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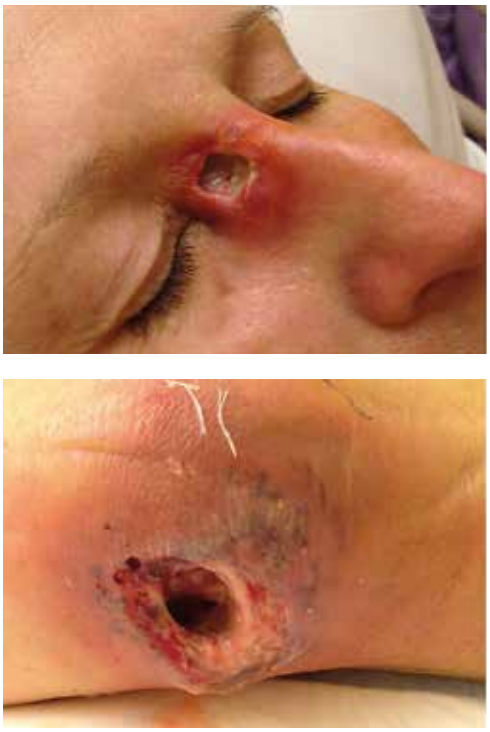

An ulcer on the nose and knee; what is your diagnosis?

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Department of Internal Medicine
's-Gravendijkwal 230
3015 CE Rotterdam
The Netherlands
Tel.: +31 (0)10-703 59 54
Fax: +31 (0)10-703 32 68
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EDITORIAL

Combination therapy of GLP-1 analogues and insulin: do the benefits outweigh the costs?

J. Versmissen

Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands,
email: j.versmissen@erasmusmc.nl

In the current issue of *the Netherlands Journal of Medicine*, Van Velsen *et al*. describe a small trial showing the benefit of adding a GLP-1 analogue to insulin in obese patients with type 2 diabetes using the maximum allowed or tolerated dosages of metformin and a sulphonylurea derivative.[1] The study clearly shows how impressive results of adding a GLP-1 analogue to insulin can be: the patients lost on average 14.3 km of weight, the HbA1C decreased by 5.5 mmol/mol (0.5 %) and patients could lower the dosage of insulin used by on average 75 IU. Patients used either a once daily, a twice daily or a full basal insulin-bolus regime. A recent meta-analysis on a regime of basal insulin and a GLP-1 analogue in a total of 4348 participants in The Lancet showed similar although less outspoken results as those of Van Velsen *et al*.: patients had lost on average 3.2 kg at the end of the study and the HbA1C decreased by 0.44% (5 mmol/mol).[2]

As stated in the accompanying editorial in The Lancet, the very first study on GLP-1 analogues included patients on insulin.[3] The lack of postprandial increase in blood glucose level was considered an artifact at first. Now, the combination of a GLP-1 analogue and insulin is more and more recognised as a potent strategy resembling normal physiology best. GLP-1 analogues simulate the incretin GLP-1 but are more resistant to degradation by dipeptidylpeptidase-4 (DPP-4): among other functions, they inhibit gastric emptying, stimulate post-prandial insulin secretion, increase satiety and suppress glucagon secretion.[4] Another argument for adding a GLP-1 analogue to insulin when oral therapy is at maximum dosage would be a protective effect on preservation of beta cell mass. However, although in rodents decline in beta cell mass can be halted and possibly even increased by GLP-1 analogues, it is difficult to confirm this in human trials.[5,6] A beneficial effect on beta cell *function* has been shown in smaller functional studies, but beta cell mass is difficult to assess.[6,7]

The combination of GLP-1 analogues with insulin has been approved by the European Medicine Agency (EMA) and the American Food and Drug Administration (FDA), but is not reimbursed in all countries. In the Netherlands, for instance, GLP-1 agonists are currently only reimbursed in obese patients (body mass index (BMI) > 35 kg/m^2) using metformin and a sulphonylurea derivative in the maximally tolerated dosage. The combination with insulin was reimbursed in the period after the introduction but redrawn in 2011. The Dutch Diabetes Patient Federation successfully fought this decision, enabling patients who already used this combination to continue. New prescriptions were no longer reimbursed. Recent studies including the meta-analysis in The Lancet might re-open this discussion.

Clearly, this discussion is about the balance between costs and (long-term) benefits: the efficacy of the combination therapy on HbA1C and especially body weight is clear, but at what price? Despite strictly limited use, the costs of GLP-1 analogues and DPP-4 inhibitors last year made up 68% of the total amount spent on non-insulin therapy for type 2 diabetes in the Netherlands.[8] When considering translation of the combination of a GLP-1 analogue and insulin to daily practice, what would be most desirable would be to add a GLP-1 analogue to once daily insulin, possibly in a fixed-dose combination limiting the burden to one subcutaneous injection a day.[3,9] Current studies showing promising results used a combination of liraglutide with insulin degludec, the most expensive insulin.[10]

Regarding the long-term benefits: first studies show that GLP-1 can be considered safe with regard to cardiovascular events and from *in vitro* and animal studies positive effects on cardiovascular outcomes are expected.[11,12] However, long-term results on decreasing microvascular and macrovascular complications have to be awaited, for instance the results of the current placebo-controlled

LEADER trial on the effect of liraglutide on cardiovascular endpoints in 9340 diabetes type 2 patients.[13]

To conclude, adding a GLP-1 analogue to insulin treatment shows clear benefits by enabling weight loss and decreasing the need for insulin. Future fixed-dose combinations might reduce the burden for patients to one subcutaneous injection with long-acting insulin and GLP-1 analogue. It has to be decided what price is acceptable to reach these advantages. Knowledge on long-term benefits such as reduction in microvascular or macrovascular complications is clearly needed to take stock of the pros and cons.

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ORIGINAL ARTICLE

High fall incidence and fracture rate in elderly dialysis patients

H.A. Polinder-Bos[1]*, M.H. Emmelot-Vonk[2], R.T. Gansevoort[1], A. Diepenbroek[1], C.A.J.M. Gaillard[1]

[1]Department of Nephrology, University Medical Centre Groningen, the Netherlands, [2]Department of Geriatric Medicine, University Medical Centre Utrecht, the Netherlands, *corresponding author: tel.: +31(0)50-3613741/+31(0)50-3616161, fax: +31(0)50-3612955, email: h.a.polinder-bos@umcg.nl

ABSTRACT

Background: Although it is recognised that the dialysis population is ageing rapidly, geriatric complications such as falls are poorly appreciated, despite the many risk factors for falls in this population. The objective of this study was to determine the incidence, complications and risk factors for falls in an elderly dialysis population.

Methods: A one-year observational study of chronic dialysis patients aged ≥ 70 years. At baseline, patient characteristics were noted and during follow-up the vital parameters and laboratory values were recorded. Patients were questioned weekly about falls, fall circumstances and consequences by trained nurses.

Results: 49 patients were included with a median age of 79.3 years (70-89 years). During follow-up 40 fall accidents occurred in 27 (55%) patients. Falls resulted in fractures in 15% of cases and in hospital admissions in 15%. In haemodialysis (HD) patients, the mean systolic blood pressure (SBP) before HD was lower in fallers compared with non-fallers (130 vs. 143 mmHg). Several patients in the lower blood pressure category received antihypertensive medication. For every 5 mmHg lower SBP (before HD) the fall risk increased by 30% (hazard ratio (HR) 1.30, 95% CI 1.03-1.65, p = 0.03). Furthermore, fall risk increased by 22% for every 10 pmol/l rise of parathyroid hormone (HR 1.22, 95% CI 1.06-1.39, p = 0.004).

Conclusions: Elderly dialysis patients have a high incidence of falls accompanied by a high fracture rate. Given the high complication rate, elderly patients at risk of falling should be identified and managed. Reduction of blood pressure-lowering medication might be a treatment strategy to reduce falls.

KEYWORDS

Falls, elderly, geriatric, fracture, dialysis

INTRODUCTION

The dialysis population is ageing rapidly in Western Europe and North America.[1-4] An ageing dialysis population is associated with specific geriatric issues such as falls.[5] Falls result in more need for long-term institutional care, functional decline and hospitalisations.[6-9] In the Netherlands, the numbers of fall-related hospital admissions among older adults more than doubled between 1981 and 2008.[10] For community-dwelling adults aged ≥ 65 years the annual fall incidence is 30%, and 15% of them fall at least twice a year.[11-13] The elderly dialysis population forms a high-risk population given the high prevalence of risk factors for falls, such as polypharmacy, multiple comorbidities including diabetes mellitus and cardiovascular disease, peripheral neuropathy, autonomic dysfunction, orthostatic hypotension, functional decline and cognitive impairment.[14-19] Nevertheless, falls in elderly dialysis patients is a poorly studied topic. Only two longer term (one year) and two shorter term (6 months) prospective studies have been performed to investigate the fall rate in the haemodialysis (HD) population. These studies suggested a fall rate of 26-47% in HD patients of different age categories,[20-23] and an increased risk of death in subjects who experienced one or more falls.[23,24]

Because of the high fall rate in the elderly dialysis population compared with the normal population and the associated adverse consequences, it is important to determine potential modifiable risk factors to define preventive strategies. We therefore started a two-centre prospective study to determine the incidence of falls and complications in an elderly HD and peritoneal dialysis (PD) population, and to identify potential modifiable risk factors for falls.

MATERIALS AND METHODS

Study participants

A prospective observational cohort study design was used for this two-centre study. All patients aged ≥ 70 years on

1 January 2011 who were receiving chronic HD or PD therapy were approached to participate in the study. The study was deemed exempt of review by the Institutional Review Board of the hospital, because of the non-interventional study design and no extra burden for the patients to be included.

Baseline assessment

Patient characteristics were collected at baseline using a formal study protocol. Data about medical history, comorbidities, causes of end-stage renal disease, type of dialysis, hours and frequency of dialysis, and medication use were abstracted from electronic chart records at baseline. Data abstraction of comorbidities was structured according to pre-specified categories, based on their known association with fall risk. The same structured method was applied for medication use, using pre-specified categories of medication that are specifically related to dialysis (phosphate-binding medication, vitamin D), or related to a higher risk of falls (all other medication categories). A structured interview with each participant was performed to record living circumstances, self-reported cognitive status (i.e. memory complaints, yes or no), fall risk factors (e.g. problems with keeping balance, yes or no) and functional status. A Barthel index measuring ten basic aspects of self-care and physical dependency was recorded.[25,26] The score ranges from 0-20, with a score of 20 meaning no limitations in activities of daily living (ADL).

Follow-up

During the follow-up of one year, vital parameters (blood pressure, heart rate, weight before and after HD, and ultrafiltration volume) were recorded on a monthly basis for routine clinical evaluation. Haemoglobin, haematocrit, creatinine, albumin, calcium, and phosphate were also recorded every month, and parathyroid hormone and 25-OH vitamin D every three months. These data were abstracted from the electronic records. Participants were monitored for accidental falls using weekly interviews in the HD units by trained dialysis nurses. The PD patients were interviewed weekly by telephone and after three months on a monthly basis. Details of falls were recorded using a pre-specified form including time, circumstances, pre-fall symptoms, (un)consciousness, injuries and any healthcare attention sought.

Definition of a fall and fall characteristics

A fall was defined as an event which resulted in a person coming to rest inadvertently on the ground or another lower level and can, for example, be due to stumbling, loss of balance, or loss of consciousness due to syncope. A fall as a consequence of paralysis as in stroke or an epileptic seizure was not included in the definition. Complications of falls were categorised in no complications, major complication (defined as a fracture), death, and minor complications (all other complications). Falls in HD patients were categorised as occurring on 'a non-HD day, 'a HD day before a HD session', or 'a HD day after a HD session'. The location of falls was categorised as at home, outside home or elsewhere.

Statistical analysis

Demographic data were summarised using the mean and standard deviation (SD) for normal distributed continuous variables, the median and interquartile range (IR) for non-normal distributed continuous variables, and percentages for categorical data. For the analyses of non-baseline measured variables, i.e. laboratory measures and dialysis-related measurements, mean values were calculated of the whole follow-up period (non-fallers), or until the first fall incident in fallers. We performed a sensitivity analysis by exclusively using the last measurement before a fall of the non-baseline measured variables in fallers. Univariate comparisons of baseline characteristics between fallers and non-fallers were made using the independent t-test or Mann-Whitney test for continuous variables. Categorical variables were analysed by univariate comparisons between fallers and non-fallers using the Fisher's exact test.

Fall incidence was determined as the number of falls that occurred during the study divided by person-years of follow-up. Potential risk factors were chosen a priori, based on acknowledged risk factors for falls in the general population, or were selected as potential dialysis-specific risk factors. Potential risk factors were first tested in a standard univariate analysis. To analyse the primary outcome (time to the first fall accident) all potential risk factors reaching a p value ≤ 0.10 were included in a multivariate Cox regression survival model. Potential risk factors were analysed stepwise in the survival analysis: 1. unadjusted; 2. adjusted for age and gender; 3. adjusted for age, gender and the other potential risk factors. Cox regression survival analysis was checked for the proportional hazard assumption. Patients who fell only once were compared with frequent fallers using a t-test or Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. All statistical analyses were performed using SPSS version 20. A p value of less than 0.05 was considered to be significant.

RESULTS

Study population

Forty-nine patients were asked to participate and all of them gave written consent. Baseline characteristics of the participating patients are presented in *table 1* overall, and stratified for fall status during follow-up. Of the 49 mainly Caucasian participants, with a median age of 79.3 years (range 70-89), 42 (86%) patients received HD and seven patients PD. One-third (16) of the patients reported

Table 1. *Baseline characteristics*

	All patients (n = 49)	Fallers (n = 27)	Non-fallers (n = 22)	P[a]
Mean age at start of study (± SD)	79.1 ± 4.4	79.0 ± 4.5	79.3 ± 4.5	0.83
Men (%)	35 (71%)	19 (70%)	16 (73%)	0.56
Type of RRT - Haemodialysis - Peritoneal dialysis	 42 7	 22 5	 20 2	 0.44
Mean duration on RRT in months (SD)	35.7 ± 32.9	38.4 ± 33.4	32.4 ± 32.9	0.73
Living circumstances - Own residency, independent - ADL and/ or IADL help - Nursing home	 19 25 5	 9 (33%) 16 (59%) 2 (7%)	 10 (46%) 9 (41%) 3 (14%)	 0.42
Cause of end-stage renal disease - Vascular - Glomerular - Interstitial - Urological - Unknown	 34 4 7 3 1	 20 (74%) 3 (11%) 3 (11%) - 1 (4%)	 14 (63%) 1 (5%) 4 (18%) 3 (14%) -	 0.22
History of - Diabetes mellitus - Hypertension - Cardiovascular disease - Peripheral vascular disease - Cerebrovascular disease - Polyneuropathy - Visual impairment - Movement disorders - History of depression	 17 30 32 6 10 5 2 10 4	 10 (37%) 17 (63%) 20 (74%) 2 (7%) 5 (19%) 2 (7%) - 5 (19%) 2 (7%)	 7 (32%) 13 (59%) 12 (55%) 4 (18%) 5 (23%) 3 (14%) 2 5 (23%) 2 (9%)	 0.77 1.0 0.23 0.39 0.74 1.0 0.20 0.74 1.0
Barthel ADL score, mean (IR)[b]	19 (2)	19 (2)	19 (2)	0.75
Subjective cognitive deficits (%)	16	9 (33%)	7 (32%)	1.0
Subjective depressive symptoms (%)	7	2 (7%)	5 (23%)	0.22
Subjective visual impairment (%)	9	5 (19%)	4 (18%)	1.0
Nutritional supplements (%)	14	7 (26%)	7 (32%)	0.76
Falls last year	13	8 (30%)	5 (23%)	0.75
Difficulties with - Balance - Walking - Standing up	 22 33 25	 14 (52%) 19 (70%) 14 (52%)	 8 (36%) 14 (64%) 11 (50%)	 0.39 0.76 1.0
Use of walking aid - None - Walking aid[#] - Wheel chair bound	 31 (63%) 15 (31%) 3	 16 (59%) 10 (37%) 1 (4%)	 15 (68%) 5 (23%) 2 (9%)	 0.46
Fear to fall	13	8 (30%)	5 (23%)	0.75
Alcohol consumption - None - 1-4 portions/ month - 1-4 portions/ week - 1-4 portions/ day	 30 3 4 12	 19 (70%) 1 (4%) 2 (7%) 5 (19%)	 11 (50%) 2 (9%) 2 (9%) 7 (32%)	 0.58
Medication use - ACE-i or ARBs - Beta blockers - Calcium channel blockers - Nitrates - Diuretics - Benzodiazepines - Opiates - Phosphate binding medication - Vitamin D - Active vitamin D	 17 28 20 8 21 19 8 38 15 42	 11 (41%) 14 (52%) 10 (37%) 6 (22%) 12 (44%) 9 (33%) 5 (19%) 21 (78%) 6 (22%) 23 (85%)	 6 (27%) 14 (64%) 10 (46%) 2 (9%) 9 (41%) 10 (46%) 3 (14%) 17 (77%) 9 (41%) 19 (86%)	 0.38 0.56 0.57 0.27 1.0 0.56 0.72 1.0 0.22 1.0

RRT = renal replacement therapy; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; ADL= basic activities of daily living; IADL= instrumental activities of daily living; # walking aid includes holding onto a subject for support; [a] P values calculated by independent samples T-test, Mann-Whitney test, Fisher's exact or chi-square to compare fallers vs. non-fallers; [b] median value and interquartile range (IR) are given because of skewed distribution.

memory defects. More than half of the patients received help with ADL or instrumental ADL activities. After two months, one patient crossed over from PD to HD, and after 11 months a patient crossed over from HD to PD. In the analysis of blood pressure, heart rate, and ultrafiltration volume, both patients who crossed over were included in the HD group because they received HD most of the study year.

