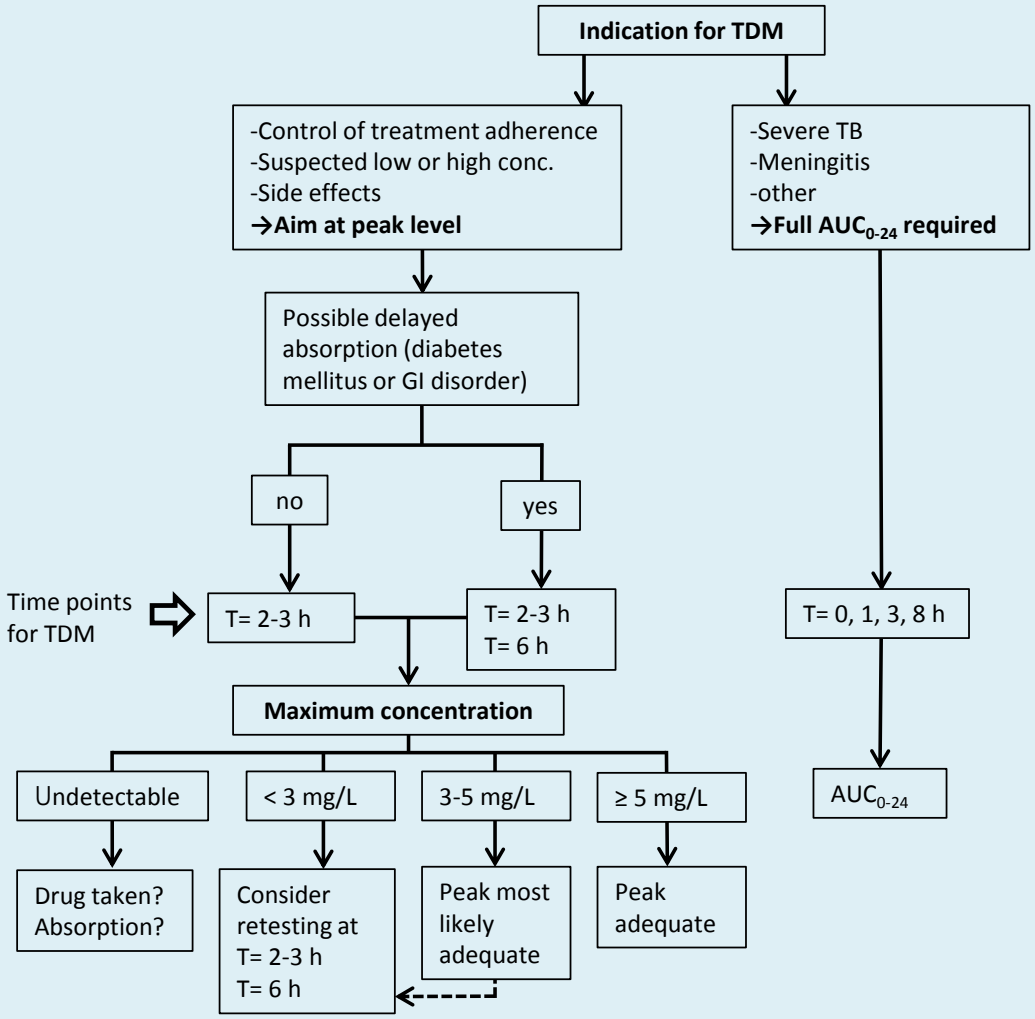
Figure 2. *Maximal rifampin levels in 12 patients in whom rifampin concentrations were measured twice*



	1*	3	5	11	12	16	29	30	31	51	55	62
□ Conc. 1	0	1.9	2.4	3.7	16.1	4.5	6.3	6.4	9.9	12.1	14.3	18.9
■ Conc. 2	19	3	5.9	11	7.6	10.9	2.2	5.8	9.6	4	8.5	12.4

Conc. 1 and Conc. 2 indicate the maximal serum rifampin concentration at the first and second measurement, respectively. Reported dose changes are indicated above the bars as dose in mg.
* The top row indicates the patient numbers corresponding to those used in *figure 1*.
** In patient 1 with initial undetectable rifampin concentrations, the maximal concentration was very high after doubling the dose, which suggested that rifampin may not have been taken at the time of first TDM.
*** In patient 29 the dose was increased from 500 mg to 600 mg based on the results of the repeated level.

Figure 3. *The selection of time points for measurement of rifampin concentrations*



The dotted line reflects the authors' opinion that retesting is generally not necessary if the clinical course is favourable but can be considered depending on the specific clinical situation

precise $AUC_{0\text{-}24}$ of rifampin is generally not needed, there are specific situations in which such information can be essential, such as in patients with extensive TB and a high bacillary load, or in patients with TB meningitis because of limited penetration. In general practice there may also be reasons to measure rifampin levels, however without the need for a precise $AUC_{0\text{-}24}$, e.g. if treatment adherence is doubted, if poor absorption is suspected or because of suspected high levels. In these situations it may suffice to measure the concentration at the time of expected peak concentration. Because there is a large inter-individual variation in pharmacokinetics the peak value can be missed if just one sample is used. However, the results of the present study showed that C_3 almost always exceeded C_6. This is in agreement with a peak between 1 and 3 hours (occasionally 4 hours) after intake in studies in which multiple time points were used, the peak being closer to 2 hours if the drug was taken without food and closer to 3 hours if taken with a light meal.[16,21] Thus, if full $AUC_{0\text{-}24}$ is not required a single measurement at 2 to 3 hours after intake may provide sufficient information. In the limited number of patients in the present study in whom C_6 exceeded C_3, more than half had a disorder associated with delayed gastric emptying such as diabetes mellitus, and including a later time point should thus be considered in that setting. In accordance with our finding, in a previous study in Indonesian patients the $AUC_{0\text{-}6}$ was about 50% lower in patients with diabetes compared with nondiabetic TB patients.[22] Trough levels were not informative and our data suggest that these could be omitted.

Combining data from the literature with those from the present study, we designed a simple and practical algorithm for the selection of time points for measurement of rifampin concentrations (*figure 3*). We think that testing rifampin concentrations at just one time point in most patients, and more frequently only on indication, could save time and money without loss of quality of care. In the LUMC, based on this study the single measurement is now implemented for routine practice, while $AUC_{0\text{-}24}$ is available if needed. Regarding the standard rifampin dose of 600 mg it has been argued that the 600 mg dose is at the lower end of the dose-response curve.[23] An update of the TDM in the treatment of tuberculosis of rifampin suggests higher doses to be more effective.[24] The pharmacokinetic profile of rifampin is nonlinear and a dose increase will result in a greater than proportional increase in AUC. Previous studies using a higher rifampin dose of 13 mg/kg or 20 mg/kg did not observe increased hepatotoxicity or other adverse events.[23,25-29] In a recent study even a 1200 mg dose was well tolerated,[30] indicating that a higher dose can probably be given without increasing the risk of side effects. Higher rifampin doses were evaluated in large clinical trials targeting C_{max} values ≥ 8 mg/l. Higher doses were associated with a better outcome and/or no increase of toxicity.[31-33] Boeree et al. even described a possibility of a shorter regimen of tuberculosis treatment with a higher dose (up to 35 mg/kg) of rifampin.[32]

A limitation of our study was the retrospective nature and the probable selection bias because rifampin levels were not routinely measured.

CONCLUSIONS

The results of this study show that in most cases a single rifampin level measured at 3 hours after intake provided sufficient information regarding adequacy of treatment. In the presence of risk factors for delayed absorption sampling at a later time point had added value. We think that a complete $AUC_{0\text{-}24}$ measurement can be limited to specific situations. Our findings could contribute to a cost-effective, rapid and patient-friendly approach to TDM of rifampin and to effective treatment. However, further studies in different populations and settings are needed to assess the generalisability of our findings.

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DISCLOSURES

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REFERENCES

1. WHO. Global tuberculosis report 2016. WHO, Geneva, Switzerland;2017.
2. Bemer-Melchior P, Bryskier A, Drugeon HB. Comparison of the in vitro activities of rifapentine and rifampicin against Mycobacterium tuberculosis complex. J Antimicrob Chemother. 2000;46:571-6.
3. Heifets LB, Iseman MD. Determination of in vitro susceptibility of mycobacteria to ansamycin. Am Rev Respir Dis. 1985;132:710-1.
4. Luna-Herrera J, Reddy MV, Gangadharam PR. In-vitro and intracellular activity of rifabutin on drug-susceptible and multiple drug-resistant (MDR) tubercle bacilli. J Antimicrob Chemother. 1995;36:355-63.
5. Cox HS, Morrow M, Deutschmann PW. Long term efficacy of DOTS regimens for tuberculosis: systematic review. BMJ. 2008;336:484-7.
6. Pasipanodya JG, McIlleron H, Burger A, et al. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. J Infect Dis. 2013;208:1464-73.
7. Srivastava S, Pasipanodya JG, Meek C, et al. Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. J Infect Dis. 2011;204:1951-9.
8. Becker C, Dressman JB, Junginger HE, et al. Biowaiver monographs for immediate release solid oral dosage forms: rifampicin. J Pharm Sci. 2009;98:2252-67.
9. Peloquin CA, Jaresko GS, Yong CL, et al. Population pharmacokinetic modeling of isoniazid, rifampin, and pyrazinamide. Antimicrob Agents Chemother. 1997;41:2670-9.
10. Ruslami R, Nijland HM, Alisjahbana B, et al. Pharmacokinetics and tolerability of a higher rifampin dose versus the standard dose in pulmonary tuberculosis patients. Antimicrob Agents Chemother. 2007;51:2546-51.
11. Smythe W, Khandelwal A, Merle C, et al. A semimechanistic pharmacokinetic-enzyme turnover model for rifampin autoinduction in adult tuberculosis patients. Antimicrob Agents Chemother. 2012;56:2091-8.
12. Wilkins JJ, Savic RM, Karlsson MO, et al. Population pharmacokinetics of rifampin in pulmonary tuberculosis patients, including a semimechanistic model to describe variable absorption. Antimicrob Agents Chemother. 2008;52:2138-48.
13. Magis-Escurra C, van den Boogaard J, Ijdema D, et al. Therapeutic drug monitoring in the treatment of tuberculosis patients. Pulm Pharmacol Ther. 2012;25:83-6.
14. Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis. Drugs. 2002;62:2169-83.
15. Prahl JB, Johansen IS, Cohen AS, et al. Clinical significance of 2 h plasma concentrations of first-line anti-tuberculosis drugs: a prospective observational study--authors' response. J Antimicrob Chemother. 2015;70:321-2.
16. Sturkenboom MG, Mulder LW, de Jager A, et al. Pharmacokinetic Modeling and Optimal Sampling Strategies for Therapeutic Drug Monitoring of Rifampin in Patients with Tuberculosis. Antimicrob Agents Chemother. 2015;59:4907-13.
17. Chandi LS, van der Sijs IH, Guchelaar H-J. Bepaling van rifampicine en desacetylrifampicine. Ziekenhuisfarmacie. 1998;1:71-2.
18. Gumbo T, Louie A, Deziel MR, et al. Concentration-dependent Mycobacterium tuberculosis killing and prevention of resistance by rifampin. Antimicrob Agents Chemother. 2007;51:3781-8.
19. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. Antimicrob Agents Chemother. 2003;47:2118-24.
20. Magis-Escurra C, Later-Nijland HM, Alffenaar JW, et al. Population pharmacokinetics and limited sampling strategy for first-line tuberculosis drugs and moxifloxacin. Int J Antimicrob Agents. 2014;44:229-34.