Follow-up of the study population

Four (8%) patients died (mean follow-up 6.8 months), and 12 (25%) patients were admitted at least once to the hospital for mainly acute care. Three patients (6%) moved from their residence to a care facility or nursing home.

Fall incidence

During the one-year follow-up period, 27 of 49 patients (55%) fell at least once. Of these fallers, 11 (41%) patients had multiple falls (range 2-3). Overall the fall incidence was 40 falls in 49 patients with 47.2 person-years of follow-up, an average of 0.85 falls/person-years of follow-up. In fallers, the fall incidence was 40 falls in 27 patients with 26.4 person-years of follow-up, an average of 1.51 falls/persons-year follow-up.

Fall characteristics

Twenty-three falls occurred at home, 14 outdoors, two in the dialysis centre and one in a nursing home. In HD patients, most falls (50%) occurred on non-HD days, nine (41%) on a HD day after dialysis and only two falls (9%) occurred on a HD day before dialysis.

Fall-related injuries and consequences

Twenty-one of the 40 (53%) falls were complicated by minor complications (e.g. wounds, bruises or contusions). Six falls (15%) were complicated by fractures (three hip fractures, two ankle fractures, and one wrist fracture). Among fallers, there was a trend that patients who experienced a fracture were longer on dialysis treatment (67.4 months vs. 31.8 months, p = 0.06). No significant differences in complications between HD and PD patients were found. Six of the 40 falls (15%) led to a visit by a general practitioner, 11 (28%) falls led to a hospital visit of which six patients were admitted to the hospital, and one patient sought medical attention in the dialysis unit; so the total number of falls that required medical help was 18 (45%).

Risk factors for falling

Data of medical history, functional status, medication use and vital and laboratory parameters were compared for fallers vs. non-fallers by univariate analysis. A significant difference was found between fallers and non-fallers for mean systolic blood pressure (SBP) before dialysis in HD patients ($p < 0.05$) (*table 2*). Medication use at baseline was compared between fallers and non-fallers as well as the total number of medications, but no significant differences were found. Five medication categories were left out of the analysis, because the number of users was < 5, namely antidepressants, anticonvulsants, digoxin, alpha blockers, and antiarrhythmic medication. A tendency towards a difference in mean parathyroid hormone (PTH) was found; however, this was not significant (p = 0.07). The fall in mean SBP after HD when compared with pre-HD was not significantly different in fallers compared with non-fallers (7 ±16 mmHg vs. 3 ±11 mmHg, p = 0.68). Using Cox regression survival analysis, in the final fully adjusted model every 5 mmHg lower mean SBP before HD increased the risk of falling by 30% (HR 1.30, CI 1.03-1.65, p = 0.03). For every 10 pmol/l higher mean PTH the risk of falling increased by 22% (HR 1.22, CI 1.06 - 1.39, p = 0.004) (*table 3*). In the sensitivity analysis, by using the last measurement before the first fall incident in fallers, the multivariate Cox regression model yielded the same results (SBP before HD: HR 1.17, CI 1.03-1.33, p = 0.02; SBP after HD: HR 0.98, CI 0.88-1.08, p = 0.63; PTH: HR 1.20, CI 1.04-1.38, p = 0.01).

Frequent fallers

Eleven patients (23%) fell more than once. Compared with patients who fell only once, these frequent fallers less often used alfacalcidol (64% vs 100%, p = 0.02) and had a higher median PTH (39.4 pmol/l-IR 60.5 pmol, vs. 22.9 pmol/l-IR 19.8 pmol/l, p = 0.03).

DISCUSSION

The main finding of this observational study among chronic HD and PD patients of ≥ 70 years is that 55% of the patients experienced at least one fall during one year of follow-up. A large number of patients, 41%, had two or more falls. Fifteen percent of the falls were complicated by a fracture. A lower SBP before dialysis (in HD patients) and a higher PTH were identified as risk factors for falling. For every 5 mmHg lower SBP before dialysis the risk of falling increased by 30% and for every 10 pmol/l higher PTH, the risk of falling increased by 22%.

The fall rate of 55% in our population with a mean age of 79 years is high, compared with the one-year fall rate of 32-41% in the community-dwelling elderly aged ≥ 80 years.[9,11,27,28] The fall incidence in our study is more similar to numbers observed in nursing home residents of 50%.[29] In a chronic HD population, Cook *et al.* found a fall rate of 47% over a median of 468 days in patients with a mean age of 74.4 years.[20] Three other prospective studies have been performed among HD patients. First, Roberts *et al.* found that 38% of the 32 patients aged ≥ 65 years fell during six months of follow-up.[21] Second, Desmet

Table 2. *Vital and laboratory measurements during follow-up*

	HD patients (n = 43)[a]	HD - Fallers (n = 23)	HD - Non-fallers (n = 20)	P[b]
Mean SBP before HD (mmHg) (±SD)	136 ± 22	130 ± 19	143 ± 22	0.04
Mean DBP before HD	70 ± 10	68 ± 11	71 ± 10	0.46
Mean SBP after HD	131± 19	127 ± 18	136 ± 19	0.10
Mean DBP after HD	66 ± 12	65 ± 13	67 ± 10	0.61
Mean HR before HD	74 ± 8	75 ± 8	74 ± 9	0.32
Mean HR after HD	76 ± 12	79 ± 13	73 ± 11	0.16
Mean ultrafiltration volume (ml)	1792 ± 582	1927 ± 635	1650 ± 496	0.12
	All patients	**Fallers**	**Non-fallers**	
Laboratory results (mean)				
- Haemoglobin (mmol/l) (±SD)	7.1 ± 0.5	7.1 ± 0.5	7.1 ± 0.4	0.75
- Haematocrit (%)	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.02	0.87
- Calcium (mmol/l)	2.32 ± 0.19	2.31 ± 0.24	2.33 ± 0.12	0.75
- Phosphate (mmol/l)	1.66 ± 0.26	1.61 ± 0.29	1.71 ± 0.23	0.21
- Creatinine (µmol/l)	820 ± 223	834 ± 222	802 ± 228	0.62
- Parathyroid hormone (pmol/l)[c]	24.7 (22)	31.2 (28.9)	20.9 (24.2)	0.07
- Albumin (g/l)	33.5 ± 2.9	33.3 ± 2.5	33.7 ± 3.5	0.59
- 25-OH Vitamin D (nmol/l)	78 ± 27	75 ± 30	81 ± 23	0.45

SBP = systolic blood pressure; DBP = diastolic blood pressure; HD = haemodialysis; HR = heart rate; [a] Total of 43 HD patients (1 patient crossed from PD to HD); [b] P values calculated by independent samples T-test or Mann-Whitney test to compare fallers vs. non-fallers; [c] median value and interquartile range are given because of skewed distribution.

Table 3. *Cox regression analyses*

	Model 1 HR (95% CI)	p	Model 2 HR (95% CI)	p	Model 3 HR (95% CI)	p
Mean SBP before dialysis (*per 5 mmHg decrease*)	1.14 (1.01-1.27)	0.03	1.16 (1.02-1.31)	0.02	1.30 (1.03-1.65)	0.03
Mean SBP after dialysis (*per 5 mmHg decrease*)	1.08 (0.98-1.20)	0.14	1.09 (0.98-1.22)	0.12	0.88 (0.72-1.09)	0.24
Mean PTH (*per 10 pmol/l increase*)	1.16 (1.03-1.30)	0.02	1.16 (1.03-1.31)	0.02	1.22 (1.06-1.39)	0.004

SBP = systolic blood pressure; PTH = parathyroid hormone; Model 1 = crude; Model 2 = adjusted for age and gender; Model 3 = adjusted for age, gender and the other potential risk factors (SBP before dialysis, SBP after dialysis, PTH).

et al. found a fall rate of 34% in older HD patients (median age 72.4 years) of the validation unit during six months of follow-up.[22] Third, Abdel-Rahman *et al.* found a fall rate of 38% in 34 older dialysis patients (mean age 74 years) during one year of follow-up.[23] Most likely, the fall rates in Cook's and our study are more representative of the actual fall risk for elderly dialysis patients. The highest fall rate in our study compared with the aforementioned studies is most likely due to the older age of the participants in our study (mean 79 years).

The fracture rate in our study (15%) is high compared with that in community-dwelling elderly people, among which only 4-6% of falls result in fractures.[30] Previously, a fourfold higher incidence of hip fracture among Caucasian patients with end-stage renal disease (ESRD) than would be expected in the general population was reported by Alem *et al.*[31] These authors found an increase in incidence the longer patients were on dialysis, suggesting that there are cumulative exposures since the initiation of renal replacement therapy that predispose patients to a hip fracture. Our results seem to point in the same direction, because we found a trend towards a higher dialysis vintage in patients who experienced a fracture after a fall compared with fallers with minor or no complications. One of the reasons for the high fracture rate might be the decrease in bone mineral density and presence of mineral bone disease among dialysis patients.[32]

An important goal of our study was to identify potential modifiable risk factors of falls. In the HD patients we found that a lower SBP before dialysis was associated with a higher risk of falls. The relation between predialysis SBP and fall risk was also found by Cook *et al.*[20] A relatively low SBP might in itself be a risk factor for falls, but could also be a sign of a worse condition and prognosis in HD patients.[33,34] When the association is causal, SBP might be an easy risk factor to modify when patients are using

BP-lowering medication. Second, review of medication with adaptation of the use of antihypertensive agents, especially in patients with a low blood pressure or patients who have already experienced a fall, might decrease the fall rate in elderly dialysis patients. Of note, there is no literature on a 'safe' systolic blood pressure in elderly ESRD patients regarding fall risk. Studies are therefore needed to elucidate what an optimal systolic blood pressure range would be in elderly dialysis patients.

In our study, among fallers with a mean SBP before HD of ≤ 130 mmHg (n = 10), the mean SBP before HD in fallers, seven of them still used BP-lowering medication. This suggests that medication use in geriatric dialysis patients can be optimised to lower their risk of falls. A second potentially modifiable risk factor is PTH, because we found that a higher PTH is also associated with a risk of falls. Muscle weakness and other neuromuscular symptoms may be present in patients with hyperparathyroidism,[35] and muscle strength and functional capacity have been shown to improve after parathyroidectomy even in 'asymptomatic patients'.[36,37] Third, a higher PTH concentration increased the risk of sarcopenia in the Longitudinal Aging Study Amsterdam.[38] Lowering PTH may therefore be a second treatment goal to lower fall incidence in elderly dialysis patients.

It is remarkable that no differences were found regarding medication use in fallers vs. non-fallers. We expected the use of psychoactive medication to be a risk factor, as is found in other studies.[9,39,40] An explanation might be that we only have baseline medication prescriptions for patients and the use of psychoactive medication might have been changed during the one-year follow-up.

The strengths of this study include the detailed information that was available regarding falls, medication use and complications. Second, in contrast to previous studies on this topic, we used Cox regression analysis to study potential risk factors for falling.[20-23] Because 'time to event' is an important factor when analysing fall risk, Cox regression analysis is to be preferred over logistic regression analysis, since this latter method does not take time-to-event into account. Third, we asked patients about falls frequently, because elderly subjects were often unable to recall falls over a longer period.[41] Fourth, this is the first study of fall incidents in a dialysis population of exclusively elderly patients.

Among the limitations of this study is the relatively small cohort size. However, even in this small cohort we found SBP and PTH as independent associated risk factors. Another limitation concerns self-reported functional status, mobility and cognition. Especially cognitive function impairment might be under-reported, because cognitive impairment often remains unrecognised in elderly dialysis patients.[42] A weakness in our analysis might be multiple testing. However, we studied pre-specified variables that are associated with fall risk in other populations, or dialysis-specific factors that might theoretically increase the risk of falls. Furthermore, only variables reaching a p value ≤ 0.10 were included in the multivariate model. Fourth, only variables collected during follow-up were significant predictors of fall risk. These measurements might be more accurate, as their mean over time was used, instead of the baseline variables that were collected only once. Because of the intrinsic variability in variables, this may result in impaired power to detect significant associations. However, repeated interviews with all of our patients during follow-up were not feasible. In addition, when we performed a sensitivity analysis using only single measurements immediately before a fall incident, similar results were obtained. This suggests that follow-up variables do indeed have more predictive value than baseline variables.

The high fall rate and high fracture rate after a fall that we found may have implications for the medical care of elderly dialysis patients. The elderly dialysis population already experiences a decline in functional status after the start of dialysis and falls increase the risk for further functional decline.[17] This makes prevention of falls and complications of falls desirable. Several single and multifactorial, healthcare-based strategies have proved to be effective in reducing the fall rate in clinical trials.[43-45] In the dialysis population only one study has been performed on fall prevention in an outpatient dialysis centre.[46] Heung *et al.* found that staff educational deficits and environmental hazards were the most significant risk factors for fall incidents. Through a targeted series of interventions, a marked reduction in fall risk was achieved.

In conclusion, elderly dialysis patients have a high fall incidence accompanied by a high fracture rate. Given the high complication rate, elderly patients at risk of falling should be identified and managed. A lower SBP before HD and a higher PTH were found to be associated risk factors for falls. Reduction in use of blood pressure-lowering medication might be a treatment strategy to reduce falls.

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DISCLOSURES

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ORIGINAL ARTICLE

High prevalence of late adverse events in malignant bone tumour survivors diagnosed at adult age

A.C.M. van de Luijtgaarden[1]*, L. Kapusta[2,6], L. Bellersen[3], J.P.M. Bokkerink[4], S.E.J. Kaal[1], Y.M.H. Versleijen-Jonkers[1], H.W.B. Schreuder[5], W.T.A. van der Graaf[1]

Departments of [1]Medical Oncology, [2]Children's Heart Centre, [3]Cardiology, [4]Paediatrics, [5]Orthopaedic Surgery, Radboud University Medical Centre, Nijmegen, the Netherlands, [6]Department of Pediatric Cardiology ,Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel, *corresponding author: tel.: +31(0)24-3610345, fax: +31(0)24-3540788, email: Addy.vandeLuijtgaarden@radboudumc.nl

ABSTRACT

Background: Late treatment-related adverse events are particularly prevalent in survivors of childhood bone cancer because of the combination of cytotoxic drugs, major surgery and radiotherapy. Existing studies for late toxicity in survivors of Ewing's sarcoma (ES) and osteosarcoma (OS) diagnosed at adult age have focused on specific sequelae. We investigated a broad spectrum of potential late effects in these patients.

Methods: Relapse-free OS and ES patients aged ≥ 16 at diagnosis and treated at the Radboud University Medical Centre (1982-2007) were invited for systematic late toxicity screening. This included history taking, physical examination, echocardiogram, bone densitometry, audiogram, and serum and urine screening for renal toxicity and infertility. Adverse events were graded according to the Common Terminology Criteria for Adverse Events version 3.0.

Results: In 24 survivors (63% male, mean age at screening 45.7 years, mean follow-up 10.9 years, 70% OS) we found a median of eight adverse events. Frequent findings included abnormal gait, osteoporosis, pain, left ventricular systolic dysfunction, obesity and nephropathy. The maximum grade of any adverse event was mild in four (17%), moderate in 11 (46%), severe in six (25%), and disabling in three cases (13%). There was a trend towards more events in patients diagnosed at an older age.

Conclusion: The incidence of late adverse events in this study of survivors of bone tumours diagnosed at adult age is higher than in any previously published childhood cancer survivorship study. Older patients seem to be particularly at risk. Our findings underscore the need for systematic screening of late effects in bone cancer survivors of adult age at diagnosis.

KEYWORDS

Bone neoplasms, drug therapy, osteosarcoma, sarcoma, Ewing's, survivors

INTRODUCTION

Bone sarcomas are rare tumours with an annual incidence of 1/100,000. Osteosarcoma (OS) and Ewing's sarcoma (ES) are well-known subtypes requiring intensive therapy. OS has a bimodal age distribution with its first peak incidence in the second and third decade of life and a smaller peak in the seventh decade; ES mainly occurs in the second decade.[1] Before the use of chemotherapy, survival was limited to only about 20% because of an almost universal development of metastases. The current treatment strategy consists of multi-agent chemotherapy and surgery. Radiotherapy may also be applied in ES. This approach yields a dramatically improved cure rate of 50-70% in patients with localised disease. Patients with metastatic disease have a poorer prognosis, but cure is still possible.[2] With such cure rates the risk of development of late therapy-related adverse events becomes relevant. While most childhood cancer survivor studies have focused on a single late adverse event, three retrospective cohort studies evaluating 14,372 (Childhood Cancer Survivor Study, CCSS),[3] 1284 (Polikliniek Late Effecten Kindertumoren, PLEK)[4] and 519 (Children's Healthcare of Atlanta, CHOA)[5] survivors of any childhood malignancy have evaluated the overall health status of study participants. In all studies, the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0, CTCAE was used to grade cancer treatment-related adverse event severity as 1 (mild), 2 (moderate), 3 (severe), 4 (disabling or life-threatening) or 5 (event-related

death).[6] All three studies conclude that late treatment-related complications are highly prevalent amongst childhood cancer survivors (*table 1*). CCSS and PLEK further show that survivors of bone tumours are especially at risk; CHOA reports similar findings for sarcoma in general. Based on these papers, lifelong medical surveillance of childhood cancer survivors is now common practice for paediatricians. Because of the specific age distribution, an important number of bone sarcoma patients are, however, cared for by medical oncologists for whom long-term follow-up for treatment sequelae of patients with bone cancer is not part of the routine. It is as yet unknown whether survivors of bone cancer diagnosed in adolescence or adulthood suffer from a comparable percentage and kind of adverse events. In this regard, though all subjects were under 20 years of age, those who were older at the time of diagnosis in the CCSS study were more likely to report any condition, conditions of grades 3-5, or multiple conditions ($p < 0.001$). In the CHOA cohort, older patients (≥ 10 years at diagnosis) were also at risk for conditions of grades 3-5 ($p < 0.01$).

We initiated the present study, aiming to characterise the point prevalence and severity of late adverse events in a cohort of relapse-free survivors of OS or ES diagnosed in adulthood. In addition, we sought to identify subpopulations at the highest risk for chronic health conditions.

PATIENTS AND METHODS

We identified all patients who had been treated for OS or ES at the Radboud University Medical Centre between 1982-2007. Patients needed to have been at least 16 years or older at diagnosis, treated at the Department of Orthopaedic Surgery, Medical Oncology (EORTC 80861: four patients; EORTC 80931: one patient; CESS86: one patient; EuroEwing99: two patients, Off-protocol cisplatin/doxorubicin: nine patients; the remainder of patients treated off-protocol individually) and/or radiotherapy, and currently alive and relapse free in order to be eligible for systematic screening for late toxicity. Details about cancer diagnosis and therapy were extracted from the patient file. The current health status and address were checked with the patient's general practitioner if more than one year had elapsed since the last visit to our hospital. The study was approved by the institutional review board and written informed consent was obtained from all participants. Screening was performed at the Medical Oncology outpatient department and consisted of physical examination and history taking, including questions on psychosocial events hampering daily life (employment, mortgage loan, life and health insurances) by a dedicated physician (AvdL), cardiac evaluation using left ventricle ejection fraction measurement with echocardiography; bone density assessment by dual energy X-ray absorptiometry (DXA; whenever applicable, femur neck bone density was measured on the unaffected side) and a pure tone audiogram to evaluate ototoxicity. Serum and urine screening were performed for renal toxicity (glomerular filtration rate, fractional calcium, magnesium and potassium excretion, urinary α1-microglobulin excretion and tubular threshold for phosphate (TmP/GFR)), fertility state (sex hormone levels and inhibin B) and metabolic syndrome trait (lipid profile, fasting glucose).

Adverse events including cardiotoxicity were graded according to the CTCAE version 3.0 unless otherwise specified.[6] Metabolic syndrome trait was defined according to the International Diabetes Federation consensus worldwide definition and graded in the Syndrome – other category as moderate,[7] Tubulopathy was defined as presence of decreased tubular phosphate reabsorption TmP/GFR < 0.80 mmol/l and increased urinary a1microglobulin excretion > 13 mg/g creatinine and graded in the Metabolic/Laboratory – Other category as mild when serum electrolyte levels were normal, moderate if electrolyte suppletion was necessary and severe if hospitalisation was needed. Male infertility was graded as mild when no sperm count was available but the serum follicle-stimulating hormone was > 10 U/l and inhibin B below the lower limit of normal,[8] otherwise the CTCAE grading was used. The prevalence of late events was calculated and treatment-related risk factors were interrogated with logistic regression analysis. Odds ratios were converted into relative risks according to the method of Zhang and Yu.[9] All analyses were performed using SPSS version 16.0 and a $p < 0.05$ was considered significant.

Table 1. *Summary of number and severity of adverse events in screening studies*

	PLEK	CCSS	CHOA	Current study
Number of adverse events per subject				
None	19.8%	37.4%	12.1%	0%
1	74.5%	62.3%	87.9%	0%
> 1	59%	37.6%	70.9%	0%
≥ 3	44.7%	23.8%	50.3%	100%
Maximum severity of adverse event				
Grade ≥ 3	36.8%	27.5%	36.4%	38%

CCSS = Childhood Cancer Survivor Study[3]; PLEK = Polikliniek Late Effecten Kindertumoren[4]; CHOA = Children's Healthcare of Atlanta[5].

RESULTS

Based on review of the patient files, 32 survivors met our eligibility criteria. Of note, of the patients who did not survive, two died because of severe cardiomyopathy.

Of the survivors five patients could not be contacted. Three patients refused to participate. Therefore, 24 of 27 contacted patients were included in our study. Characteristics of the participants are presented in *table 2*. Patients were aged 34.4 years at diagnosis (mean, range 16-63 years).

Adverse events

We scored the point prevalence of adverse events at the time of the screening visit. There were no patients without adverse events (*table 1*). The median number of events per patient was eight (range 3-12, mean 7.8). A wide range of adverse events was seen (*figure 1*). The most frequently observed events (≥ 50% of the cohort) included musculoskeletal-related symptoms, i.e. abnormal gait, abnormal joint function and pain. Osteoporosis, obesity and tubular nephropathy also affected ≥ 50% of patients. In 29% of patients, potentially treatable occult cardiomyopathy was found. The maximum severity of reported adverse events was as follows: mild in four patients (17%), moderate in 11 (46%), severe in six (25%) and disabling in three (13%). Severe or disabling late therapy-related effects (i.e. 3 grade 3) included impaired joint function, abnormal gait, obesity and low serum phosphate.

Table 2. *Demographic and treatment data*

Patient characteristics		
N		24
Sex	Male	15 (63%)
	Female	9 (38%)
Diagnosis	Osteosarcoma	17 (70%)
	Ewing's sarcoma	7 (30%)
Age at diagnosis *mean (range)*		34.4 years (16-63)
Follow-up *Mean, median (range)*		10.9 years, 6.2 years (2-27)
Age at screening *mean (range)*		45.7 years (19-66)
Treatment	Surgery only	13%
	Chemotherapy and surgery	75%
	Chemotherapy and radiotherapy	4%
	Chemotherapy, surgery and radiotherapy	8%
Chemotherapy	Anthracyclines ± other chemotherapy	46%
	Anthracyclines and alkylating agents ± other chemotherapy	42%
Cumulative anthracycline dose *median (range)*		330 mg/m² (0-518, SD 164)

Psychosocial events

Several patients reported psychosocial problems that could be attributed to cancer survival; three had difficulties obtaining a mortgage loan, three reported problems with life insurance, two had increased health insurance contributions and four were unwillingly unemployed. Because grading criteria for these events are lacking in the CTCAE criteria, these events are not included in the following analyses.

Risk factors for adverse events

Overall burden of events

Patients who were older at the time of diagnosis (OR 1.258 (95% confidence interval 1.045-1.515) RR 1.04, p = 0.016) or at follow-up (OR 1.187 (1.045-1.349), RR 1.052, p = 0.008 (with age as a continuous variable) were more likely to report more than eight adverse events. In an adjusted model combining both age parameters, age at follow-up was no longer associated with the number of reported adverse events (p = 0.952) while age at diagnosis retained a trend to significance (p = 0.083). Age was not related to the reported severity of any adverse event. Neither follow-up duration nor sex were associated with the reported number of adverse events or the maximum reported severity of any event (*table 3*).

Specific adverse events and known risk factors

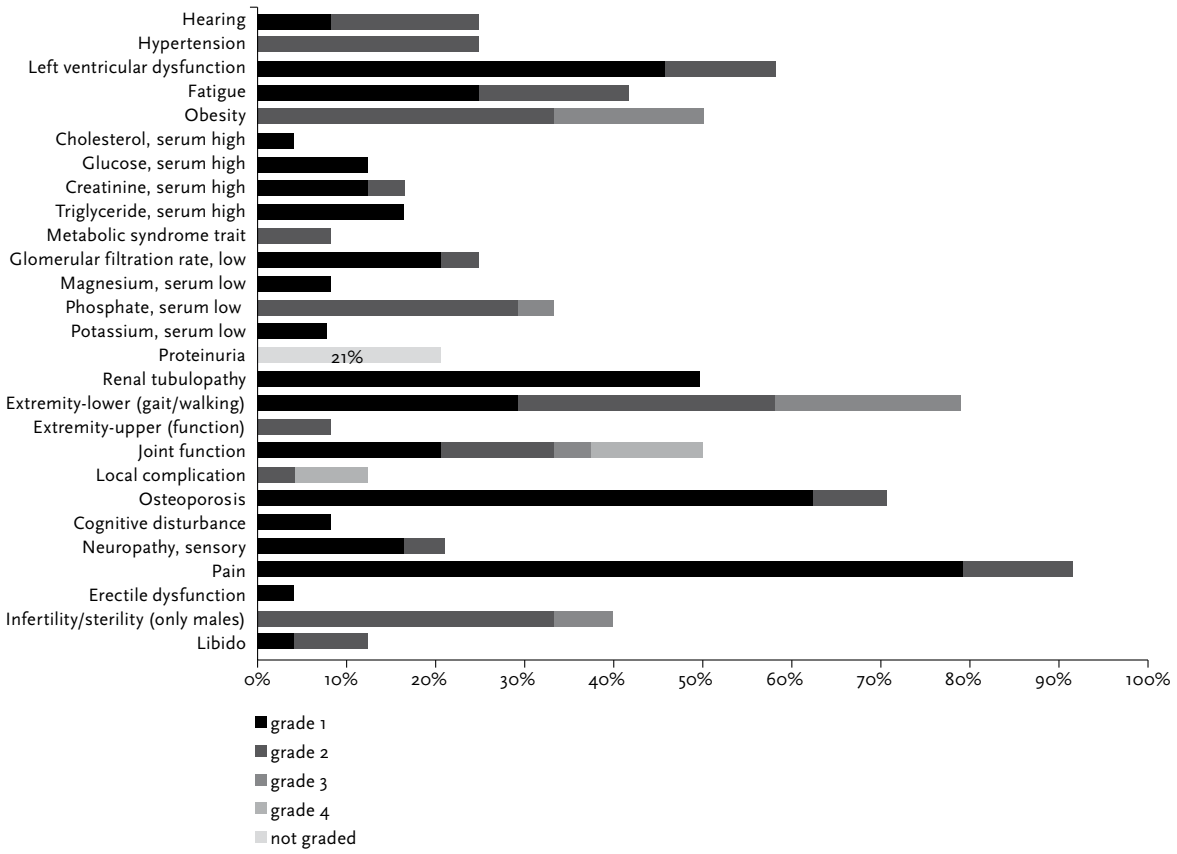
Cardiomyopathy, defined as a decreased left ventricle ejection fraction assessed by echocardiography, was more common in patients who had received a minimal dose of 350 mg/m² of doxorubicin (OR 6 (1.018-35.37), RR 2.25, p = 0.037).

We were unable to confirm other previously reported associations between specific adverse events and exposure to certain drugs, e.g. neurotoxicity and cisplatin, ifosfamide or vincristine and nephrotoxicity and cisplatin, ifosfamide or methotrexate (*table 4*).

DISCUSSION

The need for lifetime follow-up of childhood cancer survivors to timely detect and treat late adverse events is now widely recognised. Childhood cancer survivor studies show that bone cancer patients are particularly at risk for adverse events. Although bone tumours also affect adults, follow-up studies in those survivors are scarce and the available studies have focused on specific sequelae only. While our study is hampered by its retrospective inclusion and small sample size, it is the first one to assess the whole

Figure 1. *Prevalence and grade of adverse events*



Events defined and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, CTCAE;[6] Metabolic syndrome trait was defined according to the International Diabetes Federation consensus worldwide definition.[7] Tubulopathy was defined as presence of decreased tubular phosphate reabsorption TmP/GFR and increased urinary α1-microglobulin excretion.

spectrum of late adverse events in a systematic manner and with a standardised grading system in this age group. We add to the paucity of information by demonstrating that in a cohort of survivors of OS or ES diagnosed in adulthood, the point prevalence and severity of a wide variety of late adverse events is high. In fact, there were no patients without adverse events and the number of adverse events per patient was higher than in any of the previously published large childhood cancer survivorship studies (*table 1*). Of note, since 24 of 32 eligible subjects participated (two had died from cardiotoxicity, five could not be contacted and three refused to participate), toxicity in our cohort may be subject to participation bias.

In agreement with two of these childhood survivorship studies, CCSS and CHOA,[3,5] we also observed a trend that older patients are more likely to experience a multitude of late treatment sequelae. Because all but one patient had undergone surgery such as prosthetic joint replacement, arthrodesis or even limb amputation, musculoskeletal symptoms in survivors were to be expected.[10] Indeed, the most frequently observed events in our cohort included abnormal gait (79%), abnormal joint function (50%) and pain (63%). Another striking finding of our study was the exceptionally high percentage of osteopenia (63%) with a moderate prevalence of osteoporosis (8%). In survivors of osteosarcoma, osteoporosis has previously been reported in 20.8-47.5% and osteopenia in 30-43.7%;[11,12] it is notable that studies reporting high prevalence measured bone density in affected-limb femur necks. Also in heterogeneous groups of bone sarcoma and bone and soft tissue sarcoma patients the median bone density is below normal.[13,14] Various underlying mechanisms could clarify this finding: in younger patients, renal loss of minerals, nutritional deficits, physical inactivity and irregular menstrual cycles during therapy may prevent reaching a normal peak bone mass.[12] Increased loss of bone mass may be due to immobilisation or premature ovarian failure.[11,15] However, we failed to demonstrate a correlation between osteoporosis and reported difficulty in walking as a surrogate marker for immobility. No data regarding menstrual cycles, food intake or renal mineral excretion during therapy were available and the prevalence of premature ovarian failure

is undetermined in our cohort. Hence, our findings remain partially unexplained and deserve further research.

We detected renal tubulopathy, defined as a decreased tubular phosphate threshold (TmP/GFR) and increased urinary a1-microglobulin excretion in 50% of our patients. A decreased GFR was found in 25%. Other frequently found renal toxicity markers included low serum phosphate (33%) and proteinuria (21%). These findings are in line with previously reported data.[16,17] Three key chemotherapeutic agents in bone sarcoma, cisplatin, ifosfamide and methotrexate, have all been linked to renal toxicity.[16-18] Cisplatin probably potentiates ifosfamide-induced damage[19] and in itself may cause a reduction in the GFR and hypomagnesaemia.[18,20] Methotrexate has mainly tubulotoxic effects.[21] Sample size probably prevented us from confirming the aforementioned risk factors known in the literature.

Cardiotoxicity is a well-known, often dose-related adverse side effect of anthracyclines.[23] The pathogenic mechanism is not fully understood but may include anthracycline-mediated production of free oxygen radicals, cardiomyocyte apoptosis, inhibited expression of cardiomyocyte-specific genes, calcium overload caused by activation of the calcium release channel, a direct toxic effect on the adrenergic function of the myocardium and altered molecular signalling.[24,25] Cardiomyopathy, regardless of its cause, is treated with afterload reduction by angiotensin-converting enzyme (ACE) inhibition or β-blockers.[26] In breast cancer patients, ACE inhibition established a potent and long-lasting recovery and a combination of ACEi and beta-blockers achieved normalisation of left ventricle function.[27,28] It is therefore important to detect cardiomyopathy in a timely manner. Reported frequencies of subclinical cardiotoxicity after sarcoma treatment vary between 27-100% and overt heart failure is reported in 1.5-2.2% of patients.[23,29-33] Female and older patients are particularly at risk.[25,27,33,34] It has been suggested that cisplatin is also cardiotoxic and exposure to cisplatin has mainly been associated with the development of an unfavourable cardiovascular risk profile.[35-37] In our cohort, occult cardiomyopathy was found in 29% of patients while no patients suffered from overt heart failure, but it should be noted that two patients died from late diagnosed cardiomyopathy

Table 3. *Risk factors for the overall burden of adverse events*

	Number of events ≥ 8	CTCAE ≥ 2	CTCAE ≥ 3
Age at follow-up	OR 1.187 (1.045-1.349) RR 1.052 p = 0.008	p = 0.194	p = 0.538
Age at diagnosis	OR 1.258 (1.045-1.515) RR 1.04 p = 0.016	p = 0.56	p = 0.712
Follow-up duration	p = 0.146	p = 0.144	p = 0.721
Sex (female vs. male)	p = 0.831	p = 0.999	p = 0.165

OR = odds ratio (95% confidence interval); RR = relative risk; CTCAE = Common Toxicity Criteria for Adverse Events version 3.0.