21. Acocella G. Pharmacokinetics and metabolism of rifampin in humans. Rev Infect Dis. 1983;5 Suppl 3: S428-S32.
22. Nijland HM, Ruslami R, Stalenhoef JE, et al. Exposure to rifampicin is strongly reduced in patients with tuberculosis and type 2 diabetes. Clin Infect Dis. 2006;43:848-54.
23. van Ingen J, Aarnoutse RE, Donald PR, et al. Why Do We Use 600 mg of Rifampicin in Tuberculosis Treatment? Clin Infect Dis. 2011;52:e194-e9.
24. Alsultan A, Peloquin CA. Therapeutic drug monitoring in the treatment of tuberculosis:an update. Drugs. 2014;74:839-54.
25. Acocella G, Pagani V, Marchetti M, et al. Kinetic studies on rifampicin. I. Serum concentration analysis in subjects treated with different oral doses over a period of two weeks. Chemotherapy. 1971;16:356-70.
26. Curci G, Bergamini N, Delli VF, et al. Half-life of rifampicin after repeated administration of different doses in humans. Chemotherapy. 1972;17:373-81.
27. Furesz S, Scotti R, Pallanza R, Mapelli E. Rifampicin: a new rifamycin. 3. Absorption, distribution, and elimination in man. Arzneimittelforschung. 1967;17:534-7.
28. Lobue P, Menzies D. Treatment of latent tuberculosis infection: An update. Respirology. 2010;15:603-22.
29. Nitti V, Delli VF, Ninni A, Meola G. Rifampicin blood serum levels and half-life during prolonged administration in tuberculous patients. Chemotherapy. 1972;17:121-9.
30. Aarnoutse RE, Kibiki GS, Reither K, et al. Pharmacokinetics, tolerability and bacteriological response of 600, 900 and 1200 mg rifampicin daily in patients with pulmonary TB. Antimicrob Agents Chemother. 2017;61(11) pii: e01054-17.
31. Peloquin CA, Velasquez GE, Lecca L, et al. Pharmacokinetic Evidence from the HIRIF Trial To Support Increased Doses of Rifampin for Tuberculosis. Antimicrob Agents Chemother. 2017;61.
32. Boeree MJ, Heinrich N, Aarnoutse R, et al. High-dose rifampicin, moxifloxacin, and SQ109 for treating tuberculosis: a multi-arm, multi-stage randomised controlled trial. Lancet Infect Dis. 2017;17:39-49.
33. Boeree MJ, Diacon AH, Dawson R, et al. A dose-ranging trial to optimize the dose of rifampin in the treatment of tuberculosis. Am J Respir Crit Care Med. 2015;191:1058-65.

CASE REPORT

A case of late-onset systemic sclerosis with ruptured silicone breast implants

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ABSTRACT

Background: It is still unresolved whether there is a relationship between silicone breast implants (SBIs) and late-onset systemic sclerosis (SSc).
Case description: A 83-year-old female was diagnosed with limited cutaneous SSc. During follow-up the presence of ruptured SBIs was confirmed. We provide a literature review concerning SBIs and development of SSc, particularly in relation to age of onset.
Conclusion: Data about age of onset are incomplete and no details on the rupture of SBIs are reported; however, an association between SSc and SBIs possibly exists.

KEYWORDS

Systemic sclerosis, scleroderma, silicone breast implants, connective tissue diseases, late onset

What is known about this topic?
The evidence whether there is an association between SSc and SBIs is inconclusive, since individual studies are rarely adequately adjusted for potential confounders.

What does this add?
We have presented a patient with late-onset SSc and ruptured SBIs. The published data reporting on age of SSc onset in relation to time since implantation and possible rupturing of SBIs are incomplete. In patients with suspected SBI ruptures, MRI is the most accurate test to confirm SBI rupture. Replacement or removal of ruptured SBIs may result in a better health status in patients with SSc.

INTRODUCTION

The aetiology of systemic sclerosis (SSc) is unknown, but interactions between environmental factors, including silicone and silica, and epigenetic features are reported to be important.[1] At present, although many studies have been performed concerning this relationship it is still a matter of discussion whether the association between SSc and silicone breast implants (SBIs) is causal. The interest in this association started in 1964 and 290 case reports had been published by 1998.[2] However, epidemiological findings concerning this relationship are controversial.[3] Individual studies suggested an increased relative risk,[4,5] but meta-analyses only found an increased occurrence of rheumatoid arthritis and Sjogren's syndrome in patients with SBIs, but no increased risk of development of SSc.[3,6-8] Importantly, however, most studies were too small and the duration of observation too short, so no firm conclusions can be drawn as to whether an association between SSc and SBIs exists. In a recent systemic review 11 studies that examined SSc were analysed.[3] The authors concluded that the evidence remains inconclusive about any association between SSc and SBIs, since studies were rarely adequately adjusted for potential confounders.
Occurrence of SSc beyond 75 years of age is rare and called late-onset SSc.[9] The clinical course of late-onset SSc differs as compared with the clinical course in younger patients. Late-onset SSc patients more frequently present with limited cutaneous SSc (LcSSc), more often have anti-centromere antibodies (ACA) and pulmonary arterial hypertension and less often digital ulcers.[9] Late-onset SSc is associated with progressive disease and higher mortality. We observed a patient with late-onset SSc and SBIs.
The aim of our study was to describe a case of late-onset SSc in a female with ruptured SBIs and to provide a review

of the literature concerning SBIs and SSc and in particular late-onset SSc.

METHODS

Selection of studies

We carried out a PubMed search of the English language literature from 1999 to 2016. The literature before 1999 (historical studies) was considered as having been thoroughly reviewed.[6,10] In addition, we obtained the results of studies cited in meta-analyses[3,5-7,11] and other reviews.[2,10,12-15] Studies concerning SBIs and connective tissue diseases (CTDs) were selected if patients with SSc were evaluated as a separate entity.

Literature review

A review of the literature revealed six published studies, comprising four cohort studies, two case series and two case reports.[2,14,16-21] The relevant patient characteristics of these studies and previously performed studies are summarised in *table 1*. The studies mainly included Caucasian females with LcSSc. The mean age of SSc onset ranged from 35 to 83 years (current case); however, age of onset was not reported in most studies. Only one case report included a case with late-onset SSc. Levy et al. reported a case of a 79-year-old female patient with SSc and ruptured SBIs.[2]

Concerning the association of SSc and SBIs, two studies calculated relative risks, adjusted for age. Both studies performed a nested case-control study within a large cohort of females who underwent plastic surgery. Kjoller et al. performed a case-control study within a large Danish cohort and did not find an association between CTDs and SBIs, nor between SSc and SBIs (RR 3.6, 95% CI 0.3-49.7).[17] Englert et al. also did not find an association between SSc and SBIs (RR 1.5, 95% CI 0.09-23.96).[16] However, due to small sample sizes, the confidence intervals are large.

The prevalence of ruptured SBIs was not mentioned in most studies. Only five studies reported whether the SBIs were ruptured,[2,13,18,22,23] and rupturing of the SBIs occurred with a prevalence of 9-50% in SSc patients with SBIs. No studies are available concerning the follow-up of SSc patients after removal of the ruptured SBIs. The time between implantation and development of SSc was mentioned in six studies.[2,4,19,22,24,25] Mean time since implant in patients with SSc ranged from 4 months to 12.8 (SD 8.9) years.

CASE PRESENTATION

In 2010, a 83-year-old female was referred to the gastro-enterologist with abdominal complaints and disturbed

Figure 1. *New onset sclerodactyly and telangiectasia on the hands of an 83-year-old female*

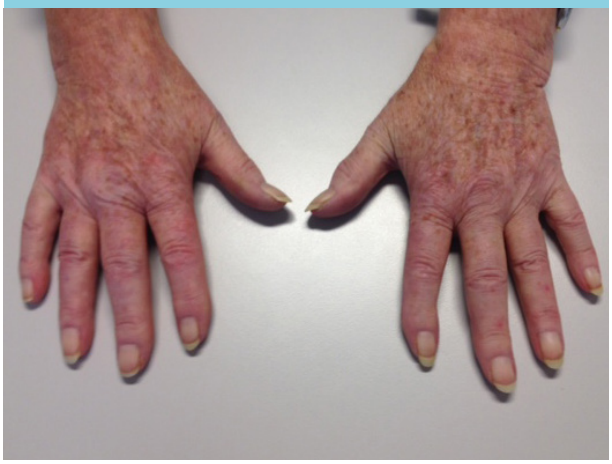

Figure 2. *New onset microstomia in an 83-year-old female*

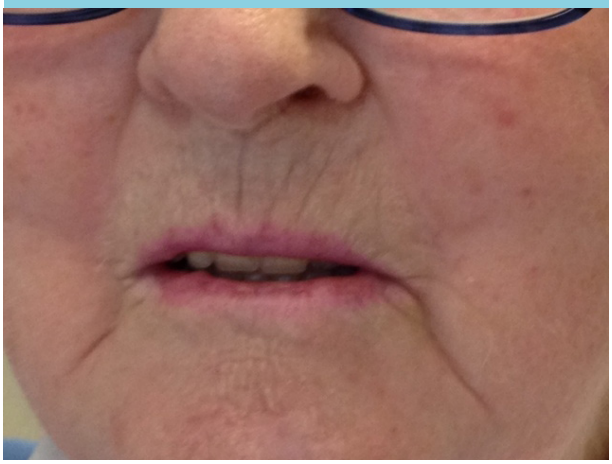

Figure 3. *Late SSc pattern on nailfold videocapillaroscopy*

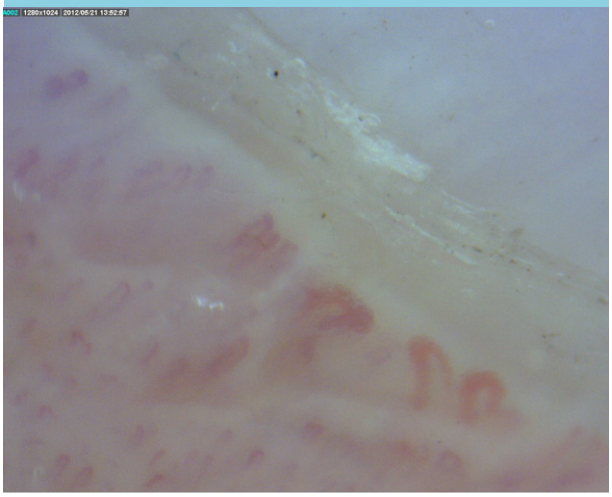

Table 1. *Studies concerning SSc and ruptured silicone breast implants, with respect to the age of onset*