Table 4. *Possible risk factors for specific adverse events*

	DOX	DOX ≥350 mg	Sex	CDDP	IFO	MTX	VCR	ALK/ DOX	Walking
Cardiomyopathy	p = 0.058	p = 0.037 OR 6 (1.018-35.37) RR 2.25	p = 0.916	p = 0.064					
Metabolic syndrome				p = 0.873					
Proteinuria				p = 0.556	p = 0.878	p = 0.112			
Tubulopathy				p = 0.921	p = 0.529	p = 0.165			
GFR				P = 0.213	p = 0.998	p = 0.998			
Magnesium				p = 0.873					
Hearing				p = 0.157					
Neuropathy				p = 0.966	p = 0.998		p = 0.997		
Male infertility								p = 0.132	
Osteoporosis									p = 0.829

Rows = late adverse event; columns = possible risk factor; cells = p value of logistic-regression analysis; OR = odds ratio (95% confidence interval); RR = relative risk; empty cells = not tested; DOX = doxorubicin; CDDP = cisplatin; IFO = ifosfamide; MTX = methotrexate, VCR = vincristine; ALK/DOX = combination of alkylating agents (i.e. cyclophosphamide and/or ifosfamide) and doxorubicin.

before the present study. We confirmed the well-known dose-dependency of anthracycline toxicity but could not confirm the significance of age and sex as risk factors for anthracycline-induced cardiotoxicity known from the existing literature. Furthermore, although obesity and hypertension were overrepresented in our cohort compared with the normal Dutch population, metabolic syndrome trait was not more prevalent in cisplatin-treated survivors, again probably due to our limited sample size.

Ototoxicity, which we found in 25% of patients, has been linked to platinum compounds, cisplatin in particular.[38,39] Peripheral neurotoxicity may be caused by ifosfamide, cisplatin (both peripheral neuropathy) and vincristine (polyneuropathy).[40,41] Of all men in our cohort, 23% were subfertile as assessed by serum tests. There is a large body of evidence that both pre- and post-pubertal testes are susceptible to cytotoxic treatment by alkylating agents.[8] Sequential alkylating agent and anthracycline-based treatment regimens carry a significant risk of infertility.[36] The combination of alkylating agents and anthracyclines did not significantly influence the risk of infertility. Because many female patients who were not postmenopausal at diagnosis were using oral contraceptives at the time of the screening visit, a reliable estimation of the incidence of female infertility in our cohort is not possible.

Though socioeconomic consequences of ES[42] and OS[43] survivorship were not qualitatively assessed in our cohort, in contrast to recent studies in long-term OS and ES childhood cancer survivors, these adverse events were also present in many of our survivors. The psychosocial consequences need to be addressed in more detail in future studies.

In conclusion, the prevalence and severity of late adverse events in survivors of bone tumours diagnosed at adult age is strikingly high and the spectrum of events remarkably broad. There is a trend towards more adverse events with increasing age at diagnosis. The most prominent adverse events included occult cardiomyopathy, osteoporosis and nephropathy, for which interventions are available and indicated, and adverse events directly related to prior orthopaedic interventions. Our findings thus underscore the need for international guidelines for the follow-up of bone cancer survivors diagnosed at adulthood, analogous to paediatric follow-up studies and recent adolescent and young adult general follow-up guidelines.[44]

DISCLOSURES

The authors declare that they have no conflict of interest. A. van de Luijtgaarden is supported by a Dr. Paul A.J. Speth Foundation research grant.

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ORIGINAL ARTICLE

A prospective study of concomitant GLP-1 analogue and insulin use in type 2 diabetes in clinical practice

E.F.S. van Velsen*, J. Lamers, V. Blok, R.J.M. van Leendert, R.M. Kiewiet-Kemper

Department of Internal Medicine and Diabetes Center, Albert Schweitzer Hospital, Dordrecht, the Netherlands, *corresponding author: tel.: +31(0)78-6541111, fax: +31(0)78-6545338, email: e.f.s.van.velsen@asz.nl

ABSTRACT

Background: A small number of studies have shown a significant reduction in HbA1c, weight and total daily insulin dose when a glucagon-like-peptide-1 (GLP-1) analogue was added in type 2 diabetes patients already on insulin treatment. Therefore, in a clinical setting, we investigated the effect of adding GLP-1 analogues in patients with type 2 diabetes already using insulin with respect to glycaemic control, body weight and insulin dose.
Methods: In this prospective hospital-based study, we included 125 patients suffering from type 2 diabetes, treated with insulin and with a body mass index ≥ 35 kg/m^2, who had started on GLP-1 analogues (liraglutide/exenatide). HbA1c, body weight, daily insulin dose, and side effects were registered at baseline, and after three, six and 12 months.
Results: HbA1c and weight decreased significantly at all the timepoints ($p \leq 0.001$ compared with baseline; HbA1c: -5.5 mmol/mol (-0.5%) and weight: -14.3 kg after 12 months), with the largest decrease in the first three months. No significant correlation was found between weight loss and HbA1c reduction, and between duration of diabetes and both weight loss and HbA1c reduction. After six and 12 months, the total daily insulin dose decreased significantly ($p < 0.001$, -75.4 IU after 12 months). Moreover, 34% of the patients were able to stop using insulin therapy after 12 months.
Conclusion: By adding a GLP-1 analogue in obese patients with type 2 diabetes already on insulin therapy, a significant reduction of HbA1c levels and body weight, and a significant reduction in insulin dose or complete discontinuation of insulin can be achieved.

KEYWORDS

Diabetes mellitus (type 2), glucagon-like peptide 1, insulin, obesity

INTRODUCTION

Glucagon-like-peptide-1 (GLP-1) analogues are a relatively new category of glucose-lowering drugs for the treatment of type 2 diabetes. These drugs have broad glucoregulatory actions, including stimulation of endogenous insulin production and secretion, and suppression of glucagon secretion, both depending on blood glucose level. Additionally, they have an effect on the brain, enhancing satiety, and on the gastrointestinal tract, delaying gastric emptying.[1]
Clinical studies showed that GLP-1 analogues are effective in improving glycaemic control in patients with suboptimal control on one or two types of oral glucose-lowering drugs.[2] Furthermore, reduction of body weight was confirmed in recent meta-analyses.[3,4]
Several randomised clinical trials showed a significantly greater reduction of HbA1c using exenatide compared with placebo when added to a regime of oral glucose-lowering drugs and long-acting insulin.[5,6] Additionally, a few reviews showed that GLP-1 analogues establish glycaemic improvement and weight loss benefit, also in patients already using insulin.[7-10] Several studies showed a significant reduction in total daily insulin dose when a GLP-1 analogue was added in patients already on insulin treatment.[11-15] Furthermore, Morrow *et al.* examined the pharmacokinetic and pharmacodynamic effects of combining liraglutide with insulin detemir, and showed that the pharmacokinetic profile of detemir was not altered by co-administration of liraglutide, and that there was an indication of an additive glucose-lowering effect when combining them.[16] Recently, the combination of a GLP-1 analogue with long-acting insulin has been approved by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) based on the aforementioned studies.

Since 2009, GLP-1 analogues have only been approved in the Netherlands for the treatment of type 2 diabetes in patients with a body mass index (BMI) > 35 kg/m² and inadequate glycaemic control while on maximal oral glucose-lowering medication.[17] The combination of insulin and GLP-1 analogues is not reimbursed in the Netherlands, which implies that GLP-1 analogues are not available for patients with type 2 diabetes using insulin. However, in several cases in clinical practice, we observed that starting GLP-1 analogues in patients already on insulin therapy led to improvement of glycaemic control, and a reduction of total daily insulin dose. Therefore, the current Dutch guidelines might lead to unavailability of an effective therapy for patients with type 2 diabetes already using insulin.
In a clinical setting, we investigated the effect of adding GLP-1 analogues in patients with type 2 diabetes and obesity already using insulin with respect to glycaemic control, body weight and total daily insulin dose.

METHODS

Study population

From April 2010 until May 2012 we included all patients starting treatment with GLP-1 analogues (liraglutide once daily or exenatide once weekly) at the Department of Internal Medicine of our hospital. All patients suffered from type 2 diabetes, were at least 18 years of age, had suboptimal glycaemic control (HbA1c > 53 mmol/mol or with frequent complications of insulin use) despite lifestyle modifications and maximum allowed or tolerated doses of oral glucose-lowering drugs, were using insulin (one, two or four times daily), and had a BMI ≥ 35 kg/m².

Procedures

When starting liraglutide or exenatide, the insulin dosage was adjusted based on the insulin frequency, i.e. 1) in patients using once- or twice-daily insulin, the doses were reduced by 50%, and 2) in patients using insulin four times daily, the short-acting insulin was stopped and the long-acting dose was reduced by 50%. Thereafter, the total insulin dose was tapered by the doctor using the observed glucose values with the intention to reduce the insulin dose towards zero. Liraglutide was uptitrated from 0.6 mg per day to 1.2 mg, and, if indicated, to 1.8 mg per day. Each titration had to be completed for at least one week.
At baseline, and after three, six and 12 months, HbA1c and body weight were registered.
HbA1c was measured in mmol/mol, and the values were afterwards converted towards National Glycohaemoglobin Standardisation Program (NGSP)-derived units (%). Weight was measured in kilograms, and BMI was calculated by dividing weight (kg) by the square of the height (m²). Furthermore, at baseline and after six and 12 months medication (oral medication, daily insulin dose in IU) was recorded.

Statistical analysis

For analyses, we used longitudinal analysis using repeated measures analysis with an unstructured covariance matrix to model the covariance structure. Analyses were performed for HbA1c and weight comparing baseline with three, six and 12 months, and for insulin dose at six and 12 months. These analyses were adjusted for age and sex. Furthermore, the correlation between HbA1c and weight change, between duration of diabetes and HbA1c and weight change, and between weight change and insulin dosage change was calculated at three, six and 12 months. In addition, adverse events and complications during the study period were registered. All analyses were performed using SPSS for Windows.

Results

During the study period we included 125 patients. *Table 1* lists the characteristics of the study population. The mean age was 59.3 years, and 61 (49%) patients were women. At baseline, mean HbA1c was 68.3 mmol/mol (8.4%),

Table 1. *Characteristics of the study population*

	Total (n = 125)
Age (years)	59.3 ± 10.0
Gender	
- Men (%)	64 (51)
- Women (%)	61 (49)
Weight (kg)	121.4 ± 19.1
BMI (kg/m²)	41.5 ± 5.1
HbA1c (%)	8.4 ± 1.2
HbA1c (mmol/mol)	68.3 ± 12.6
Insulin dose (IU)	114 ± 68
- Long acting (IU)	63 ± 33
- Short acting (IU)	49 ± 49
Duration of diabetes (years)	12.6 ± 7.5
Insulin daily frequency	
- Once (%)	27 (22%)
- Twice (%)	21 (17%)
- Four times (%)	74 (59%)
- Insulin pump (%)	3 (2%)
GLP-1 analogue	
- Liraglutide (%)	121 (96.8)
- Exenatide (%)	4 (3.2)

Values are means (± standard deviation) or numbers (percentages).

while mean BMI and mean body weight were 41.5 kg/m² and 121.4 kg, respectively. Furthermore, the average daily insulin therapy dose was 114 IU. A total of 121 patients (97%) started liraglutide, the others used exenatide.

Table 2, figure 1 and *figure 2* show HbA1c and weight over time. HbA1c decreased significantly at all three timepoints (-4.1 mmol/mol (-0.4%), -5.4 mmol/mol (-0.5%), and -5.5 mmol/mol (-0.5%) at three, six and 12 months respectively, $p \leq 0.001$ compared with baseline), with the largest decrease in the first three months. Similarly, a significant weight reduction was seen at all timepoints (-9.7 kg, -12.5 kg, and -14.3 kg at three, six and 12 months respectively, $p < 0.001$ compared with baseline), with the largest decrease in the first three months as well.

After both six and 12 months, the total daily insulin dose decreased significantly (-75.0 IU and -75.4 IU respectively, $p < 0.001$). Moreover, 36 patients (29%) were able to discontinue any form of insulin therapy at six months, while at 12 months 42 patients (34%) were able to do this.

There was no significant correlation between HbA1c and weight change at three, six and 12 months. Additionally, no significant correlation between duration of diabetes and HbA1c or weight change, respectively, was found. A significant correlation between weight change and insulin dosage change ($p < 0.001$) at six and 12 months was found.

Table 2. *Change from baseline for HbA1c, weight and insulin dosage*

	3 months	6 months	12 months
Weight (kg)	-9.7 ± 6.6 *	-12.5 ± 8.4 *	-14.3 ± 9.5 *
HbA1c (%)	-0.4 ± 1.2 *	-0.5 ± 1.4 *	-0.5 ± 1.3 *
HbA1c (mmol/mol)	-4.1 ± 12.9 *	-5.4 ± 15.8 *	-5.5 ± 14.2 *
Insulin dosage (IU)		-75.0 ± 55.8 *	-75.4 ± 60.3 *
- Long acting (IU)		-25.6 ± 29.0 *	-26.6 ± 31.6 *
- Short acting (IU)		-43.5 ± 45.8 *	-37.5 ± 40.2 *
Correlation between change of weight and change of HbA1c (r)	-0.11	-0.11	-0.04
Correlation between change of weight and duration of diabetes (r)	-0.08	-0.20	-0.16
Correlation between change of HbA1c and duration of diabetes (r)	0.05	0.07	0.10
Correlation between change of weight and change of insulin dosage (r)		0.38	0.44

Values are means (± standard deviation) or correlation coefficient.
*** p-value ≤ 0.001 compared with baseline adjusted for age and sex.**

Figure 1. *Bar graphs showing HbA1c change over time*

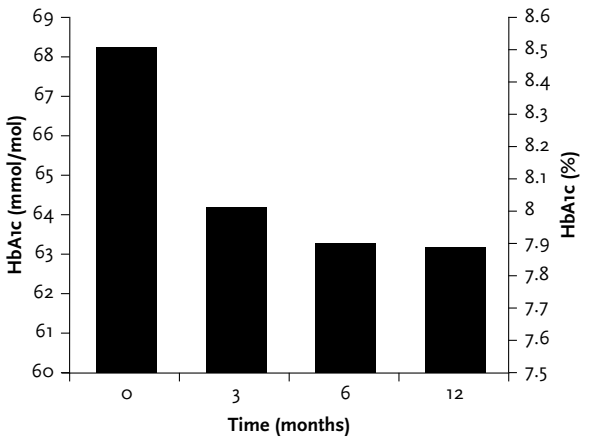
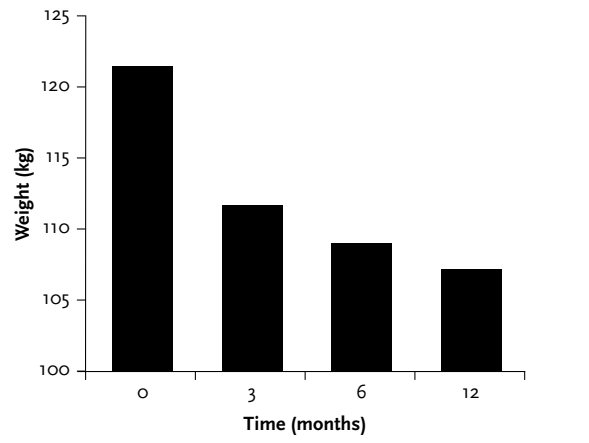


Figure 2. *Bar graphs showing weight change over time*



During the study, 19 patients (15%) discontinued the GLP-1 analogues due to lack of effect on glycaemic control, while five patients (4%) stopped due to intolerable side effects. No pancreatitis or diabetic ketoacidosis was observed during the study period.

DISCUSSION

We have shown that patients with type 2 diabetes already on insulin therapy benefit from adding GLP-1 analogues, as both HbA1c levels and body weight decreased despite a significant reduction or discontinuation of the insulin dose. Remarkably, no significant correlation between weight loss and HbA1c reduction could be observed.

During the study period no diabetic ketoacidosis was observed. This is probably due to the method that insulin therapy was not discontinued at the start, but tapered based on the glucose values.

We found that HbA1c decreased significantly at three, six and 12 months, with the decrease in the first three months being the largest. Previous clinical studies, also including patients on insulin therapy, showed a decrease in HbA1c of 0.9% after 13 weeks,[18] 0.55% after 12 months and 0.54% after 27 months,[12] and 0.32% after 12 months.[11] Our result of 5.5 mmol/mol (0.5%) after 12 months is therefore comparable with other clinical studies.

We observed a significant weight reduction at all timepoints, with the largest decrease in the first three months leading to a total reduction of 14 kg (12%) after 12 months. In clinical studies evaluating patients on insulin therapy, Mulligan *et al.* retrospectively showed a mean weight reduction of 2.4 kg (2%) after 13 weeks.[18] Yoon *et al.* showed a decrease of 4.3 kg (4%) and Thong *et al.* of 7.6 kg (7%) after 12 months in patients also using insulin.[11,12] In a prospective study, Nayak *et al.* showed a weight decrease of 12.8 kg (11%) after 12 months.[13] Our observations are in line with the observations of this last-mentioned prospective clinical study. However, the total weight loss observed in our study population was considerably higher than reported by Thong *et al.*, Yoon *et al.*, and Mulligan *et al.*[11,12,18] This difference might be explained by our inclusion criterion of a BMI ≥ 35 kg/m^2 which is considerably higher than other studies. Therefore, our patients had more excessive weight and therefore one might argue that there is potentially more weight that can be lost.