Study	Year	Country	Study design	Outcome	Systemic sclerosis					Silicone breast implants				RR (95% CI)
					No. of patients	Caucasian N (%)	Age onset mean (SD)	LcSSc N (%)	ANA N (%)	No. of patients	Age implant mean (SD)	Ruptured N (%)	Time since implant mean (SD)	of SSc
McLaughlin	1994	Denmark	Cohort study	Medical records	2	Unknown	Unknown	Unknown	Unknown	2	Unknown	Unknown	7.5 (4.9) years	27.7 (3.1-99.8)
Claman	1994	USA	Case-control	Self-reported	6	Unknown	49.9 (10.7)*	3 (50)	4 (67)	6	unknown	Unknown	15.3 (4.3) years*	NA
Burns	1996	USA	Case-control	Medical records	274	228 (83.2)	49.9	Unknown	Unknown	2	Unknown	1 (50)	6.5 (7.7) years	1.30 (0.27-6.23)[γ]
Englert	1996	Australia	Case-control	Medical records	556	Unknown	47.3 (9.5)	3 (100)	Unknown	3	41.3 (10.2)	Unknown	6 (3.6) years	1.00 (0.16-6.16)[¶]
Hennekens	1996	USA	Cohort study	Self-reported	324	Unknown	Unknown[†]	Unknown	Unknown	10	Unknown[†]	Unknown	Unknown	1.84 (0.98-3.46)[‡]
Hochberg	1996	USA	Case-control	Clinical diagnosis	837	757 (90.4)	55.3 (12.9)	Unknown	Unknown	11	Unknown[†]	1 (9)	Unknown	1.08 (0.53-2.17)[§]
Englert	2001	Australia	Cohort study	classification criteria	2	Unknown	Unknown	Unknown	Unknown	2	Unknown	Unknown	Unknown	1.50 (0.09-23.96)
Brown	2001	USA	Cohort study	Self-reported	6	Unknown	Unknown	Unknown	Unknown	6	Unknown	3 (50)	Unknown	NA
Kjoller	2001	Denmark	Cohort study	Medical records	6	Unknown	Unknown	Unknown	Unknown	6	Unknown	Unknown	Unknown	3.6 (0.3-49.7)
Levy	2009	Israel	Case series	Clinical diagnosis	4	4 (100)	57.5 (17.2)	3 (75)	4 (100)	4	44.8 (10)	1 (25)	12.8 (8.9) years	NA
Maijers	2013	NL	Cohort study	Self-reported	1	Unknown	Unknown	Unknown	Unknown	1	Unknown	Unknown	Unknown	NA
Tervaert	2013	NL	Case series	Classification criteria	1	1 (100)	60	0 (0); DcSSc 1 (100)	1 (100)	1	Unknown	Unknown	Unknown	NA
Aranji	2014	New Zeeland	Case report	Clinical diagnosis	1	Unknown	47	0 (0)	1 (100)	1	Unknown	1 (100)	Unknown	NA
Psarras	2014	Greece	Case report	Classification criteria	1	Unknown	35	1 (100)	0 (0)	1	35	Unknown	4 months	NA
Colaris	2016	NL	Cohort study	Unknown	2	Unknown	Unknown	Unknown	Unknown	2	Unknown	Unknown	Unknown	NA
Meijs	2017	NL	Case report	Classification criteria	1	1 (100)	83	1 (100)	1 (100)	1	40	1 (100)	43 years	NA

No = number; LcSSc = limited cutaneous SSc; ANA = antinuclear antibodies; ACA = anticentromere antibodies; Anti-Scl-70 = anti-topoisomerase; RR = relative risk; SSc = systemic sclerosis; NL = the Netherlands; NA = not applicable.
* Mean of 11 women including 5 cases with SSc
[γ] Adjusted for age, race and date of birth
[¶] Adjusted for socioeconomic status, age, ethnicity
[†] Not possible to calculate
[‡] Adjusted for age
[§] Adjusted for age, race and geographic site

defecation. Endoscopic examination did not show any abnormalities, but the gastroenterologist noticed typical clinical features of SSc and referred the patient to one of us [K.H.]. During clinical evaluation it was noticed that she had Raynaud's phenomenon, which had been present since 2006, telangiectasia, puffy fingers, sclerodactyly (*figure 1*), scleroderma of the face, microstomia (*figure 2*) and arthralgia. Antinuclear antibody (fine speckled pattern) and anti-ENA were positive, while auto-antibodies against SS-A (anti-RO60), ACA and anti-Scl-70 (anti-topoisomerase) were negative. Sicca complaints were absent, but she used eye drops twice a day because of a previous cornea transplantation as indicated for bilateral corneal degeneration based on Fuchs endothelial corneal dystrophy.

The diagnosis LcSSc was made based on Raynaud's phenomenon, limited skin involvement, positive auto-antibodies, and a late SSc pattern on nailfold videocapillaroscopy (NVC) (*figure 3*). Furthermore, she fulfilled the ACR/EULAR criteria[26] based on the presence of sclerodactyly (score 4), telangiectasia (score 2), abnormal NVC pattern (score 2) and Raynaud's phenomenon (score 3).

Treatment with nonsteroidal anti-inflammatory drugs and low-dose prednisone was started.

In 2012, during a regular visit to the hospital, the patient said that she had followed a discussion on national television about SBIs and a possible association with SSc. She wondered if in her case an association could be present since she had had SBIs implanted at the age of 40 for cosmetic reasons. A mammography was performed and showed a ruptured SBI on the left side, whereas the SBI on the right side was still intact. Both SBIs were removed by a plastic surgeon. Afterwards regular follow-up was continued by her referring and treating internist.

Three years after ablation of the SBIs, the patient was doing very well, with stable disease and no cardiopulmonary involvement. In January 2016, however, she died due to old age at 89 years; an autopsy was not performed.

DISCUSSION

We describe a patient with late-onset SSc and ruptured SBI. A literature search was performed concerning age of onset of SSc and time since implant in patients with SSc and SBIs (*table 1*).

Based on case reports a causal relation between the occurrence of SSc and SBIs is suspected. A causal relation between either polyarthritis or lupus and silicone implants has been suggested in animal models.[27,28] However, such studies are lacking for SSc. Furthermore, cohort studies on this topic could not demonstrate a significant association.[16,17] Also meta-analyses did not demonstrate a significant association.[3,6-8,11] The role of ruptured SBIs could not, however, be established in these meta-analyses since the individual studies did not provide adequate data on rupture or leakage of implants.[6] Furthermore, critical remarks can be made on the quality of these studies, e.g., the lack of quality and power to demonstrate an association. In addition, most studies used the number of self-reported SSc as outcome parameter, without using any classification criteria. In our case, the diagnosis of SSc was clinically confirmed and the patient fulfilled the ACR/EULAR 2013 criteria.[26]

Importantly, we have additionally collected data from all SSc patients included in the Leiden Systemic Sclerosis Cohort, which is a prospective cohort study in patients with SSc who participate in an annual two-day multidisciplinary healthcare program aiming to structure screening for organ involvement and to provide multidisciplinary care for patients with SSc.[29] Patients were included if they had a diagnosis of SSc according to either the American Rheumatism Association,[30] the LeRoy criteria,[31] or the ACR/EULAR 2013 classification criteria.[26] Between April 2009 and January 2014, 278 females were referred, including nine with SBIs, mean age 57 (ranged 22-69) years. In eight patients the SBIs were placed before the development of SSc; the time since SBIs and development was a mean of 16 (ranged 1-40) years. We consider a prevalence of 3% (8 out of 278 female patients) as a high frequency.

Rupture is a well-known complication of SBIs. It is known that the risk of implant rupture increases with implant age.[32] Whereas in the past about 50% of SBIs were ruptured after a mean follow-up of 10 years,[33] in 2002 about 15% of the modern implants rupture between the third and tenth year after implantation.[34] The prevalence of rupture among the controversial Poly Implant Prothèse, however, was higher than that of other SBIs suggesting that SBI products are not completely comparable.[35] The data reported on age of SSc onset in relation to time since implantation and possible rupturing of SBIs are incomplete. This could be important since rupture increases silicone exposure to the body. Leakage of silicones i.e. so-called 'bleeding' through intact implants, however, also occurs and may also result in the development in symptomatic disease.[36]

Several case reports describing SBI rupture from compression during a mammogram have been described.[33-35,37-44] Brown et al. collected data on adverse events reported to the Food and Drug Administration (FDA) concerning mammography and found 41 cases that experienced rupture during mammography.[33] It is likely that in women who had intracapsular ruptures before their mammogram, compression during mammography results in breaking of the fibrous capsule around the implant. Therefore, if a rupture is expected, magnetic resonance imaging seems to be the most accurate method

for identification of the SBI rupture.[12] Also the FDA advises follow-up MRIs in patients with SBIs. Our patient did very well after removal of the SBIs. Her disease did not progress and the patient felt well.

Few reports are available concerning the follow-up of SSc patients after removal of SBIs.[45] In a recent review, it was found that removal of SBIs in symptomatic patients resulted in an improvement of 76% (457 of 603 patients).[46] In patients with SBIs and autoimmune diseases, however, improvement occurred in only 16% of the patients. Therefore, it is still unknown if replacement or removal of ruptured SBIs leads to a better health status in patients with SSc.[15] The current advice is to remove damaged SBIs, regardless of whether a leakage or rupture is present.[46] However, more studies on this subject are needed.

In summary, ruptured SBIs are reported in a low number of published SSc cases. It is still unclear whether an association between SSc and ruptured SBIs is present since epidemiological studies are inconclusive. Here, we present a case of a patient with late-onset SSc and ruptured SBIs. The advanced age of our patient and the absence of disease progression after removal of the SBIs raised the suspicion of a possible association. More research is warranted to clarify the putative relation between SBIs and late-onset SSc and the follow-up of the disease status within SSc females after removal of ruptured SBIs.

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DISCLOSURES

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REFERENCES

1. Nietert PJ, Silver RM. Systemic sclerosis: environmental and occupational risk factors. Curr Opin Rheumatol. 2000;12:520-6.
2. Levy Y, Rotman-Pikielny P, Ehrenfeld M, Shoenfeld Y. Silicone breast implantation-induced scleroderma: description of four patients and a critical review of the literature. Lupus. 2009;18:1226-32.
3. Balk EM, Earley A, Avendano EA, Raman G. Long-Term Health Outcomes in Women With Silicone Gel Breast Implants: A Systematic Review. Ann Intern Med. 20162;164:164-75.
4. Hennekens CH, Lee IM, Cook NR, et al. Self-reported breast implants and connective-tissue diseases in female health professionals. A retrospective cohort study. JAMA. 1996;275:616-21.
5. McLaughlin JK, Fraumeni JF, Jr., Olsen J, Mellemkjaer L. Re: Breast implants, cancer, and systemic sclerosis. J Natl Cancer Inst. 1994;86:1424.
6. Hochberg MC, Perlmutter DL. The association of augmentation mammoplasty with connective tissue disease, including systematic sclerosis (scleroderma): a meta-analysis. Curr Top Microbiol Immunol. 1996;210:411-7.
7. Janowsky EC, Kupper LL, Hulka BS. Meta-analyses of the relation between silicone breast implants and the risk of connective-tissue diseases. N Engl J Med. 2000;342:781-90.
8. Perkins LL, Clark BD, Klein PJ, Cook RR. A meta-analysis of breast implants and connective tissue disease. Ann Plast Surg. 1995;35:561-70.
9. Hugle T, Schuetz P, Daikeler T, et al. Late-onset systemic sclerosis--a systematic survey of the EULAR scleroderma trials and research group database. Rheumatology (Oxford). 2011;50:161-5.
10. Holmich LR, Lipworth L, McLaughlin JK, Friis S. Breast implant rupture and connective tissue disease: a review of the literature. Plast Reconstr Surg. 2007;120:62S-9S.
11. Whorton D, Wong O. Scleroderma and silicone breast implants. West J Med. 1997;167:159-65.
12. Di Benedetto G, Cecchini S, Grassetti L, et al. Comparative study of breast implant rupture using mammography, sonography, and magnetic resonance imaging: correlation with surgical findings. Breast J. 2008;14:532-7.
13. Lipworth L, Tarone RE, McLaughlin JK. Silicone breast implants and connective tissue disease: an updated review of the epidemiologic evidence. Ann Plast Surg. 2004;52:598-601.
14. Al Aranji G, White D, Solanki K. Scleroderma renal crisis following silicone breast implant rupture: a case report and review of the literature. Clin Exp Rheumatol. 2014;32:262-6.
15. Lidar M, Agmon-Levin N, Langevitz P, Shoenfeld Y. Silicone and scleroderma revisited. Lupus. 2012;21:121-7.
16. Englert H, Joyner E, McGill N, et al. Women's health after plastic surgery. Intern Med J. 2001;31:77-89.
17. Kjoller K, Friis S, Mellemkjaer L, et al. Connective tissue disease and other rheumatic conditions following cosmetic breast implantation in Denmark. Arch Intern Med. 2001; 9;161:973-9.
18. Lipworth L, Holmich LR, McLaughlin JK. Silicone breast implants and connective tissue disease: no association. Semin Immunopathol. 2011;33:287-94.
19. Brown SL, Pennello G, Berg WA, Soo MS, Middleton MS. Silicone gel breast implant rupture, extracapsular silicone, and health status in a population of women. J Rheumatol. 2001;28:996-1003.
20. Psarras A, Gkougkourelas I, Tselios K, Sarantopoulos A, Boura P. Systemic sclerosis and silicone breast implant: a case report and review of the literature. Case Rep Rheumatol. 2014;2014:809629.
21. Maijers MC, de Blok CJ, Niessen FB, et al. Women with silicone breast implants and unexplained systemic symptoms: a descriptive cohort study. Neth J Med. 2013;71:534-40.
22. Burns CJ, Laing TJ, Gillespie BW, et al. The epidemiology of scleroderma among women: assessment of risk from exposure to silicone and silica. J Rheumatol. 1996;23:1904-11.
23. Hochberg MC, Perlmutter DL, Medsger TA, Jr., et al. Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). Arthritis Rheum. 1996; 39:1125-31.
24. Claman HN, Robertson AD. Antinuclear antibodies and breast implants. West J Med. 1994;160:225-8.
25. Englert H, Morris D, March L. Scleroderma and silicone gel breast prostheses--the Sydney study revisited. Aust N Z J Med. 1996;26:349-55.
26. Van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis. 2013;72:1747-55.
27. Schaefer CJ, Wooley PH. The influence of silicone implantation on murine lupus in MRL lpr/lpr mice. J Rheumatol. 1999;26:2215-21.
28. Schaefer CJ, Lawrence WD, Wooley PH. Influence of long term silicone implantation on type II collagen induced arthritis in mice. Ann Rheum Dis. 1999;58:503-9.
29. Meijs J, Schouffoer AA, Ajmone MN, et al. A prediction model for progressive disease in systemic sclerosis. RMD Open. 2015;1:e000113.
30. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American

Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Arthritis Rheum. 1980;23:581-90.

31. Leroy EC, Medsger TA, Jr. Criteria for the classification of early systemic sclerosis. J Rheumatol. 2001;28:1573-6.
32. Holmich LR, Friis S, Fryzek JP, et al. Incidence of silicone breast implant rupture. Arch Surg. 2003;138:801-6.
33. Brown SL, Todd JF, Luu HM. Breast implant adverse events during mammography: reports to the Food and Drug Administration. J Womens Health (Larchmt). 2004;13:371-8.
34. Kjoller K, Holmich LR, Jacobsen PH, et al. Epidemiological investigation of local complications after cosmetic breast implant surgery in Denmark. Ann Plast Surg. 2002;48:229-37.
35. Maijers MC, Niessen FB. Prevalence of rupture in poly implant Prothese silicone breast implants, recalled from the European market in 2010. Plast Reconstr Surg. 2012;129:1372-8.
36. Cohen Tervaert JW, Kappel RM. Silicone implant incompatibility syndrome (SIIS): a frequent cause of ASIA (Shoenfeld's syndrome). Immunol Res. 2013;56:293-8.
37. James NJ. Mammography as a cause of firmness after augmentation mammaplasty. Plast Reconstr Surg. 1989;83:577.
38. Andersen B, Hawtof D, Alani H, Kapetansky D. The diagnosis of ruptured breast implants. Plast Reconstr Surg. 1989;84:903-7.
39. Hawes DR. Collapse of a breast implant after mammography. AJR Am J Roentgenol. 1990;154:1345-6.
40. Renfrew DL, Franken EA, Jr., Berbaum KS, Weigelt FH, Abu-Yousef MM. Error in radiology: classification and lessons in 182 cases presented at a problem case conference. Radiology. 1992;183:145-50.
41. De Camara DL, Sheridan JM, Kammer BA. Rupture and aging of silicone gel breast implants. Plast Reconstr Surg. 1993;91:828-34.
42. Pickford MA, Webster MH. Implant rupture by mammography. Br J Plast Surg. 1994;47:512.
43. Beraka GJ. Rupture of implants following mammography. Plast Reconstr Surg. 1995;95:936-7.
44. Pay AD, Kenealy J. Breast implant rupture following contralateral mammography. Plast Reconstr Surg. 1997;99:1734-5.
45. Cohen Tervaert JW, Colaris MJ, Van der Hulst RR. Silicone breast implants and autoimmune rheumatic diseases: myth or reality. Curr Opin Rheumatol. 2017 Apr 4. doi: 10.1097/BOR.0000000000000391. [Epub ahead of print]
46. De Boer M, Colaris M, Van der Hulst R, Cohen Tervaert JW. Is explantation of silicone breast implants useful in patients with complaints? Immunol Res. 2017;65:25-36.

CASE REPORT

'Transformation' from amyloid light chain amyloidosis to symptomatic multiple myeloma

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ABSTRACT

Amyloid light chain (AL) amyloidosis and multiple myeloma (MM) are both clonal plasma cell disorders, and may be concurrently present in patients. However, symptomatic MM seldom develops in patients with AL amyloidosis, while the other way around is common. With this case report, we discuss the difficulties in the differential diagnosis between AL amyloidosis and MM, and extend on the possible mechanisms involved in the development of these overlapping disorders. In addition, we provide clinicians with tools that may help improve their management and monitoring of such patients.

KEYWORDS

Amyloidosis, plasma cell dyscrasia, multiple myeloma

What was known about this topic?
AL amyloidosis and MM are both clonal plasma cell disorders. Although both may be concurrently present in a single patient, the development of symptomatic myeloma in AL amyloidosis patients is very rare.

What does this add?
Improved survival of patients with AL amyloidosis may result in the progression of smouldering to symptomatic MM. It is important for clinicians to be aware of this possibility. Especially bone pain should raise awareness and CT or MRI imaging should be used to detect collapsed vertebrae with or not yet with lytic bone lesions.