Insulin dose was decreased significantly during the study, both at six and 12 months (64% after 12 months) with almost all the decrease seen in the first six months. In a retrospective study with 27 months of follow-up, Yoon *et al.* also showed a significant decrease in total daily insulin dose in the first 12 months with respect to baseline.[12] The largest reduction was seen in the first six months, however; thereafter insulin doses increased again but stayed below the baseline dose, even after 27 months. Moreover, several other studies showed a significant reduction in daily insulin dose when a GLP-1 analogue was added to insulin treatment.[11,13-15]

Of our patients, 34% were able to discontinue any form of insulin. After adding a GLP-1 analogue to insulin, Thong *et al.* found that 16.6% of their patients were able to stop using insulin and for Lind *et al.* this was 8%.[11,15] This is lower than the percentage observed in our study, which might be caused by the fact that our study was designed to decrease or discontinue insulin, while their studies were retrospective.

Four percent of our study patients discontinued GLP-1 analogues due to intolerable side effects. Gastrointestinal side effects are common in GLP-1 analogues; Buse *et al.* reported nausea in 21% of patients using liraglutide and in 9% of patients using exenatide. Furthermore, diarrhoea was seen by them in 13% and 6%, respectively.[19]

Due to lack of glycaemic control, 15% of our study population discontinued GLP-1 analogues. Davis *et al.* showed that 18% of their patients treated with exenatide lost glycaemic control before week 16, and that pre-treatment C-peptide levels and baseline body weight were the best estimators of successful glycaemic control.[20]

We observed no significant correlation between HbA1c reduction and weight loss after three, six and 12 months. Yoon *et al.* did not observe a significant correlation between HbA1c and weight change after six and 12 months either.[12] This absence of correlation is an interesting observation as it was thought that these two go together.[21,22] Furthermore, we found no correlation between HbA1c reduction and duration of diabetes, and between weight change and duration of diabetes. It could be hypothesised that differences in endogenous GLP-1 production or insulin rest capacity play a crucial role in these inter-individual differences. Further studies are needed to confirm our findings.

A significant correlation between weight change and insulin dosage change was found. This might be explained by the fact that reducing weight leads to decreasing insulin resistance and therefore less insulin is needed, but it might also be the other way around.

The prospective character of our study is one of its main strengths since most clinical studies have a retrospective design. Additionally, its clinical setting gives a direction for daily practice. Limitations include its single-arm design, so no conclusions can be drawn about the benefits of combination therapy over insulin therapy alone, and factors other than GLP-analogue treatment affecting weight and HbA1c reduction cannot be ruled out, e.g. positive impulse by initial weight loss resulting in lifestyle change. Further research might include a prospective double-arm design, comparing combination treatment with GLP-1 analogues and insulin with insulin treatment only to overcome these difficulties. Moreover, starting a GLP-1 analogue, and then inclusion in the study, was a decision made by the patient's clinician, resulting in the fact that he/she only included a patient when benefit was expected; this might lead to confounding by indication. However, it might also be that we omitted patients who would have benefited from GLP-1 analogues, and this type of inclusion is how clinical practice works.

In conclusion, our study shows that obese patients with type 2 diabetes already on insulin therapy benefit from GLP-1 analogues: despite significant reduction or discontinuation of insulin dose, HbA1c levels and body weight decreased. Therefore, we suggest that the present indication for the use of GLP-1 analogues in

the Netherlands should be reconsidered. Further studies are needed to find an explanation why reduction in body weight and change in HbA1c are not correlated.

DISCLOSURES

The authors declare no conflicts of interest.

Statement of Human Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

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ORIGINAL ARTICLE

Quantitative ultrasound of the heel as triage test to measure bone mineral density compared with dual energy X-ray absorptiometry in men with prostate cancer commencing with androgen deprivation therapy

C. van Casteren-Messidoro[1]*, A.M. Huisman[1], E. Birnie[2,3], M. van Gelder[4], F.E. van de Geijn[1], P. Hamberg[4,5]

Departments of [1]Rheumatology [2]Statistics, [4]Internal Medicine, [5]Prostate Cancer Center, Sint Franciscus Gasthuis, the Netherlands, [3]Institute of Health Policy and Management, Erasmus University, Rotterdam, the Netherlands, *corresponding author: tel.: +31(0)10-4616150, email: chiaramessidoro@hotmail.com

ABSTRACT

Objective: Androgen deprivation therapy (ADT) puts patients at an increased risk of developing osteoporosis. Assessment of bone mineral density (BMD) is most commonly performed by dual energy X-ray absorptiometry (DXA). Alternative ways of estimating BMD, such as quantitative ultrasound (QUS) measurement of the heel, are explored as DXA is expensive, non-portable and uses ionising radiation. We therefore investigated the diagnostic value of QUS as compared with DXA in patients commencing ADT.

Methods: In this cross-sectional study of 60 patients with prostate cancer who were about to start ADT, BMD was measured with DXA and QUS. The fracture risk score, as implemented by the Dutch National Osteoporosis Guideline, was also measured.

Results: No significant correlations were found between the separate DXA T scores and worst DXA T score, and the QUS T scores. Correlations between DXA T scores/QUS scores and fracture risk score were also non-significant. If QUS had been used as a screening tool, with a threshold of T ≤ -0.5 to perform DXA, then relevant osteopenia/osteoporosis (worst DXA T score ≤ -2.0) would have been missed in 1/18 (5.6%) patients. The negative predictive value is 0.95. Using QUS as a screening test prior to DXA and a QUS threshold T score ≤ -0.5 would avoid 21 (35%) DXA scans at the cost of missing one (5.6%) case.

Conclusion: QUS testing cannot replace DXA scans fully as a diagnostic test. However, QUS can be incorporated as triage test prior to DXA to reduce the need for unnecessary DXA scans and the associated costs.

KEYWORDS

Androgen deprivation therapy, osteoporosis, bone mineral density, dual energy x-ray absorptiometry, quantitative ultrasound

INTRODUCTION

Androgen deprivation therapy (ADT), frequently used as part of the treatment of prostate cancer, puts patients at an increased risk for developing osteoporosis, resulting from therapy-induced hypogonadism. Androgen deprivation can be achieved by eliminating the secretion of testicular androgens by surgical castration or by reducing circulating androgens by inhibiting the hypothalamic-hypophyseal-gonadal axis. Hormonal therapy using luteinising hormone releasing hormone (LHRH) agonists or antagonists is currently the main way to achieve medical castration.[1] Hormonal therapy is currently part of the curative treatment of prostate cancer in patients with high-risk prostate cancer who receive a combination of radiotherapy and hormonal therapy, the so-called Bolla schedule;[2] hormonal therapy is also used as palliative treatment in metastatic disease.

During long-term therapy, ADT reduces bone mineral density (BMD) and increases the risk of clinical fractures. During initial ADT, BMD on the hip and spine decrease by approximately 3% per year. Most studies have reported that BMD continues to decline steadily during long-term therapy.[3]

Assessment of BMD is considered the standard evaluation of patients commencing ADT, according to the international guidelines. BMD should be measured every two years if the initial T score is < 1.0, or every year if the T score is between 1.0-2.5, in the absence of associated risk factors. Otherwise, active protective bone treatment should have started at the initiation of ADT.[1,4]

The World Health Organisation defined osteoporosis based upon dual energy X-ray absorptiometry (DXA) measurements, as this is the most widely used method for measuring BMD. DXA provides accurate measurements at clinically relevant sites, i.e. those with major clinical consequences when a fracture occurs. The major disadvantages of DXA are that the instrument is large (not portable), relatively expensive compared with alternative peripheral technologies, and uses radiation albeit at a low dose. Alternative techniques to evaluate bone status at peripheral sites have been developed, e.g. quantitative ultrasound (QUS) measurement of the heel, considered one of the best alternative methods currently available for the assessment of fracture risk.[5]

Replacing DXA measurement by QUS measurements seems attractive as QUS is inexpensive, transportable and free of ionising radiation.[6] Full replacement of a DXA-based diagnostic strategy by QUS measurements is only desirable when the validity of QUS in patients treated with ADT has been demonstrated. In this paper we investigate whether BMD can be validly assessed at the onset of ADT by QUS compared with DXA as reference test. Moreover, if QUS as stand-alone test cannot fully replace DXA, we explore the possibilities of a test strategy that is partially based on QUS. Also, we investigate the value of the fracture risk score compared with DXA and QUS.

PATIENTS AND METHODS

Patients and design

Included in this cross-sectional study were patients who started ADT as treatment for prostate cancer in our clinic between March 2011 and March 2012. There were no exclusion criteria. BMD was measured by DXA scan as well as by QUS, in random order, preferably before and otherwise after initiation of ADT.

BMD measurement by DXA scan was performed in terms of three T scores: at the spine (level L1-L4) and both hips (femoral necks or radius/ulna if hip measurement was impossible due to hip replacement), using a Hologic Discovery DXA scan. We added the worst T score of each DXA measurement. QUS was performed using an Achilles Bone Ultrasonogram (GE Healthcare) which measures the bone stiffness index and calculates a T score. We also recorded the fracture risk score, a tool based on clinical parameters identifying patients at risk for osteoporosis, as implemented by the Dutch National Osteoporosis Guideline (*table 1*).[7] This risk score ranges between 0-12; a higher score indicates higher risk. A DXA scan is advised when the risk score is ≥ 4 points.

Table 1. *Patient's characteristics and distribution of fracture risk score (n = 60)*

Characteristic	
Setting	
Bolla	44 (73.3%)
Metastatic prostate cancer	16 (26.7%)
Type of androgen deprivation	
Gosereline	50 (83.3%)
Degarelix	4 (6.7%)
Busereline	4 (6.7%)
Other	2 (3.4%)
Total fracture risk score (see under)	
0	1 (1.7%)
1	20 (33.3%)
2	25 (41.7%)
3	10 (16.7%)
4+	4 (6.7%)
Median (IQR / range)	2 (1-2/ 0-7)
Weight < 60 kg and/or BMI <20 kg/m² (1 point)	0
Age > 60 years (1 point)	58 (96.6%)
Previous fracture (1 point)	5 (8.4%)
Hip fracture in a parent (1 point)	9 (15%)
Reduced mobility (1 point)	8 (13.4%)
Rheumatoid arthritis (1 point)	0
Secondary osteoporosis (1 point)	9 (15%)
Corticosteroids use (4 points)	2 (3.4%)

Items on fracture risk score:

Weight < 60 kg and/or BMI < 20 kg/m²	1 point
Age > 60 years	1 point
Age > 70 years	2 points
Previous fracture after the age of 50 years	1 point
Hip fracture in a parent	1 point
Reduced mobility	1 point
Rheumatoid arthritis	1 point
Disease or condition associated with secondary osteoporosis (see under)	1 point
Corticosteroids use (> 3 months; 7.5 mg/day)	4 points

Disease or condition associated with secondary osteoporosis:

- Inflammatory bowel disease: Crohn's disease and ulcerative colitis
- Malabsorption
- Chronic inflammatory disorders such spondylarthropathy (ankylosing spondylitis), SLE, sarcoidosis
- Organ transplantation
- Diabetes mellitus
- Untreated hyperthyroidism
- Use of anticonvulsants
- Untreated hyperparathyroidism
- COPD
- Pernicious anaemia
- Low sun exposure

Diagnostic strategies

We compared the validity of the following diagnostic strategies: 1) QUS as stand-alone test was compared with DXA T scores considered as reference test; 2) QUS as screening test with the presence of severe osteopenia as reference (defined as DXA T score ≤ -2.0); 3) fracture risk score as screening test with the presence of severe osteopenia as reference (defined as DXA worst T score ≤ -2.0); and 4) a diagnostic index based on the weighted QUS T score and the fracture risk with the presence of osteoporosis as reference.

Statistical analysis

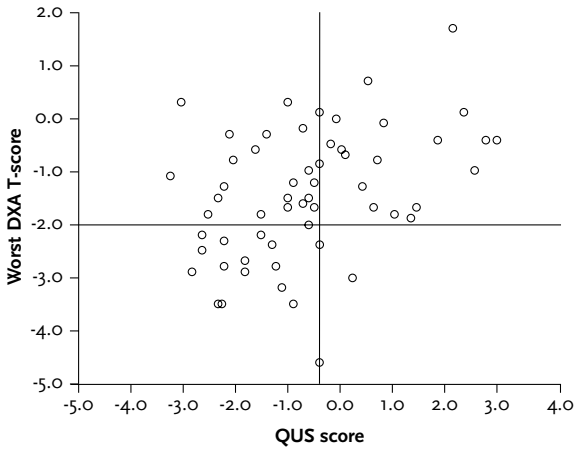
Patient, disease and treatment characteristics at baseline, the QUS and DXA T scores and the differences in T scores between QUS and DXA were described with conventional descriptive statistics: n (%) for nominal and ordinal variables, mean (SD) for quantitative variables with approximate normal distributions and median (interquartile range [IQR]) for quantitative variables with skewed distributions. Intraclass correlation coefficients (ICCs, two way mixed, single measures) were used to quantify the correlations between the QUS T score and the single T scores and worst T score. The strength of associations between the DXA and QUS T scores on the one hand and the fracture risk score was estimated with linear regression analyses and expressed as adjusted R2. The diagnostic strategies were evaluated compared with their reference in terms of the proportion of correct predictions, sensitivity, specificity, positive and negative predictive values (all with 95% CIs). The value of the fracture risk score in addition to the QUS T score was estimated with binary logistic regression and expressed as the change in diagnostic accuracy and the change in the -2log likelihood (-2LL) goodness of fit measure. A p value (two-tailed) < 0.05 was considered a statistically significant difference.

Table 2. *Description of quantitative ultrasound and dual energy X-ray absorptiometry values*

	Mean (SD)	Median	Range Min / Max	IQR
QUS	-0.72 (1.50)	-0.75	-3.3 / 2.8	-1.90 / 0.08
DXA				
T score right hip	-0.40 (0.91)	-0.45	-2.8 / 1.7	-0.90 / 0.30
T score left hip	-0.33 (0.94)	-0.35	-3.1 / 1.7	-0.98 / 0.18
T score LVB	-0.49 (1.59)	-0.70	-3.5 / 3.9	-1.60 / 0.50
T score worst	-1.42 (1.21)	-1.40	-4.6 / 1.7	-2.28 / -0.53

LVB = lumbar vertebral body.

Figure 1. *Scatter plot (worst DXA T score and QUS score)*



RESULTS

The characteristics of the 60 included patients are shown in *table 1*.

Comparison of T scores

The mean QUS value was -0.72. The mean DXA T scores for right hip, left hip and lumbar spine were -0.40, -0.33 and -0.49, respectively. The mean value of the worst DXA T score was -1.42 (*table 2*). The ICCs between all the separate T scores as well as the worst T score with the QUS-derived score were low and not significant. The QUS T score is neither a precise nor a valid estimate of the worst DXA T score (*figure 1*).

Comparison of test characteristics

If QUS had been used as screening tool, with a threshold of T ≤ -0.5 to perform a DXA, then relevant osteopenia (worst DXA T score ≤ -2.0) would have been missed in 1/18 (5.6%, 95% CI 0.1-27.3%) patients, with a negative predictive value (NPV) of 0.95 (95% CI 75.1-99.9%, *table 3*). At a threshold of QUS of T ≤ -0.7 the NPV would be 0.89 (95% CI 70.8-97.7%). Using QUS as triage test prior to DXA and a QUS threshold T score ≤ -0.5, 35% (95% CI 23.1-48.4%) of the DXA scans would have been avoided (*table 3*).

The majority of patients (58.4%) had a low-intermediate fracture risk score of 1-2 or 3 points (scale: 0-12). Only four patients (6.7%) had a risk score of ≥ 4 points (*table 1*). ICCs between DXA T scores/QUS score and fracture risk score resulted in adjusted R2 between -0.014 and -0.017 (all non-significant). The proportion of correct predictions using the fracture risk score of 4 as a cut-off level was 22/60 (36.7%, 95% CI 24.6-50.1%) with a sensitivity of 0/18 (0%, 95% CI 0.0-18.5%), a specificity of 38/42 (90.5%,

Table 3. *Relevant osteopenia/osteoporosis cases with QUS threshold T= -0.5*

	Osteoporosis + (DXA T score ≤ -2.0)	Osteoporosis – (DXA T score > -2.0)	Total
QUS T score ≤ -0.5	17	22	39
QUS T score > -0.5	1	20	21
Total	18	42	60

Sensitivity: 17/18 (94%, 95% CI 73-99%); specificity 20/42 (47%, 95% CI 32-63%).

Table 4. *Relevant osteopenia/osteoporosis cases with fracture risk score*

	Osteoporosis + (DXA worst T score ≤ -2.0)	Osteoporosis – (DXA worst T score ≥ -2.0)	Total
Fracture risk score 0-3	18	38	56
Fracture risk score ≥4	0	4	4
Total	18	42	60

Positive predictive value = 0/4 (0%, 95% CI 0.0-60.2%).

95% CI 0.774-0.973) and an NPV of 38/56 (67.9%, 95% CI 54.0-79.7%) (*table 4*). Hence, QUS does outperform the fracture risk score as triage test.