INTRODUCTION

Amyloid light chain (AL) amyloidosis and multiple myeloma (MM) are both part of the spectrum of plasma cell dyscrasias, in which malignant bone marrow residing plasma cells produce monoclonal proteins, sometimes composed of light chains only. AL amyloidosis is characterised by precipitation of monoclonal immunoglobulin light chain fragments as extracellular amyloid depositions in various tissues, causing organ dysfunction and subsequent disease manifestations.[1] MM is characterised by the CRAB criteria, consisting of hypercalcaemia, nephropathy, anaemia and bone disease.[2] Patients may present with both disorders simultaneously, and the distinction between the two may be very difficult. According to the literature, around 8% of the patients with AL amyloidosis also fulfill the CRAB criteria fitting the diagnosis of MM at the time the diagnosis AL amyloidosis is made, while 1 to 38% of patients diagnosed with MM may have amyloid deposits.[4,5] However, isolated amyloid detected in bone marrow or abdominal fat biopsy but without systemic amyloid deposits with organ dysfunction seldom develops into systemic AL amyloidosis and should therefore not to be considered as AL amyloidosis.[6] In the disease trajectory of patients with MM, approximately 10-15% will eventually develop AL amyloidosis,[7] while patients with AL amyloidosis very rarely develop symptomatic MM.

CASE REPORT

A 56-year-old man who presented with severe weight loss (30 kg in one year), diarrhoea, peripheral sensory neuropathy and orthostatic hypotension was diagnosed with AL amyloidosis. Kappa free light chains (FLC) were elevated to 384 mg/l (normal 3.30-19.40 mg/l) and the FLC ratio was 28.66 (normal 0.26-1.65). M protein was not detected. Amyloid depositions could be confirmed in myocardium, kidney, bone marrow and abdominal fat biopsies. Bone marrow examination showed a kappa positive plasmacytosis of 10%. At diagnosis no lytic bone lesions

or collapsed vertebrae were seen on low dose CT-whole body scan and also no other CRAB criteria were present. Nevertheless, the presence of 10% monoclonal plasma cells may suggest smouldering MM with associated AL amyloidosis. Treatment of the patient with bortezomib and dexamethasone resulted in a very good partial response.

Two and a half years later, the patient presented with severe back pain and shorter statue. At this time point, low dose CT-whole body scan showed diffuse osteopenia and five collapsed vertebrae, additionally confirmed by total spine MRI scan. Bone marrow examination showed a plasmacytosis of 20% and kappa FLC was increased to 56.80 mg/l. He had no anaemia or hypercalcaemia, but progressive renal insufficiency and non-Bence Jones proteinuria were present. We concluded that the vertebral collapses were most likely due to development of symptomatic MM, although no lytic bone lesions were seen. He received melphalan and dexamethasone, combined with bisphosphonate therapy and again a very good partial response was achieved. Another five and a half years later his renal insufficiency further progressed (creatinine level 368 µmol/l, normal 64-104 µmol/l) and a second biopsy of the kidney confirmed increased amyloid depositions compared with the first biopsy. CT-whole body scan showed, apart from diffuse osteopenia, also a lytic lesion in vertebrae C5. Third-line treatment with endoxan and prednisone was started.

DISCUSSION AND CONCLUSION

In our case, without a confirming biopsy, it remains unclear whether the vertebral collapses were due to symptomatic MM or amyloid deposits in the bone. However, collapsed vertebrae due to AL amyloidosis is very rare, whereas it is a common complication in MM. Moreover, the lytic bone lesion seen some years later strengthens our hypothesis that our patient developed symptomatic MM, while initially having smouldering MM with an associated AL amyloidosis.

In the literature, we found eleven similar case reports of patients with AL amyloidosis who 'transformed' to MM.[8-10] The mean time between the diagnosis of AL amyloidosis and MM in these studies was 35 (range, 9-81) months and survival time after the diagnosis of MM ranged from less than a month to more than 48 months. There are similarities between the progression of monoclonal gammopathy of undetermined significance (MGUS) to MM and the progression of AL amyloidosis to MM.[8] MGUS always precedes MM, but can exist for years without clinical manifestation. Importantly, light chain MGUS is also capable of producing amyloid, and precipitation of these light chain fragments in tissues will result in tissue damage and clinical symptoms of amyloidosis, before abundant clonal proliferation of MM occurs.

In our patient we may conclude the possibility of a smouldering MM (eventually slowly progressing to MM) at the time of diagnosing AL amyloidosis. Difficulties in the differential diagnosis between AL amyloidosis and MM are due to partial overlap in diagnostic criteria. Patients with AL amyloidosis may present with a small number of monoclonal plasma cells in the bone marrow examination, but almost 40% of patients have more than 10% monoclonal plasma cells at the time of diagnosis and thus may be considered as having (smouldering) MM.

With improvement of the survival of patients with AL amyloidosis with the currently available treatment modalities, in the near future more patients may live long enough to develop symptomatic MM. Clinicians should be aware of this possibility and in addition to monitoring AL amyloidosis associated symptoms, also monitor patients for development of MM-induced organ damage such as bone fractures.

DISCLOSURES

The authors declare no conflict of interest.

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Karlijn H.G. Rutten is a medical student who is participating in the Honours program of the Faculty of Medicine of the University Medical Center Utrecht, the Netherlands.

REFERENCES

1. Palladini G, Merlini G. What is new in diagnosis and management of light chain amyloidosis? Blood. 2016;128:159-68.
2. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-48.
3. Kourelis TV, Kumar SK, Gertz MA, et al. Coexistent multiple myeloma or increased bone marrow plasma cells define equally high-risk populations in patients with immunoglobulin light chain amyloidosis. J Clin Oncol. 2013;31:4319-23.
4. Siragusa S, Morice W, Gertz MA, et al. Asymptomatic immunoglobulin light chain amyloidosis (AL) at the time of diagnostic bone marrow biopsy in newly diagnosed patients with multiple myeloma and smoldering myeloma. A series of 144 cases and a review of the literature. Ann Hematol. 2011;90:101-6.
5. Desikan KR, Dhodapkar MV, Hough A, et al. Incidence and impact of light chain associated (AL) amyloidosis on the prognosis of patients with multiple myeloma treated with autologous transplantation. Leuk Lymphoma. 1997;27:315-9.
6. Chakraborty R, Gertz MA, Disperzieri A. Natural history of amyloidosis isolated to fat and bone marrow aspirate. Br J Haematol. 2017;179:170-2.
7. Kyle RA. Multiple myeloma and other plasma cell disorders. In: Hematology: basic principles and practice. 2[nd] edition. 1995. p. 1354-74.
8. Rajkumar SV, Gertz MA, Kyle RA. Primary systemic amyloidosis with delayed progression to multiple myeloma. Cancer. 1998;82:1501-5.
9. Hoffman J. AL amyloidosis and progression to multiple myeloma with gain (1q). Br J Haematol. 2008;144:963-4.
10. Rodrigues L, Neves M, Sá H, et al. Clinical challenges of an oligosecretory plasma cell dyscrasia. Br Med J Case Rep. 2013;1-4.

CASE REPORT

Cytomegalovirus-associated thrombosis

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ABSTRACT

Cytomegalovirus (CMV) infections are often asymptomatic, but symptoms can range from a mononucleosis-like syndrome to a severe, disseminated infection in immunocompromised patients. We present two cases of immunocompetent patients with acute CMV infection directly followed by portal vein thrombosis in one and pulmonary embolism in the other patient. Thromboembolism may be a severe complication of acute CMV infection, with possible therapeutic implications.

KEYWORDS

Acute CMV infection, cytomegalovirus, portal vein thrombosis, pulmonary embolism, venous thromboembolism

What was known on this topic?
Cytomegalovirus infection is often asymptomatic or causes a mononucleosis-like syndrome. Immunocompromised patients are especially at risk for a severe, disseminated infection with complications.

What does this add?
Acute CMV infection is a risk factor for arterial and venous thromboembolism, even in immunocompetent patients and in the absence of other thrombophilic factors. CMV-associated thrombosis mainly occurs in adults and appears to be an underestimated complication; recognition may have implications for treatment. The virus interferes directly in the coagulation pathways and is more strongly associated with thrombosis than other herpes viruses.

INTRODUCTION

Approximately 65% of the general population will become infected with the cytomegalovirus (CMV), in 50% during adulthood. The manifestation of CMV infection varies from asymptomatic or mild mononucleosis-like syndrome to a severe, disseminated infection in immunocompromised patients.[1] It is not generally known that acute CMV infection is associated with arterial and venous thromboembolism (VTE).

CASE PRESENTATIONS

Patient A, a 66-year-old man, presented to the emergency department (ED) with abdominal pain. In the last month he had been investigated elsewhere because of fever, anorexia, fatigue and mildly elevated liver enzymes. Because of hypotension, tachypnoea and lactic acidosis upon ED presentation, an abdominal computed tomography scan with intravenous contrast was performed that showed intestinal ischaemia due to extensive portal vein thrombosis. Anticoagulant therapy was started and partial resection of the small intestine was performed, followed by a long and complicated stay in the intensive care department. The portal vein thrombosis was regarded as unprovoked and both the family history and thrombophilia testing were negative. Eventually, the results of the serological tests (which were requested before the time of presentation at the emergency department) became available and showed evidence for acute CMV infection (*table 1*). Apart from the acute CMV infection, he had no thrombophilic risk factors. Anticoagulant therapy was stopped after 15 months during follow-up in the outpatient clinic and he is currently rehabilitating in a nursing home.

Patient B, a 60-year-old woman, presented to the ED with chest pain, fever and dyspnoea. The initial chest X-ray and electrocardiogram showed no explanation for her symptoms. Three weeks earlier, she had visited our outpatient clinic with fatigue, fever and muscle pain. CMV testing at that time revealed an acute CMV infection as an explanation for her symptoms. Additionally, the family history revealed that her mother had died postpartum of venous thromboembolism. A CT scan was

Table 1. *CMV serology of patient A and B*

Test	Patient A T1	T2 *(after 1 year)*	Patient B T1	T2 *(after 2 weeks)*
CMV IgM	Positive	Negative	Positive	Positive
CMV IgM index	4.68	*	7.73	12.70
CMV IgG	Positive	Positive	Inconclusive	Positive
CMV IgG titre	18	*	5	23
CMV avidity IgG	Low	*	Inconclusive	Low

* Not measured; T1 = first measurement; T2 = second measurement.
Patient A was diagnosed with VTE at T1. Serology shows IgM and IgG antibodies with a low avidity at T1 (proving an acute infection) with complete seroconversion at T2.
Patient B was diagnosed with VTE at T2, and had his first symptoms at T1. The serological tests show an acute infection at T1 which is confirmed by the increase in index (= a derivative of the titre) and avidity at T2.

Table 2. *Hypercoagulation tests in patient A and B*

Test	Patient A	Patient B
Anticardiolipin IgG*	< 10 GPL-U/ml (negative)	< 10 GPL-U/ml (negative)
Anticardiolipin IgM*	< 10 MPL-U/ml (negative)	< 10 MPL-U/ml (negative)
Anti-beta2-glycoprotein IgG*	< 10 U/ml (negative)	< 10 U/ml (negative)
Anti-beta2-glycoprotein IgM*	< 10 U/ml (negative)	< 10 U/ml (negative)
Lupus anticoagulants*	Negative	Negative
Protein C activity**	84% (normal)	Not performed
Protein S activity**	98% (normal)	Not performed
Factor V Leiden mutation	No aberration	Heterozygote
Antithrombin activity	95% (normal)	121% (slightly elevated)
Prothrombin mutation	Absent	Absent
Factor VIII activity**	238% (elevated)	176% (elevated)
JAK2 V617 exon 14 mutation	Negative	Not performed
JAK2 exon 12 mutation	Negative	Not performed
Calreticulin mutation	Negative	Not performed

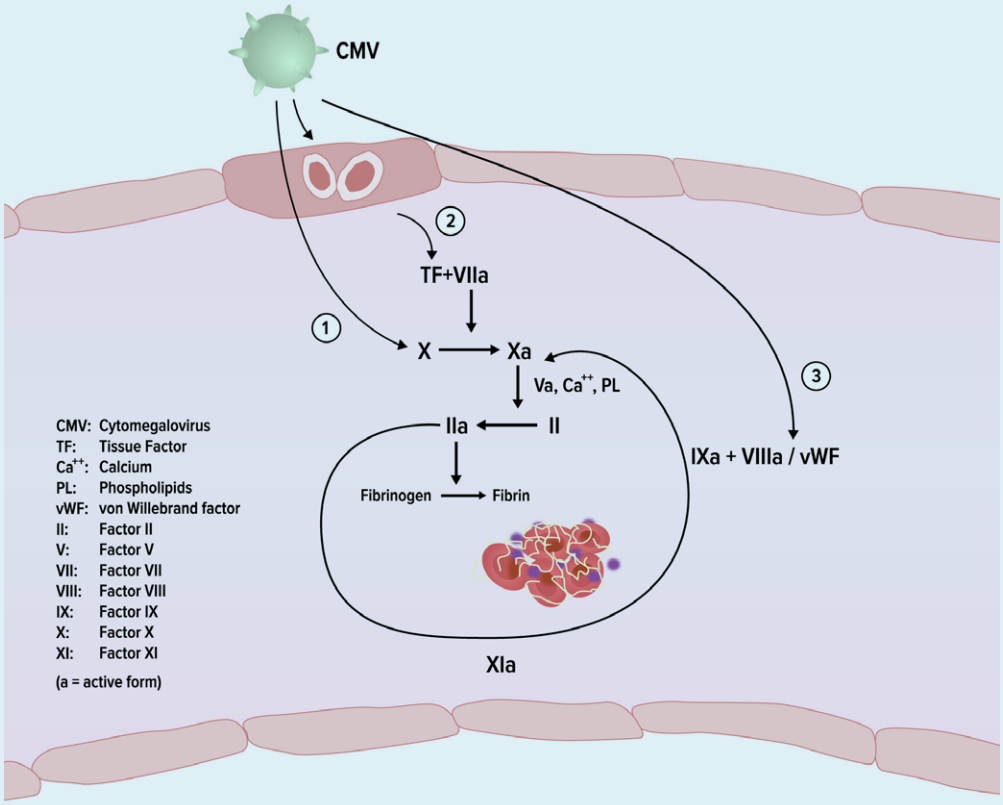
* Testing is repeated after 12 weeks to confirm values
** Protein C and S and factor VIII activity ca not be evaluated during anticoagulation therapy

performed and showed massive pulmonary embolism together with a pulmonary infarction. She became haemodynamically unstable and was transferred to the intensive care unit to initiate thrombolytic therapy, followed by treatment with anticoagulant therapy. Hypercoagulability testing revealed heterozygosity for the factor V Leiden mutation (*table 2*). Patient B is being treated with lifetime anticoagulation (direct-acting oral anticoagulants) because of the positive family history, the severity of the thrombotic event and the heterozygosity for the factor V Leiden mutation. She was discharged home after a short hospitalisation and has recovered completely.

DISCUSSION

Although more than 100 patients with both venous and arterial CMV-associated thromboembolism are reported in the current literature, this association is not generally known. Based on the results of several studies, which are discussed below, it seems that CMV forms a risk factor for arterial and venous thromboembolism. However, this is not mentioned in the current Dutch guidelines. In our two immunocompetent patients we performed additional tests for hypercoagulability because of the extensive thrombosis and severity of symptoms (*table 1*). Serological

Figure 1. *Mechanisms of how cytomegalovirus interferes with coagulation pathways. 1) Activation of factor X; 2) Systemic endotheliitis and tissue factor expression; 3) Increased production of factor VIII and von Willebrand factor*



tests for CMV were performed in both patients because they initially presented with mononucleosis-like syndrome (*table 2*).

Epidemiology

In a large meta-analysis reporting 97 patients, thrombosis incidence of 6.4% is reported in hospitalised adult patients with an acute CMV infection. Conversely, an acute CMV infection is detectable in 2-9% of the hospitalised patients with VTE, versus 1.6% CMV infections in the control group without VTE. Although patients are usually exposed to CMV during childhood and adolescence, CMV-associated thrombosis occurs mainly in adults, as in our patients. In this meta-analysis reporting on 97 patients with CMV-associated thrombosis, the mean age was 40 years (25-55 years), the most common sites were deep vein, pulmonary or splanchnic vein thrombosis and immunocompetent patients more often had a genetic or acquired predisposing thrombophilic factor relative to immunocompromised patients.[2] CMV may be the last trigger needed to provoke thrombosis in these patients. In other studies, CMV was the only detectable risk factor for thrombosis in immunocompetent patients.[3]

Pathogenesis

Viral hepatitis can induce a procoagulant state due to inflammation. However, CMV is more strongly associated with venous thromboembolism than other herpes viruses that can lead to hepatitis, such as the Epstein-Barr virus for instance.[4] CMV seems to interfere directly with haemostasis and several pathogenetic mechanisms have been described explaining its role in thrombosis (*figure 1*). CMV activates in vitro factor X and stimulates the production of factor VIII and von Willebrand factor. CMV binds to platelets via Toll Like Receptor 2, thereby presenting the adhesion molecule P selectin on the cell surface. In addition, CMV causes systemic endotheliitis at various sites in the body, leading to expression of tissue factor. These three mechanisms result in platelet and leukocyte aggregation, adhesion and thrombin formation. In vivo, a transient increase in antiphospholipid antibodies can be observed. Also, decreased protein C activation has been described.[5-8]

Implications for clinical practice

The Dutch national guideline 'Antithrombotic therapy', published in 2016, differentiates for treatment in

patients with provoked or unprovoked (idiopathic) venous thromboembolism.[9] CMV has specific thrombogenic characteristics and appears to be a trigger for thrombosis in patients, either in the presence or absence of other predisposing factors for thrombophilia. However, CMV is not recognised as a risk factor for thrombosis. Therefore, we advocate to perform serological testing if patients present with thrombosis and mononucleosis-like syndrome. We recommend to prospectively follow this patient group in order to determine predisposing thrombophilic factors, the relative risk of CMV and thrombosis relapse risk. The estimated relative risk can be evaluated in order to determine the extent of secondary prophylaxis.

CONCLUSION

Acute CMV infection is a transient risk factor for both arterial and venous thromboembolism and can occur in immunocompetent patients in the absence of other thrombophilic factors. Based on the literature, thrombosis appears to be an underestimated complication of CMV infection. Recognition of symptoms leads to early diagnosis and treatment of complications. More research is needed to determine the relative risk of CMV as a provoking factor for thrombosis, the duration of treatment and the recurrence rate.

DISCLOSURES

The authors declare no conflict of interest. No funding or financial support was received.

REFERENCES

1. Richtlijn CMV-infectie, Rijksinstituut voor volksgezondheid en milieu, authorised January 2015, last consulted 14 October 2017. https://lci.rivm.nl/richtlijnen/cmv-infectie.
2. Justo D, Finn T, Atzmony L, et al. Thrombosis associated with acute cytomegalovirus infection: a meta-analysis. Eur J Int Med. 2011;22:195-9.
3. Atzmony L, Grosfeld A, Saar N, Justo D. Inherited and acquired predispositions for thrombosis in immunocompetent patients with cytomegalovirus-associated thrombosis. Eur J Int Med. 2010;21:2-5.
4. Squizzato A, Gerdes VEA. Viral Hepatitis and Thrombosis: A Narrative Review. Semin Thromb Hemost. 2012;38:530-4.
5. Pichenot M, Morell-Dubois S, et al. Acute cytomegalovirus infection as a transient risk factor for thrombosis: report of three cases and focus on specific coagulation pathways. Thromb Res. 2013;132:145-7.
6. Mijnhout GS, Groeneveld PHP. Acute cytomegalovirus infection complicated by venous thrombosis and pulmonary embolism. Tijdschr Infect. 2014;9:77-80.
7. Goeijenbier M, van Wissen M, van de Weg C, et al. Review: viral infections and mechanisms of thrombosis and bleeding. J Med Virol. 2012;84;1680-96.
8. Assinger A. Platelets and infection – an emerging role of platelets in viral infections. Front Immunol. 2014;5;1-9.
9. Richtlijn Antitrombotisch beleid, authorised 21 April 2016, last consulted on 14 July 2017. http://richtlijnendatabase.nl/module/antitrombotisch_beleid_-_algemeen.html.