A diagnostic index based on the fracture risk score and the QUS T score jointly did not improve the diagnostic accuracy nor the -2LL goodness of fit measure compared with a model without the fracture risk score (proportion of correct predictions: 42/60 for both models; -2LL 61.1 vs. 61.5).

DISCUSSION

In our cohort, QUS appears unable to reflect the BMD as generated by DXA, the reference test for the detection of osteoporosis. One plausible explanation for the low correlation is the fact that both methods structurally evaluate different parameters of BMD. DXA measures bone mineral content and bone area and estimates areal BMD. BMD measured by QUS, in contrast, provides no actual measurement of BMD but is the result of measurements derived from the transmission of ultrasound through bone. Measurements are most commonly made at the calcaneus, a skeletal site composed primarily of cancellous (trabecular) bone, similar to the spine. However, the correlation between DXA lumbar spine T score and QUS score in our cohort was one of the lowest and therefore does not support that view. A study of 230 postmenopausal women also showed lower correlation coefficients between QUS and DXA T scores of the lumbar spine compared with the hips, which might be caused by using only one composite T score for the lumbar spine instead of four different values (L1, L2, L3 and L4).[8] A review of comparative studies between bone densitometry and QUS of the calcaneus in osteoporosis reported a sensitivity of QUS compared with DXA in detecting osteoporosis of 65-67% when compared with spine BMD and 72-74% when compared with hip BMD.[9]

As the DXA scan delivers T scores from different sites with a higher likelihood of detecting osteoporosis, the outcome of an alternative screening method should be compared with the worst T score from the DXA scan. In our study the correlation between QUS T score and the worst DXA T score appeared to be the lowest of all, confirming the lack of potential of QUS to assess BMD in our cohort, when compared with the gold standard (*figure 1*).

Many studies have demonstrated that low BMD measured by DXA at any skeletal site (spine, hip of forearm) is predictive for an osteoporotic fracture.[10-12] QUS, however, has also been shown to be a good predictor of osteoporotic fracture risk.[13-22] Advantages of QUS include lower expense, portability and lack of radiation exposure. However, a point of controversy with regard to earlier studies is the cut-off point for the diagnostic determination of osteoporosis with the QUS method. No agreement could be found between the threshold accepted for DXA (a T score < -2.5 for osteoporosis) and a QUS threshold for detecting osteoporosis.[9] A meta-analysis of 25 studies that evaluated the sensitivity and specificity of calcaneus ultrasound for identifying patients with DXA T scores < -2.5 concluded that the currently used ultrasound cut-off thresholds do not have sufficiently high sensitivity or specificity to definitively exclude or confirm DXA diagnosed osteoporosis.[23] However, our cohort concerned patients with a low risk of osteoporosis (due to the low fracture risk score). The majority of patients (62%) had their BMD measurement prior to or within the first two months of ADT, and this makes an ADT-induced effect on BMD less likely.

We also estimated the test characteristics of QUS for different QUS T score thresholds to perform a DXA, with the idea of using QUS as a screening tool. With a QUS threshold of T ≤ -0.5 to perform a DXA we found a high NPV of 0.95, meaning that relevant osteopenia/osteoporosis would have rarely been missed. Using QUS as screening or triage test prior to DXA and a QUS threshold T score ≤ -0.5 would avoid 35% of DXA scans at the cost of missing one (5.6%) relevant case of osteopenia/osteoporosis. A previous review of seven studies comparing different QUS thresholds and DXA T scores resulted in QUS sensitivity ranging from 79-93% and specificity from 28-90% (when at a lower threshold). QUS thresholds had a

variability of -1.7 and -2.4, and a T score of < -3.65 for QUS was equivalent to a T score < -2.5 for DXA.[9]

Besides a benefit in patient comfort by using the mobile device as a screening tool, there is also a relevant cost benefit. A true cost analysis is hampered by the lack of a standardised price for a QUS measurement. An estimate of price based upon a unit price of a QUS machine (€ 16,000) with a full depreciation in ten years, yearly service costs and costs for staff (15 minutes per measurement) is € 13 per measurement. A set price for conventional DXA is € 167 per measurement.

If the standard strategy had been used in our 60 patients, then the costs of the BMD measurement for the whole group would have been € 10,020. The screening strategy would have resulted in 60 QUS measurements and 39 DXA measurements, with total costs of € 7293, rendering a cost reduction of 27%.

This fracture risk score aimed to identify the patients at risk for osteoporotic fractures on the basis of clinical parameters appeared to be a poor predictor of worst DXA T score. We found very weak correlations between the risk score and the DXA T scores and QUS scores. Moreover, the fracture risk score did not identify any of the patients with a relevant osteopenia based on worst DXA T score in our cohort. In summary, the risk score seems to be a poor test, worse than the lower threshold QUS. Jointly, the fracture risk score and the QUS T score did not improve the diagnostic accuracy.

This study has a number of limitations, such as the relatively small number of included patients. Also, we correlated the DXA T scores with the QUS T scores, while there is no consensus on the QUS threshold to predict osteoporosis. Thus the choice to test the sensitivity of QUS with different thresholds, compared with DXA-proven severe osteopenia, which showed a very good NPV of QUS used as a screening tool prior to DXA. This is a retrospective cohort, but prospective studies analysing whether the BMD decline of patients as measured by DXA is predictable by QUS are needed. Also the further role of QUS as a possible screening tool needs to be further elucidated.

In conclusion, QUS is not suitable to detect osteoporosis at the start of ADT treatment in men with prostate cancer, compared with the gold standard (DXA), and therefore cannot replace DXA fully. However, QUS can be incorporated in the diagnostic strategy as triage test prior to DXA to reduce the need for DXA scans and performs better than a risk score based on clinical parameters.

DISCLOSURES

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ORIGINAL ARTICLE

Determinants of plasma 25-hydroxyvitamin D levels in healthy adults in the Netherlands

G. van Grootheest[1]*, Y. Milaneschi[1], P.T.A.M. Lips[2], A.C. Heijboer[3], J.H. Smit[1], B.W.J.H. Penninx[1]

[1]GGZ inGeest and Department of Psychiatry, EMGO+ Institute and Neuroscience Campus Amsterdam, [2]Endocrine Section, Department of Internal Medicine, [3]Endocrine Laboratory, Department of Clinical Chemistry, VU University Medical Center, the Netherlands, *corresponding author: tel.: +31 (0)20-788 5678, email: g.vangrootheest@ggzingeest.nl

ABSTRACT

Background: Vitamin D plays a key role in maintaining skeletal health, but is also related to various non-skeletal health issues. Several determinants have been identified that influence blood plasma levels of 25-hydroxyvitamin D (25(OH)D), often in specific patients or elderly populations. This paper aims to replicate these findings in a healthy population.

Methods: Plasma levels of 25(OH)D were measured using tandem mass spectrometry. We examined the cross-sectional association of sociodemographic, health, lifestyle and sampling characteristics with 25(OH)D in a group of 539 adults, who were healthy control subjects in the NESDA study in the Netherlands (latitude 52 °N).

Results: Mean 25(OH)D levels were 68.0 (±27.2) nmol/l. Levels under 50 nmol/l occurred in 27% of the population; 40% reached levels above 75 nmol/l. Women had higher levels than men, and the use of oral contraceptives showed a significant positive association among females. Subjects with non-European ancestry had dramatically lower 25(OH)D levels. Other factors that were negatively associated were body mass index and the renal estimated glomerular filtration rate (eGFR). Meteorological data replaced season as a significant determinant. Moderate alcohol consumption and sports showed a positive association, while physical activity and the hepatic marker gamma-glutamyl transferase did not. Our results disconfirm the influence of age in this population of under 65 year olds.

Conclusion: Insufficient 25(OH)D levels were common in a healthy population. The set of eight variables that were significant in a multiple regression model (sex, ancestry, oral contraceptives, eGFR, BMI, sports, alcohol, sunshine) explained 29.5% of the variance.

KEYWORDS

25(OH)D, determinant, vitamin D

INTRODUCTION

In recent years, the interest in vitamin D has increased within several fields of medicine. Vitamin D is best known for its key role in maintaining the body's calcium homeostasis and thereby its involvement in skeletal health. Deficiency increases the risk of developing osteoporosis and, in severe cases, causes rickets or osteomalacia.[1,2] Vitamin D receptors are found in tissues throughout the entire human body, and recent findings indicate an involvement of vitamin D deficiency in other diseases, such as autoimmune diseases, certain types of cancer, cardiovascular diseases, and the metabolic syndrome.[3-5] Furthermore, vitamin D deficiency has been shown to be associated with psychiatric disorders such as depression.[6-9] Rickets and osteomalacia became increasingly common in European cities during the period of urbanisation and industrialisation from the 17th until the early 20th century, but were largely eradicated after the influence of sunlight exposure and cod liver oil was discovered.[10,11] These days, some foods are fortified with vitamin D. The only vitamin D-supplemented food in the Netherlands is margarine but in the United States and Canada, fortification of products such as cereals and milk is usual practice, resulting in increased circulating vitamin D levels in the population.[12] Nevertheless, vitamin D insufficiency and deficiency still commonly occur in environments with sufficient sunlight and dietary sources of vitamin D, primarily among vulnerable groups in the winter.[13,14]

Vitamin D occurs in the form of D2 and D3. Vitamin D2 is synthesised in plants; vitamin D3 is synthesised in humans and animals under the influence of ultraviolet radiation. Both forms are obtained by humans through the dietary intake of vegetables, eggs, dairy products, meat and fish, but the main source of vitamin D3 in the human body is *de novo* synthesis in the skin. Vitamin D is hydroxylised in the liver and stored as 25-hydroxyvitamin D (also called calcidiol, 25(OH)D or D25). In the kidneys, 25(OH)D is

converted into the hormonal form 1,25-dihydroxyvitamin D (also called calcitriol or 1,25$(OH)_2$D). Whereas 1,25$(OH)_2$D is the active form of vitamin D, the most reliable marker of vitamin D status is 25(OH)D (15). With a half-life of 2-3 weeks, 25(OH)D is much more stable than its derivative 1,25$(OH)_2$D, which has a half-life of only six hours.[16]

Although diet and sunlight exposure play an established role in vitamin D physiology, other determinants of vitamin D status have been identified. These include physical and sociodemographic characteristics (sex, age, sex hormones, ethnicity, socioeconomic status, urbanisation) and lifestyle (body mass index (BMI), physical activity, consumption of tobacco and alcohol, vitamin supplementation).[17,18] Further, as the liver and the kidneys play a crucial role in vitamin D physiology it can be expected that markers of hepatic and renal function, such as gamma-glutamyl transferase (GGT) and the estimated glomerular filtration rate (eGFR), are associated with plasma 25(OH)D levels. Finally, circumstantial sampling variables such as season and plasma storage time may influence 25(OH)D levels.

There has been a great interest in vitamin D and its involvement in various health problems in recent years. Vitamin D status is often studied in specific groups that have an increased risk of vitamin D deficiency or osteoporosis, such as hospitalised or elderly people. In such groups, confounding of variables makes it difficult to translate findings to the general population. Whereas several studies have described vitamin D determinants in healthy populations, countries differ in climate, dietary intake and supplementation of vitamin D. The aim of the current paper is to verify the independent importance of a wide range of sociodemographic, health, lifestyle and sampling characteristics for plasma levels of 25-hydroxyvitamin D in a group of healthy adults without clinically overt psychiatric or somatic diseases in the Netherlands (latitude 52 °N).

METHODS

Subjects

We used data from the baseline assessment of the Netherlands Study of Depression and Anxiety (NESDA),[19] an ongoing longitudinal cohort study, focusing on the course and determinants of depression and anxiety. The presence of depressive and anxiety disorders in this study was determined according to DSM-IV criteria using the World Health Organisation Composite International Diagnostic Interview (CIDI, version 2.1). The study was approved by our institution's medical ethics board and respondents signed a written informed consent form.

The NESDA cohort included 2981 subjects, aged 18-65, who visited one of our five research sites for a detailed interview. A total of 652 subjects did not have a lifetime history of anxiety, major depressive or dysthymic disorders as determined by the CIDI. For the present analyses, we used data from these control subjects only. Of these, 105 respondents were excluded because they reported clinically overt disease of the thyroid gland, kidney or liver, osteoporosis, stroke, lung emphysema, cancer, rheumatoid arthritis, multiple sclerosis, HIV or any other autoimmune disease. Furthermore, subjects were excluded due to unsuccessful blood draw (n = 7) or an extreme plasma 25(OH)D value (> 4 SD above the mean, n = 1). These exclusion criteria led to a total of 539 qualified subjects with a valid 25(OH)D measurement.

Vitamin D status

Fasting blood was collected at the time of baseline interview, between 8.15-10.10 hours. Vacutainers with EDTA (BD Diagnostics, NJ, USA) were used to collect plasma for later assessment of 25(OH)D and creatinine. Samples were spun down within one hour after blood draw and cryovials with portions of 500 µl of EDTA plasma were stored at -80 °C.

Measurements of 25(OH)D were performed at the Endocrine Laboratory of the VU University Medical Center. 25(OH)D was measured by isotope dilution/online solid-phase extraction liquid chromatography/tandem mass spectrometry (ID-XLC-MS/MS).[20] First, 25(OH)D was released from its binding proteins with a proprietary protein disruption buffer. Deuterated internal standard (IS) 25(OH)D3-d6 was added and samples were mixed. Samples were extracted and analysed by XLC-MS/MS (a Symbiosis online SPE system, Spark Holland) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corporation). We quantified plasma 25(OH)D by relating analyte/IS peak area ratios in patient plasma to analyte/IS peak area ratios in BSA-PBS buffer spiked with 25(OH)D2 and 25(OH)D3 at concentrations ranging from 0-100 µg/l (0-250 nmol/l) and IS at a fixed concentration. We established the accuracy of 25-hydroxyvitamin D results by measuring the standard and a control with a reference method.[21] 25(OH)D2 and 25(OH)D3 were measured separately but combined for the analyses.

We used continuous 25(OH)D measures for the majority of our analyses, but also describe the study population by classification into four groups: < 25 nmol/l, 25-50 nmol/l, 50-75 nmol/l and ≥ 75 nmol/l. Although there is no consensus on 25(OH)D classification, levels under 50 nmol/l are generally considered insufficient or deficient; levels of at least 75 nmol/l are recommended by e.g. the Institute of Medicine.[2,22,23]

DETERMINANTS

Sociodemographics

Sex, age, country of birth and educational level were assessed during the interview. We also obtained the birth

country of both biological parents for 501 respondents, allowing us to distinguish those with one and those with two non-European parents. We considered the educational level an indicator of socioeconomic status. The degree of urbanisation was based on the postal code of residence. Areas were considered urban when the surrounding address density is 1500 or more per square kilometre (Statistics Netherlands, www.cbs.nl).

Health and lifestyle indicators

Menopausal status and the use of oral contraceptives were assessed during the interview. Creatinine levels were measured at the Clinical Chemistry Laboratory of the University Medical Center Groningen with an enzymatic method (Roche Hitachi Modular, Roche Diagnostics, Basel, Switzerland). Estimated GFR (ml/min/1.73 m^2) was calculated using the CKD-EPI equation.[24] GGT was measured at each research site's local laboratory with an enzymatic method (Roche Hitachi Modular). Body height and weight were measured by the research assistant and used to calculate the body mass index (BMI = weight (kg) / height $(m)^2$).

Alcohol consumption (number of drinks/day) was reported by means of the written Alcohol Use Disorders Identification Test (AUDIT).[25] Consumption of up to 14 standard glasses per week for women or 21 for men was labelled as moderate; a greater consumption was considered heavy drinking. Physical activity was expressed in weekly Metabolic Equivalent of Task (MET) hours, based on the International Physical Activity Questionnaire (IPAQ)[26] and the frequency of sporting activities was assessed in the questionnaire. During the interview, participants were asked whether they smoked and whether they had used any vitamin supplements during the past 30 days.

Sampling

We regarded the period from October to March as winter and the period from April to September as summer. Although the season is an established determinant of vitamin D status, it is a rather crude measure of (potential) sunlight exposure. We therefore calculated the mean amount of sunshine during the ten-week period prior to each interview and at the relevant research site, using publicly accessible data from the Royal Netherlands Meteorological Institute (KNMI, www.knmi.nl). The weekly number of sunlight hours was defined as the sum of all sub-periods in that week for which the solar irradiance exceeded 120 W/m^2.

Statistical analyses

Univariate associations between individual characteristics and the continuous 25(OH)D variable were established using linear regression. All candidate predictors were entered into a multiple linear regression model in order to examine the independence of determinants. Sex and seasonal differences in the prevalence of vitamin D insufficiency were tested using chi-square tests. Data were analysed using IBM SPSS Statistics for Windows version 20 (IBM Corporation, Armonk, NY, USA).