PHOTO QUIZ

A case of progressive abdominal angina

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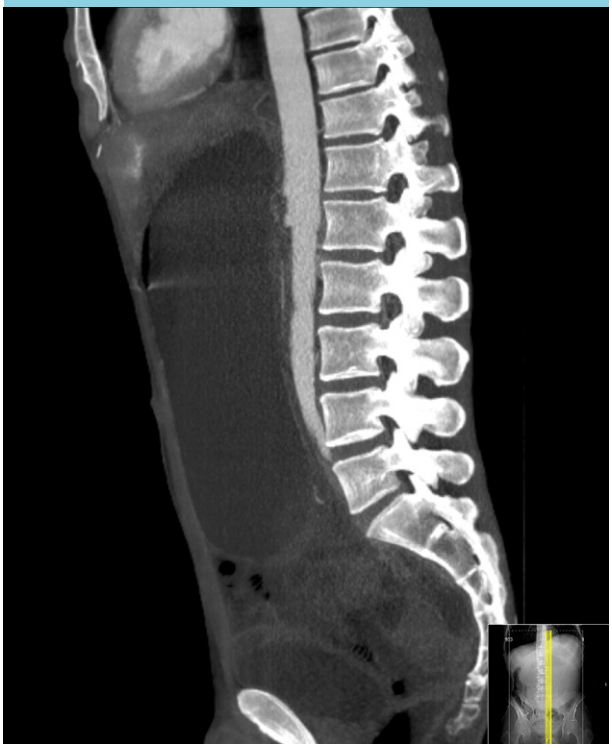
CASE REPORT

A 38-year-old patient was admitted to our hospital with signs of progressive abdominal angina, nausea, vomiting and cachexia, which had started four months earlier. His medical history reported seminoma 10 years before, for which he underwent orchiectomy followed by para-aortic radiotherapy (13 fractions of 26Gy). Within one year he presented with a local recurrence of the seminoma, which was successfully treated with chemotherapy. Further medical history included paranoid schizophrenia and drug abuse. There was no history of blood coagulation disorders or a family history of occlusive arterial disease.

The patient was referred to the psychiatric ward of our hospital by the psychiatrist from the mental health centre where he lived. The initial diagnosis was nonspecific abdominal pain combined with a delusional disorder, linked to his psychiatric and drug-related history. Thirty-eight days after presentation, a CT angiography was performed due to unsatisfactory clinical improvement, progressive abdominal pain and a high CRP (420 mg/l) (*figure 1*).

Figure 1. *CT angiography showing significant occlusion of the superior mesenteric artery (SMA) and the celiac trunk.*



WHAT IS YOUR DIAGNOSIS?

See page 256 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 255)

A CASE OF PROGRESSIVE ABDOMINAL ANGINA

DIAGNOSIS

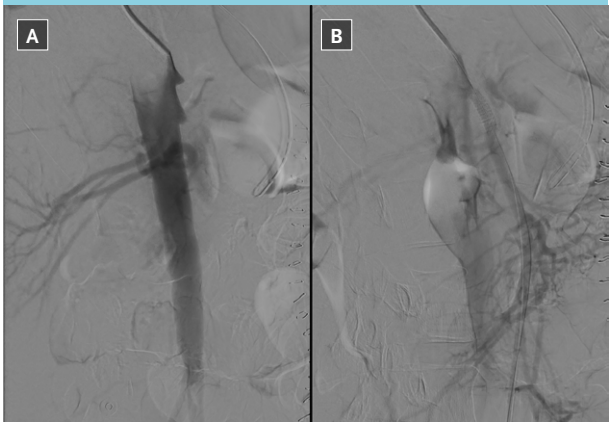
Radiotherapy-induced arterial occlusion of the superior mesenteric artery (SMA) and the celiac trunk.

The absence of abnormalities of the vessel walls in other arteries, the specific location of occlusion in the radiated tissue and the age of the patient suggested that the arterial occlusion was a late complication of radiotherapy which he underwent ten years earlier. The history of nicotine and drug abuse and psychiatric comorbidity suggested that in the early stage, the abdominal pain was caused by a psychiatric disturbance, and this delayed the further diagnostic process. Smoking and intravenous drug abuse may have accelerated the process, but are unlikely to be the sole cause of localised abdominal arterial occlusion. The intervention-radiologist performed a difficult percutaneous transluminal angioplasty with stent placement (Express SD 6x19mm, Boston Scientific) in the SMA the next day (*figure 2*) through brachial access. During this long procedure the patient was conscious and because of agitation we decided stop after stenting the SMA.

Radiotherapy-induced arterial damage was first described in 1899.[1] Studies show that arterial occlusion is rare and can develop many years after radiotherapy, intervals ranging from 4 to 44 years after treatment.[2] It is notable that advanced local occlusion can be present without any signs of arterial damage in other large arteries. Arterial occlusion in the irradiated area, with sparing of the non-radiated areas, is highly suggestive of radiotherapy-induced arterial occlusion. The pathogenesis of radiotherapy-induced arterial stenosis is similar to that of accelerated atherosclerosis and can be seen in young patients many years after radiotherapy.[3]

Early revascularisation is important to prevent bowel ischaemia. Most patients with coronary or femoral occlusion after radiotherapy show slowly progressive nonspecific symptoms.[5] When found early, revascularisation is successful in most cases.[5] Intervention can be difficult, due to fibrotic changes and a slow recovery of the vessel wall in the radiated area. Surgical intervention of arterial occlusion is often complicated due to early re-occlusion. Stenting of the artery can be challenging, but seems the appropriate and preferable treatment of radiation-induced arterial occlusion.[6]

Figure 2. *Angioplasty before the intervention. (A) shows total occlusion of the SMA and celiac trunk. The SMA was successfully stented (B)*



CONCLUSION

Although arterial occlusion is rarely seen as a result of radiotherapy, it can be a serious and potentially lethal complication. It needs to be stressed that although other diseases may be distracting, it is important to be certain of the full medical history of the patient to prevent early mortality in young patients who have undergone radiotherapy.

DISCLOSURES

All authors declare no conflict of interest.

REFERENCES

1. Gassmann A. Zur histologie der Roentgenulcer. Ann Intern Med. 1899:199-211.
2. Scott AS, Parr LA, Johnstone PA. Risk of cerebrovascular events after neck and supraclavicular radiotherapy: a systematic review. Radiother Oncol. 2009;90:163-5.
3. Jurado JA, Bashir R, Burket MW. Radiation-induced peripheral artery disease. Cathet Cardiovasc Interv. 2008;72:563-8.
4. Piedbois P, Becquemin JP, Blanc I, et al. Arterial occlusive disease after radiotherapy: a report of fourteen cases. Radiother Oncol. 1990;17:133-40.
5. Melliere D, Desgranges P, Berrahal D, et al. [Radiation-induced aorto-ilio-femoral arterial arteritis. Mediocrity of the long-term results after conventional surgery]. J Mal Vasc. 2000;25:332-5.
6. Hassen-Khodja R, Kieffer E. Radiotherapy-induced supra-aortic trunk disease: early and long-term results of surgical and endovascular reconstruction. J Vasc Surg. 2004;40:254-61.

PHOTO QUIZ

Traumatic occlusion of the renal artery

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CASE REPORT

A 25-year-old male was referred to our emergency department because of haematuria and flank pain after falling off his all-terrain bike in the woods earlier that day. Despite some abdominal discomfort and a slight dizziness immediately after the fall, he decided to ride his bike home. After several hours he experienced gross haematuria which led him to seek medical attention. At presentation his vital signs were normal. He showed some tenderness over the left lower ribs and left-sided abdomen and some superficial skin abrasions were visible.

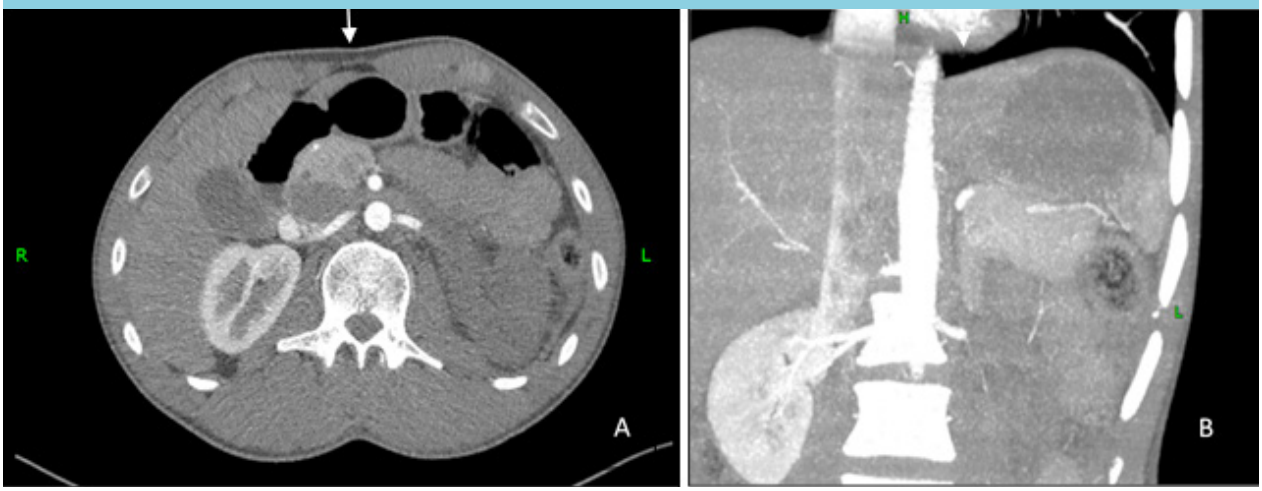
An abdominal CT scan was performed which showed complete occlusion of the left renal artery with a lack of contrast enhancement of the left kidney and a small amount of free fluid in the pouch of Douglas (*figure 1*). Due to the delayed clinical presentation (> 6 hours) the avascular left kidney could no longer be saved.

After a 24-hour observation period on the ICU the patient was discharged home uneventfully. Outpatient follow-up three months after the accident showed a normal blood pressure and normal function of the remaining right kidney on subsequent renogram studies.

WHAT IS YOUR DIAGNOSIS?

See page 258 for the answer to this photo quiz.

Figure 1. *Cross-sectional abdominal CT scan after IV contrast administration*



ANSWER TO PHOTO QUIZ (PAGE 257)

TRAUMATIC OCCLUSION OF THE RENAL ARTERY

DIAGNOSIS

Injuries of the renal artery are a rare complication of blunt abdominal trauma and occur mainly in young males.[1] The reported incidence of blunt renal injuries ranges from 0.05% to 4% of all abdominal injuries[2] and generally effects the left kidney, probably due to its hypermobility because of the longer pedicle.[1-3] During blunt trauma the sudden acceleration probably causes traction to the renal artery which leads to a subintimal dissection 1 to 2 cm from the aorta, because of the maximal angulation, followed by thrombosis of the artery.[1,2] Besides abdominal or flank pain, haematuria is seen in 76% of the cases.[1]

In general there are three options to manage renal artery thrombosis: observation, immediate revascularisation and prophylactic nephrectomy.[2] Unfortunately, revascularisation is seldom successful due to the prolonged warm ischaemia time because of patient delay in seeking help but the literature about revascularisation is scarce. In a study by Haas et al. and the study by Knudson et al. approximately 25% of revascularisation attempts in patients with unilateral renal artery injury were successful.[1,3] Cases studies report successful revascularisation 12 to 19 hours post injury.[2] Revascularisation should therefore always be considered, especially in patients with bilateral renal artery occlusion.

After hospital discharge, outpatient follow-up of these patients is important because of the high chance of developing arterial hypertension. Hypertension occurs in 32-50% of the patients without revascularisation[1,4] and can be treated by either nephrectomy of the damaged kidney or with medication alone.

CONCLUSION

Posttraumatic occlusion of the renal artery is an uncommon but serious injury which mostly affects young males with blunt abdominal trauma. In the management of traumatic occlusion of the renal artery revascularisation should always be considered.

REFERENCES

1. Haas CA, Dinchman KH, Nasrallah PF, Spirnak JPM. Traumatic Renal Artery Occlusion: A 15-Year Review. J Trauma Inj Infect Crit Care. 1998;45:557-61.
2. Sangthong B, Demetriades D, Martin M, et al. Management and Hospital Outcomes of Blunt Renal Artery Injuries: Analysis of 517 Patients from the National Trauma Data Bank. J Am Coll Surg. 2006;203:612-7.
3. Knudson MM, Harrison PB, Hoyt DB et al. Outcome after Major Renovascular Injuries: A Western Trauma Association Multicenter Report. J Trauma Inj Infect Crit Care. 2000;49:1116-22.
4. Stables DP, Fouche RF, de Villiers van Niekerk JP, Cremin BJ, Holt SA, Peterson NE. Traumatic renal artery occlusion: 21 cases. J Urol. 1976;115:229-33.

Figure 2. *Axial (A) and coronal reconstructions (B) show complete occlusion of the left renal artery (black arrow). Also note the absent contrast attenuation of the left kidney as compared with the right kidney, most likely secondary to the presence of a traumatic dissection in the left renal artery*

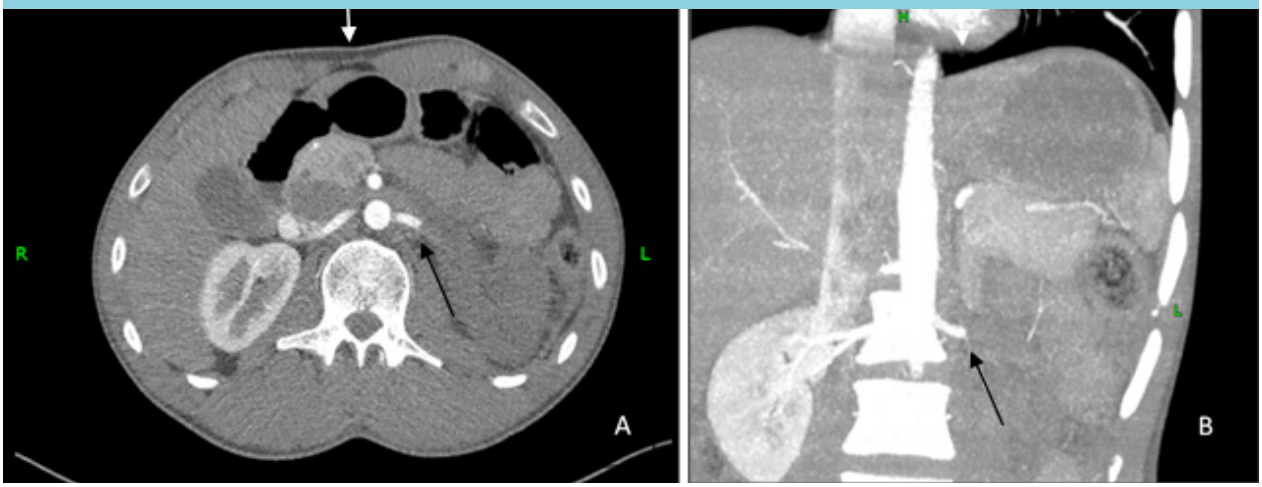


PHOTO QUIZ

An unusual cause of hyperammonaemia

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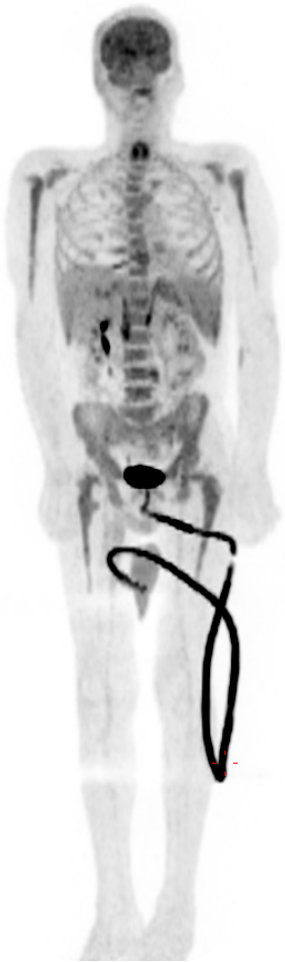
CASE REPORT

A 52-year-old man with a history of IL-12/23Rb1 deficiency presented to the emergency room with impaired consciousness. Laboratory investigations showed normal plasma glucose and electrolytes, but respiratory alkalosis, increased inflammatory parameters and pancytopenia. Lumbar puncture and head MRI did not show any abnormalities. An electroencephalogram revealed triphasic waves. Since these signs are often seen in metabolic encephalopathy, we checked plasma ammonia levels, which were elevated up to 216 µmol/l (reference 14-43 µmol/l). Liver function tests (i.e. clotting times and bilirubin levels) were normal. Moreover, abdominal ultrasound and CT scan excluded the presence of liver cirrhosis or flow anomalies. Blood, faeces, sputum and urine cultures and PCR all remained negative. Plasma amino acids (in particular glutamine), carnitine profiles and urinary orotic acid were all normal. A FDG PET-CT showed extensive FDG uptake in the bone marrow (*figure 1*). Bone marrow aspirate and biopsy were obtained (*figure 2*).

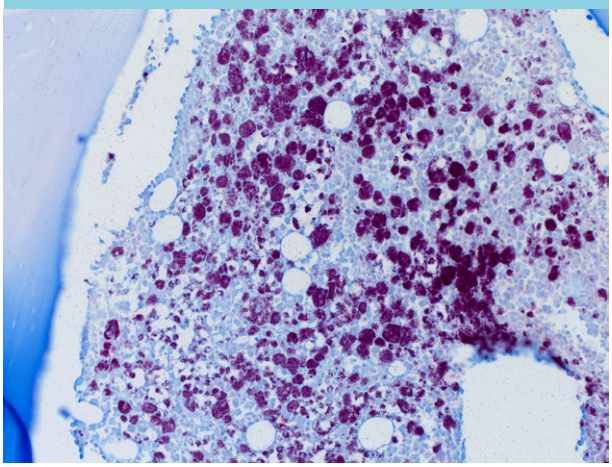
Figure 1. *Overview PET image shows physiological uptake in brain and bladder and diffuse bone marrow FDG uptake*



WHAT IS YOUR DIAGNOSIS?

See page 260 for the answer to this photo quiz

Figure 2. *Axial bone marrow biopsy stained by using the Ziehl-Neelsen method. Magnification x10*



ANSWER TO PHOTO QUIZ (PAGE 259)

AN UNUSUAL CAUSE OF HYPERAMMONAEMIA

DIAGNOSIS

In adults, unrecognised end-stage liver disease is the most likely cause of high ammonia levels.[1] In this case liver function tests, abdominal ultrasound and CT scan ruled out the presence of liver cirrhosis and portal flow anomalies. Our patient was not using any drugs that might interfere with the urea cycle, such as valproate, which inhibits the synthesis of N-acetylglutamate, a cofactor that promotes the first step in the detoxification of ammonia.[1] A normal amino acid profiling and normal urine orotic acid levels render a defect in the urea cycle unlikely.

Infections with urea-producing bacteria, often present in complex urinary tract infections, may cause hyperammonaemia by hydrolysis of urea into carbon dioxide and ammonia.[1] Patients with an IL-12/23Rb1-deficiency are, however, particularly prone to develop atypical mycobacterial infections because of an inability to produce interferon gamma.[2] As a consequence, we suspected an atypical mycobacterium to be the cause of the hyperammonaemic encephalopathy in this patient. By means of FDG PET-CT the infection was localised in the bone marrow which, in retrospect, explains the pancytopenia. Targeted bone marrow biopsy, showing positive PCRs and Ziehl-Neelsen stains, confirmed the presence of atypical mycobacteria. Cultures ultimately showed *Mycobacterium genavense* (*figure 2*). Although this infection has been reported in patients who are immunocompromised (e.g. after solid organ transplantation), there has been only one published case of *M. genavense* presenting with hyperammonaemia.[3] Of interest, previous studies have shown that this mycobacterium has urease activity,[4] which renders this microorganism the most likely cause of the hyperammonaemia in the present case.

During the diagnostic workup, the patient was treated with interferon gamma. Furthermore, treatment of hyperammonaemia was initiated by administering lactulose, rifaximin, continuous infusion of dextrose 10% solution, restriction of dietary protein intake, and eventually continuous veno-venous haemofiltration. Once the diagnosis was confirmed triple therapy with rifabutin, isoniazid and clarithromycin was started.

Despite the early start with ammonia-lowering therapy and the initial improvement of laboratory parameters, the patient's consciousness fluctuated during admission. Unfortunately, he eventually died from a disseminated infection.

DISCLOSURES

All authors declare no conflict of interest. No funding or financial support was received.

REFERENCES

1. Rabier D. Hyperammonemia. In: Hollak CEM, Lachman RH, editors. Inherited Metabolic Disease in Adults, a Clinical Guide. Oxford: Oxford University Press; 2016. p. 515-520.
2. Potjewijd J, de Paus RA, Van Wengen A, et al. Disseminated Mycobacterium genavense infection in a patient with a novel partial interleukin-12/23 receptor β1 deficiency. Clin Immunol. 2012;144:83-6.
3. Nurmohamed S, Weenink A, Moeniralam H, Visser C, Bemelman F. Hyperammonemia in generalized Mycobacterium genavense infection after renal transplantation. Am J Transplant. 2007;7:722-3.
4. Coyle MB, Carlson LC, Wallis CK, et al. Laboratory aspects of "Mycobacterium genavense," a proposed species isolated from AIDS patients. J Clin Microbiol. 1992;30:3206-12.