Table 1. *Characteristics of the study population (n = 539)*

	Percentage or mean (± SD)
25-Hydroxyvitamin D levels	
Mean 25(OH)D (nmol/l)	68.0 (± 27.2)
Sufficiency:	
- < 25 nmol/l (<10 ng/ml)	5.0%
- 25-50 nmol/l (10 – 20 ng/ml)	21.5%
- 50-75 nmol/l (20 – 30 ng/ml)	33.4%
- > 75 nmol/l (>30 ng/ml)	40.1%
Sociodemographics	
Sex (female)	62.7%
Mean age (years)	39.2 (± 14.6)
Non-European ancestry:	
- One parent	4.45%
- Both parents	2.97%
Urban (> 1500/km^2)	72.6%
High educational level (college/university)	41.6%
Health and lifestyle characteristics	
Menopause (only among women)	30.1%
Oral contraceptives (only among women)	33.7%
Mean estimated GFR (ml/min/1.73 m^2)	106 (± 16.8)
Mean GGT (U/l)	24.2 (± 27.5)
Mean BMI	24.8 (± 4.53)
Mean sports activities (times/month)	3.80 (± 3.14)
Mean physical activity (MET-hours/week)	65.0 (± 52.0)
Mean alcohol use (drinks/week)	7.22 (± 9.13)
Smoking (yes)	27.3%
Vitamin supplements (yes)	13.0%
Sampling variables	
Mean storage of biobank sample (years)	5.22 (± 0.62)
Mean sunshine (hours/week over 10 weeks)	50.7 (± 19.3)
Season (winter)	49.0%

BMI = body mass index; GFR = glomerular filtration rate; GGT = gamma-glutamyl transferase; MET = metabolic equivalent task.

RESULTS

Description of the population

Table 1 shows the characteristics of the 539 subjects, of whom 62.7% were female. The mean age was 39 years (SD 14.6) and the mean level of 25(OH)D was 68.0 nmol/l (SD 27.2). Levels above 75 nmol/l were detected in 40.1% of our sample. Levels below 50 nmol/l were observed in 26.5%, and occurred more frequently in men (33.4%) than in women (22.5%, p = 0.006).

Determinants of 25(OH)D levels

Table 2 lists the association of the variables with 25(OH) D levels, both in a univariate and in a multiple regression analysis. The multiple regression model explained 30.2% of the variance; the variance explained by only those eight variables that were significant was 29.5%.

Sociodemographics

Mean levels of 25(OH)D were 61.8 nmol/l in men and 71.6 nmol/l in women, which was a significant difference ($F_{1,538}$ = 16.9, p < 0.001). In the multiple regression model, sex remained highly significant (p = 0.001). Whereas age is commonly considered a determinant of vitamin D levels, no such association was found in the univariate analysis (B = -0.123, p = 0.125). As age might have a differential effect in men and women, we explored the association in both sexes separately using a regression model including sex, age, and their interaction. We found that 25(OH)D decreased with age in women (B = -0.274, p = 0.009), but not in men (B = 0.139, p = 0.244). There was a significant interaction between sex and age (p = 0.011).

We further examined whether the effect of age in women was driven by menopausal status or the use of oral contraceptives. No significant difference was found between premenopausal and postmenopausal women ($F_{1,331}$ = 1.33, p = 0.250). Women who used oral contraceptives, however, had significantly higher 25(OH)D levels than those who did not (80.6 nmol/l vs. 67.1 nmol/l, $F_{1,337}$ = 19.0, p < 0.001) and they were also younger (29 years vs. 44 years, $F_{1,337}$ = 113.8, p < 0.001). After adjustment for the use of oral contraceptives, age was no longer significantly associated with 25(OH)D in women (p = 0.578). In the multiple model with all predictors, neither age (p = 0.831) nor the sex x age interaction (p = 0.167) remained significant.

Respondents with two non-European biological parents had significantly lower levels of 25(OH)D (34.6 nmol/l vs. 69.6 nmol/l respectively, $F_{1,514}$ = 27.4, p < 0.001) than those with two European parents. Respondents who were the child of a mixed couple had intermediate levels (57.3 nmol/l), differing significantly from both the European (F1,522 = 4.89, p = 0.027) and the non-European group ($F_{1,39}$ = 7.30, p = 0.010). Being the child of one or two non-European parents remained a significant predictor in the multiple regression analysis (p < 0.001). Respondents living in urban areas had lower 25(OH)D levels than those in less urban areas ($F_{1,535}$ = 9.19, p = 0.003). This association, however, lost significance in the multiple model (p = 0.175). Having a college or university degree was not related to 25(OH)D levels ($F_{1,537}$ = 0.15, p = 0.698).

Health and lifestyle indicators

A negative association of 25(OH)D with GGT was initially found (p = 0.001), but it did not remain significant in the multiple model (p = 0.122). The negative association of 25(OH)D with eGFR, which is calculated from creatinine levels, age, and sex, was not significant in the univariate analysis (p = 0.493), but highly in the multiple regression analysis (p = 0.005).

The BMI was significantly associated with lower 25(OH)D, both in the univariate and the multiple analyses (p < 0.001). The frequency of doing sports activities was a significant factor in both the univariate and the multiple regression analyses (p values < 0.001), whereas physical activity expressed in weekly MET-hours was not (p = 0.568 and p = 0.823). The consumption of alcohol appeared to have a positive association with 25(OH)D in the multivariate model only (p = 0.001). We further examined whether elevated levels of 25(OH)D could be found in moderate as well as in heavy drinkers. Both moderate and heavy alcohol users had 25(OH)D levels that were significantly higher than subjects who drank no more than one glass per week (p < 0.001 and p = 0.026, respectively). Vitamin D status did not differ between moderate and heavy drinkers (p = 0.959). Smoking was not associated with 25(OH)D (p = 0.664). The use of vitamin supplements was correlated with higher 25(OH) D levels (p = 0.023), but did not remain significant in the multiple regression (p = 0.124).

Sampling

Plasma samples were collected over a period of two and a half years and 25(OH)D levels were assessed four years after collection of the last sample. We found no evidence of degradation over time (p = 0.756). Concentrations of 25(OH)D were higher in summer than in winter (73.9 nmol/l vs. 62.3 nmol/l, respectively, p < 0.001). Levels of 25(OH)D were highest in August, when the amount of sunshine is at its peak (*figure 1*). The amount of sunshine during the ten weeks prior to blood draw was highly significant in the multiple model (p < 0.001).

DISCUSSION

Among a group of healthy adults in the Netherlands, at a northern latitude (52 °N), 26% had plasma 25(OH)D under 50 nmol/l and 40% had levels above 75 nmol/l. We identified eight determinants of plasma levels of 25(OH)

Table 2. *Associations of sociodemographic, health, lifestyle and sampling variables with 25(OH)D levels*

	Univariate			Multiple regression		
	B	**Significance**		**B**	**Significance**	
Sociodemographics						
Sex (female)	9.797	< 0.001	*	13.86	0.046	*
Age	-0.123	0.125		0.032	0.831	
Interaction age x sex	-	-		-0.218	0.167	
Non-European ancestry (per parent)	-16.12	< 0.001	*	-12.78	< 0.001	*
Urban (> 1500/km^2)	-7.904	0.003	*	-3.538	0.175	
High educational level (college/university)	0.921	0.698		-1.231	0.589	
Health and lifestyle indicators						
Oral contraceptives among women	13.58	< 0.001	*	8.587	0.007	*
eGFR (ml/min/1.73m^2)	-0.048	0.493		-0.250	0.005	*
GGT (U/l)	-0.142	0.001	*	-0.063	0.122	
BMI	-1.139	< 0.001	*	-0.921	< 0.001	*
Sports activities (times/month)	1.945	< 0.001	*	1.429	< 0.001	*
Physical activity (MET-hours/week)	0.013	0.568		-0.005	0.823	
Alcohol use (drinks/week)	0.053	0.678		0.403	0.001	*
Smoking	-1.141	0.664		-1.835	0.443	
Vitamin supplements	7.904	0.023	*	4.832	0.124	
Sampling variables						
Storage of biobank sample (years)	-0.718	0.703		0.540	0.756	
Sunshine (hours/week over 10 weeks)	0.521	< 0.001	*	0.485	< 0.001	*

BMI = body mass index; eGFR = estimated glomerular filtration rate; GGT = gamma-glutamyl transferase; MET = metabolic equivalent task; * p value < 0.05.

D: sex, oral contraceptive use, birth country of the parents, renal function, BMI, engagement in sports activities, alcohol consumption and the available amount of sunshine were independent significant determinants in a multiple regression model.

Sociodemographics

Since osteoporosis is most prevalent among postmenopausal women, it makes sense to examine the influence of sex and age on vitamin D status. Compared with several other European studies,[18,27,28] we found relatively high levels of circulating 25(OH)D and, surprisingly, levels were higher in women than in men. The higher levels in our population may be explained by the fact that we selected only healthy subjects instead of a random draw from the general population. Moreover, one study sampled in the winter,[28] when levels are lowest. Our finding that women had higher levels than men may be explained by the distribution of age in our sample: 42% of our subjects were under the age of 35, whereas the aforementioned studies included subjects with a minimum age of 35 years. Our result that 25(OH)D levels were higher in women than in men applied to these younger subjects, but not to the group above 35 years of age (results not shown).

Although it has been established that the ageing skin produces less vitamin D,[29] it does not explain the interaction of age with sex. The use of oral contraceptives, however, is age-dependent but also associated with 25(OH) D levels.[30] This finding is confirmed by our results: 25(OH)D levels were 20% higher in oral contraceptive users. Correspondingly, age was no longer significantly correlated with 25(OH)D when adjusting for the use of oral contraceptives, indicating that the age effect in women was driven by the use of oral contraceptives.

The association between the use of oral contraceptives and 25(OH)D levels has been shown repeatedly.[30-33] In addition to these cross-sectional studies, Harris also found that 25(OH)D dropped in women who discontinued oral contraceptives, although their number was small.[34] The inverse effect of vitamin D on oestrogen levels, however, is more controversial. A stimulating influence of 1,25$(OH)_2$D on oestrogen production in women has been found,[35] while others reported a negative association between the two.[18,36]

Clearly, the mechanisms of interplay between vitamin D and oestrogens needs further investigation.

Our results confirm that in the Netherlands, people with a non-European ancestry have significantly lower 25(OH)D levels than native Europeans, and that respondents with a mixed background have intermediate levels. Because skin colour was not recorded in the NESDA cohort, this finding must be interpreted with caution. But as the most commonly reported birth countries in this group were Surinam, Indonesia and the Dutch Antilles, it may be assumed that their average skin tone was darker than the average in the Netherlands. It has been recognised that a darker skin synthesises less vitamin D upon the same exposure to UV light,[37] but other explanations for the decreased 25(OH)D levels may be found in diet and sun-avoiding behaviour.[14,38]

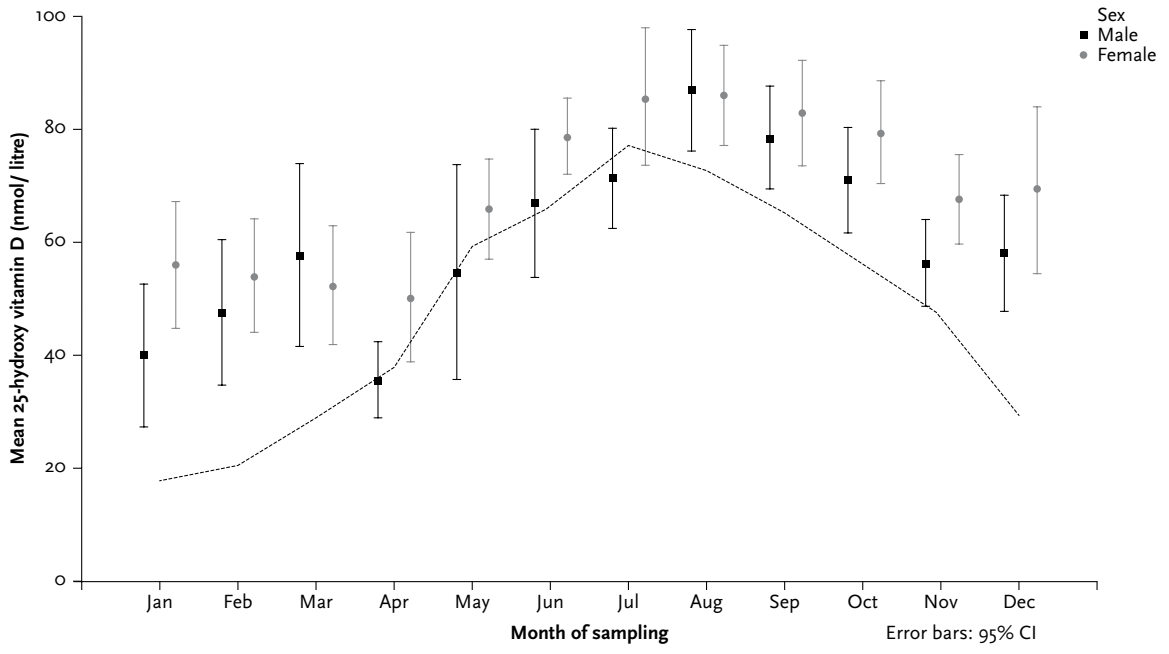
Clinical hypovitaminosis used to occur majorly at the time of industrialisation when people in the cities were insufficiently exposed to sunlight. In our study sample, urbanisation was not significantly correlated with 25(OH)D levels when ancestry was entered into the multiple regression model. Respondents with one or more non-European parents were more likely to live in urban areas than those with a European origin (97.5% vs. 71.7% respectively).

Socioeconomic status is generally considered to be a predictor for different health indicators. Whereas it has been reported that low-income groups have decreased 25(OH)D levels,[39] a recent study found an association with income but not educational level.[40] Similarly, our results do not reveal an association of 25(OH)D with educational attainment.

Health and lifestyle indicators

As the liver and kidney play an important role in the metabolism of vitamin D, we measured GGT and the eGFR as indicators of hepatic and renal function, respectively. Increased GGT levels can indicate impaired hepatic function, which might relate to a decreased production of 25(OH)D. This association was confirmed in the univariate analysis, but did not remain significant in the multiple regression model. Whereas impaired renal function is related to a decreased 25(OH)D,[41] our results show that an increased eGFR may correlate with decreased levels of 25(OH)D within healthy subjects. We suggest that an elevated eGFR may indicate a higher hydroxylation rate of 25(OH)D into 1,25$(OH)_2$D, resulting in lower 25(OH)D values, but an increase of readily available 1,25$(OH)_2$D. Support for this hypothesis can be found in a recent study that showed a direct relation between eGFR and circulating levels of 1,25$(OH)_2$D.[42]

Figure 1. *Fluctuation of 25(OH)D levels over the months of the year in men (n = 201) and women (n = 338). The grey line shows the mean weekly hours of sunshine in the ten weeks prior to sampling, ranging from 17 hours/week in January to 76 hours/week in July*



Evidence suggests that vitamin D status is associated with physical fitness. We confirm earlier findings that people with an increased BMI have lower 25(OH)D levels,[43,44] but the relation between 25(OH)D and physical activity is not so straightforward. Our results show that respondents who did sports had higher levels of 25(OH)D, while the association with physical activity expressed in weekly MET-hours was not significant. This corresponds with earlier indications that vigorous, but not moderate physical activity, is associated with a higher 25(OH)D.[45] Correspondingly, an association with physical fitness but not physical activity has been found,[46] although such cross-sectional results may be caused by the positive effect of vitamin D on muscular health.[47] Furthermore, as people who do sports might spend more time outdoors or have a healthier diet, we must conclude that the current results provide insufficient evidence to disentangle whether physical activity has a direct or indirect link with 25(OH)D. Although alcoholism is associated with a decrease in 25(OH)D, moderate consumption has been reported to have a positive influence on bone mineral density and 25(OH)D.[48] Correspondingly, we found that consumers of alcohol had higher 25(OH)D than those who drank no more than one glass per week, resulting in a positive effect of alcohol consumption in the multiple analysis. In spite of the fact that 10.8% of the study sample were labelled as heavy drinkers, excessive alcohol use was rare in our sample (1.1% showed alcohol dependence according to the AUDIT). Therefore, these data do not provide sufficient information to speculate about the consequences of alcoholism on vitamin D status.

Our results do not confirm previous findings that indicated an association between smoking and vitamin D intake and circulating levels, and bone density.[49-51] In general, however, these associations have only been found in postmenopausal women, while the majority of our sample consisted of men and premenopausal women.

The use of vitamin supplements was correlated with increased plasma 25(OH)D, but did not remain significant in the multiple model. It would, however, be premature to conclude that the use of vitamin supplements has no effect on vitamin D status at all. One limitation in the ascertainment of vitamin use is the fact that multivitamin tablets were included, while the presence and amount of vitamin D in such preparations was unknown. Also, since no more than 13% of the study population used these supplements, the statistical power is limited.

Sampling

Our data confirm earlier findings that samples do not show deterioration while stored at -80 °C for an extended period of time, but that the season is an important determinant.[52] In the summer months, 80.3% had levels of at least 50 nmol/l. This percentage was significantly lower in winter (66.9%, $p < 0.001$). As *figure 1* shows, the number of sunshine hours is around four times higher in July than in January but, in addition, the intensity of UV radiation is higher in the summer months, more time is being spent outside, and less skin is generally covered by clothes.

Strengths and limitations

Whereas vitamin D status is commonly studied in elderly populations, the present study examined determinants of plasma 25(OH)D in healthy adults between 18-65 years of age. The strengths of our analyses are the fact that mental and physical diseases were absent, and the simultaneous ascertainment of sociodemographic, health and lifestyle indicators, and sampling variables. In particular, the use of a quantified measure for availability of sunshine proved to be a much stronger predictor for 25(OH)D levels than merely the season. A limitation, however, was the lack of information about actual time spent in the sunshine and about dietary intake and supplementation of vitamin D.

CONCLUSION

Within a group of healthy adults at a northern latitude, 27% had insufficient 25(OH)D levels (< 50 nmol/l) and only 40% reached the recommended levels (> 75 nmol/l). A wide range of determinants influenced 25-hydroxyvitamin D levels: male gender, non-European ancestry, an elevated BMI, an increased eGFR and sampling in a season with less available sunlight were all associated with lower 25(OH)D levels. Those who participated in sports, consumed moderate amounts of alcohol and women who used oral contraceptives had higher 25(OH)D levels. This set of eight variables explained 29.5% of the variance. Our results confirm the role of these predictors, but disconfirm the influence of age and urbanisation in a healthy adult population under 65 years of age.

DISCLOSURES

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SPECIAL REPORT

The diagnostic tangle of pyoderma gangrenosum: a case report and review of the literature

E. Bakelants[1], J. van der Hilst[1,4], L. Corluy[2], R. Achten[3,4], I. Gyssens[1,4,5], P. Messiaen[1]*

Departments of [1]Infectious Diseases and Immunity, [2]Rheumatology, [3]Pathology, Jessa Hospital Hasselt, Belgium, [4]Hasselt University, Hasselt Belgium, [5]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, the Netherlands, *corresponding author: email: peter.messiaen@jessazh.be

ABSTRACT

This report describes a 55-year-old patient with the rare inflammatory dermatosis pyoderma gangrenosum. It is an often misdiagnosed condition of unclear origin and pathogenesis. There is an association with underlying systemic disorders such as inflammatory bowel disease, haematological disorders, rheumatological disease or solid malignancies, although this last association is still under investigation. The diagnosis can be challenging and treatment depends upon the severity of the lesions. The long-term prognosis is unpredictable.

KEYWORDS

Pyoderma gangrenosum, ulcerative dermatosis, sterile necrosis

What was known on this topic?
Pyoderma gangrenosum is a rare, non-infectious and painful ulcerative disease, belonging to the spectrum of neutrophilic dermatoses, which is often mistaken for an infection. It can be associated with a variety of severe comorbid conditions.

What does this add?
This well-illustrated case aims at increasing the physician's awareness for this condition. The diagnostic features and current therapeutic recommendations are briefly reviewed.

INTRODUCTION

Pyoderma gangrenosum is a rare inflammatory neutrophilic dermatosis with poorly understood aetiology and pathogenesis. In up to 50% of cases, the disorder is associated with an underlying systemic disease. It presents as an inflammatory papule rapidly evolving into an ulcer with undermined borders with a necrotic and mucopurulent base. It is an often misdiagnosed condition.[1-3]

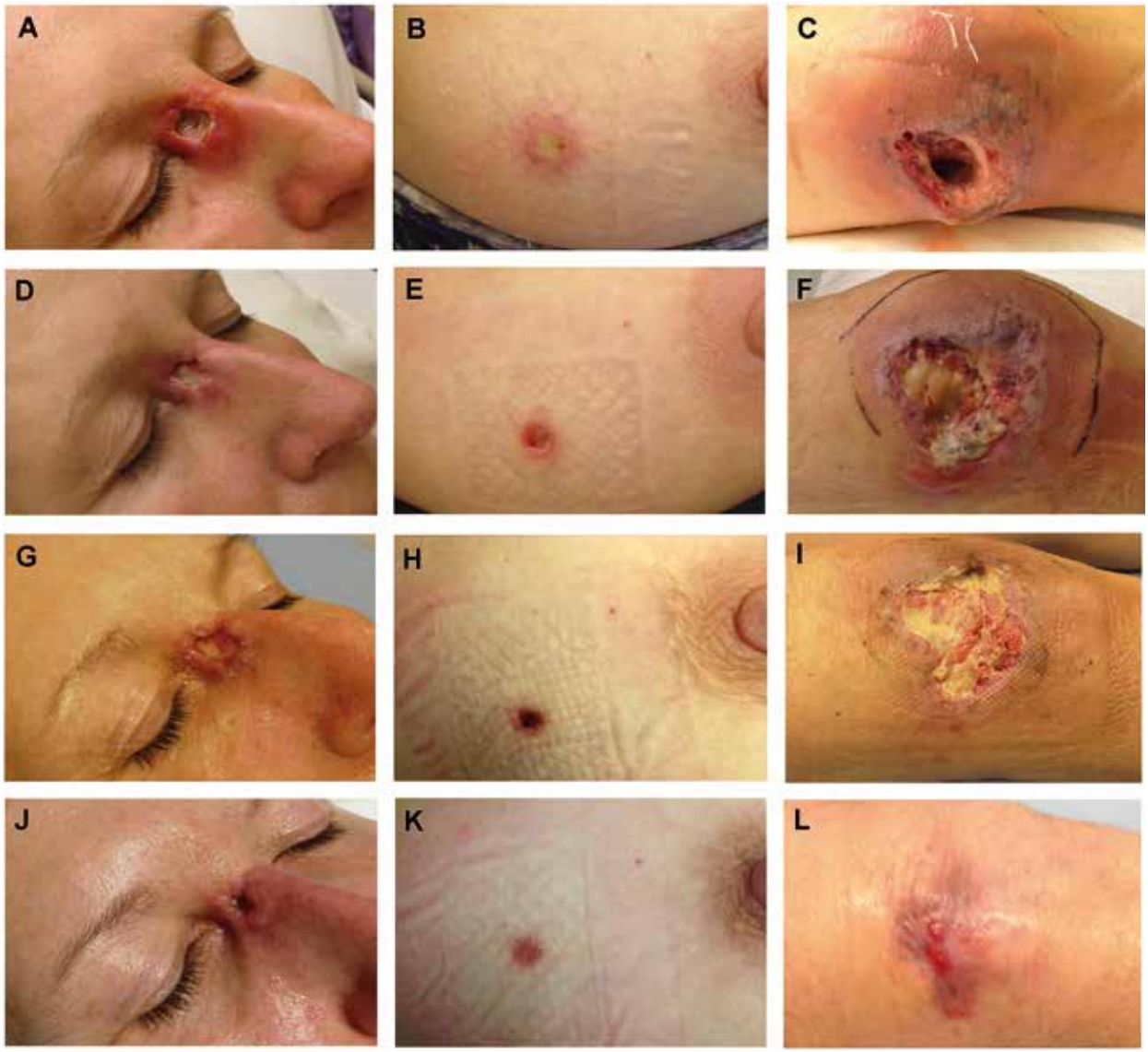
This report describes a 55-year-old patient with pyoderma gangrenosum, illustrated with sequential clinical pictures spanning the complete disease course towards resolution. We briefly review the current understanding, diagnosis, and management of this disease.

CASE REPORT

A 55-year-old woman with a history of ulcerative colitis was admitted to hospital because of suspected arthritis of the right knee starting 14 days earlier. She had taken NSAIDs with only temporary relief. Three days before admission a synovial fluid aspiration was performed because of persistent pain and swelling of the right knee. The aspirate contained few leukocytes, no crystals and bacterial cultures remained negative. Given the lack of clinical improvement and the inconclusive results of the aspiration, she was admitted to hospital. Temperature at admission was 37.3 °C. On clinical examination, her right knee was red, swollen, and tender. In addition, a small red furuncle-like nodule was observed on the nasal bridge.

The haemoglobin was 10.5 g/dl, leukocyte count 7.4 x 10^9/ml without left shift and C-reactive protein was 340 mg/l. The laboratory values for liver and kidney function as well as the electrolyte panel were unremarkable. There were no bone lesions visible on X-ray of the right knee. Ultrasound examination showed a significant amount of intra-articular fluid and a new puncture was performed. The differential diagnosis at that time included gout, rheumatological systemic disorder and infectious causes such as septic arthritis, *Borrelia burgdorferi* or gonococcal infection. Also, on account of a recent flare-up of underlying ulcerative colitis, inflammatory bowel disease-associated arthropathy was considered. Because of the limited response to paracetamol and NSAID so far, an empirical therapy with cefuroxime 1.5 g three times a day intravenously (TID IV) was started immediately after the puncture. Again the aspirate yielded no crystals, a negative Gram staining and 2250 leukocytes per µl. Bacterial cultures remained negative. One day later, a blister of 3 cm developed at the puncture site, spontaneously draining purulent haemorrhagic fluid. Within a few hours a necrotic ulcer developed with blue edges (*figure 1C*). Underneath the ulcer there was a large zone of subcutaneous fat necrosis reaching the fascia. On suspicion of fasciitis necroticans, cultures were taken from the wound and the antibiotic therapy was switched to meropenem (1g TID IV) and clindamycin (600 mg TID IV). Meanwhile, the lesion on the nasal bridge had enlarged and yielded a large amount

Figure 1. *Consecutive clinical pictures of the three lesions*



Clinical presentation of the pyoderma gangrenosum lesions of the nasal bridge (left), right breast (middle) and right knee (right) three days after admission (A-C), and one week (D-F), two weeks (G-I) and four weeks (J-L), respectively, after starting high-dose corticosteroid treatment. Informed consent was obtained from the patient for the use of these clinical photos.

of pus at incision (*figure 1A*). Shortly thereafter a new lesion developed on the right breast (*figure 1B*).

Given the negative blood, tissue and synovial fluid cultures and the lack of clinical and biochemical improvement despite broad-spectrum antibiotics, the development of a new plane ulcer on the right breast and the underlying inflammatory bowel disease, the diagnosis of pyoderma gangrenosum was considered. High-dose methylprednisolone (1 mg/kg) was administered intravenously after which progressive healing of the ulcers ensued (*figure 1D-L*). The anatomopathological examination of a punch biopsy of the knee lesion sampled before the administration of corticosteroids showed an infiltrate with a mixed composition, associated with signs of vasculitis and subepidermal oedema. These were compatible with the diagnosis of pyoderma gangrenosum.

DISCUSSION

Initially described as painful enlarging necrotic ulcers by Brunsting and colleagues in 1930, pyoderma gangrenosum was believed to be the spread of a bacterial infection leading to cutaneous gangrene.[4] According to current insights, it is a rare inflammatory skin disorder and part of the spectrum of neutrophilic dermatoses. Up to 50-70% of cases are associated with an underlying systemic disease such as inflammatory bowel disease, haematological disorders, rheumatological disease or solid malignancies, although some debate remains on the last-mentioned association.[3,6,10,11] The incidence is estimated at 3-10 patients per million population per year, with a peak incidence between 20-50 years and a slight female predominance.[2,3,5,6] Patients can present with a sterile inflammatory papule, pustule or vesicle, either 'de novo' or after (minimal) trauma or surgery. Often these lesions remain unrecognised as pyoderma gangrenosum because they look unimpressive at first sight.[7-9] The lesions expand and form an ulcer, producing a purulent and haemorrhagic exudate. The ulcers show an irregular necrotic or mucopurulent base with an erythematous radiance. Patients have mostly single and rarely multiple lesions, all in different stages of development.[3,5-7] An exacerbation of pyoderma on sites of trauma such as biopsy, surgery, insect bites, or injections is commonly known as *pathergy*. This phenomenon is seen in 20% of patients.[3,5-9]

Our patient presented with a nodule on the nasal bridge draining pus on incision, mimicking a furuncle but rapidly evolving into a widening ulcer. Development of the large ulcers after puncture, as well as the development of a new lesion on the breast, together with sterile cultures eventually led to the diagnosis.

Pyoderma gangrenosum is a diagnosis of exclusion. There is no specific biochemical test available to confirm the diagnosis. Diagnostic criteria have been proposed (*table 1*). The diagnosis can be supported if two major criteria and at least two minor criteria are present.[3,10] Biopsy is indispensable in ruling out alternative diagnoses and this outweighs the risk of possible local disease progression (pathergy).[3,5,10]

The treatment of pyoderma gangrenosum is challenging since no randomised controlled trials are available to guide therapy (*table 2*). In milder forms, topical treatment with corticosteroid or tacrolimus ointment may suffice.[6,11,12] In more severe cases systemic treatment is necessary and will depend on the associated underlying disorder. Current recommendations state high doses of corticosteroids (e.g. methylprednisolone 0.5-1.5 mg/kg orally per day) to induce remission, with gradual tapering over 4-6 weeks.[3,10,12,13] Cyclosporine A has become an accepted treatment for widespread pyoderma gangrenosum after initial steroids or in combination with steroids, starting at 4-5 mg/kg/day orally. The drug induces an early response but has no impact on the incidence of recurrences.[12,13] Azathioprine (100-150 mg/day) in combination with steroids for induction therapy has to be considered when the underlying disease is ulcerative colitis or Crohn's disease.[12,13] Complete withdrawal of therapeutics may be possible, but a low-dose maintenance therapy is often required and the long-term outcome is unpredictable.[11-14] Sulpha drugs are useful in milder cases: the combination of steroids with dapsone up to 200 mg daily has been

Table 1. *Diagnostic criteria for pyoderma gangrenosum (adapted from Su et al. and Al Ghazal et al.[3,10])*

Major criteria
1. Sterile pustule or ulcer with a violaceous and undermined border
2. Other relevant differential diagnoses have been excluded (this usually involves skin biopsy).
Minor criteria
1. History suggestive of pathergy
2. Histopathological findings showing sterile dermal neutrophilia and/or mixed inflammation and/or lymphocytic vasculitis
3. Underlying systemic disease associated with pyoderma gangrenosum
4. Response to systemic immunosuppressive therapy
5. Extremely painful ulcer (out of proportion to size of ulceration, VAS > 4)
Major and minor diagnostic criteria which can be used to support the diagnosis of pyoderma gangrenosum. Two major criteria and at least two minor criteria are required. VAS = visual analogue score.

Table 2. *Current treatment recommendations for pyoderma gangrenosum*

Disease stadium dose	Treatment	Recommended dose
Limited disease	Topical corticosteroid	Clobetasol 0.05% ointment
	Topical tacrolimus	Tacrolimus 0.03-0.1% ointment
Extensive disease	*First-line induction therapy*	
	Systemic glucocorticoids	Prednisone 0.5-1.5 mg/kg/day
	± Cyclosporine	4-5 mg/kg/day
	± Azathioprine	100-150 mg/day
	Second-line	
	Cyclosporine	4-5 mg/kg/day
	In conjunction with corticosteroids:	
	Thalidomide	
	Dapsone	200 mg/day
	Methotrexate	
	Infliximab	5 mg/kg single dose
	Anakinra	

Overview of the current treatment regimens for different stages of pyoderma gangrenosum. When available, the recommended dose is mentioned.

described.[13,14] Methotrexate and thalidomide have been used as well but are generally more effective as adjunctive therapy rather than first-line agents.[13-15] Recent studies show favourable results with anakinra, an IL-1 receptor antagonist and single-dose infliximab, etanercept and adalimumab, monoclonal chimeric antibodies against tumour necrosis factor alpha, although worsening of pyoderma gangrenosum has been described after the use of the last two as well.[13-15]

CONCLUSION

This case shows that pyoderma gangrenosum is a challenging condition which needs to be considered in patients with unusual skin ulcerations and sterile necrosis not responding to systemic antibiotic treatment. The pathogenesis is poorly understood and validated diagnostic tests are lacking. Clinicians should be aware of this condition in order to improve recognition and treatment. Abstaining from surgical debridement results in optimal chances of healing without residual scarring.

DISCLOSURES

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CASE REPORT

Hypocitraturia: a common but not well-known cause of nephrolithiasis

S. Bos[1]*, R.R.H. Nap[2], R.S.M.E. Wouters[3], F.G.H. van der Kleij[4]

Departments of [1,2,3,4]Internal Medicine, [2,4]Nephrology, [3]Endocrinology, Scheper Hospital Emmen, the Netherlands, *corresponding author: tel.: +31(0)59-1691360, email: sarah.bos@sze.nl

ABSTRACT

Nephrolithiasis is a frequent problem that can cause serious morbidity. When associated with an underlying metabolic disorder the recurrence rate is higher. Hypocitraturia is estimated to be present in 20-60% of cases. Several secondary causes are known. Potassium citrate is the primary treatment. In the case we present here we emphasise the need for metabolic screening, focussing on hypocitraturia, a less well-known cause of nephrolithiasis.

KEYWORDS

Hypocitraturia, metabolic screening, nephrolithiasis

What was known on this topic?
Nephrolithiasis is a frequent problem that occurs worldwide and can cause serious morbidity. Obstruction, infection and deterioration of kidney function were not uncommon a few decades ago. Although new therapies have been introduced, high recurrence rates still put patients at risk for these complications. When renal stones are the symptom of an underlying metabolic disorder there is a higher risk of recurrence.

What does this case add?
This case underlines the importance of early metabolic screening for patients with recurrent nephrolithiasis, and awareness of the diagnosis of hypocitraturia. This approach will help to predict recurrent stone formation and further complications.

BACKGROUND

Nephrolithiasis is a frequent problem that can cause serious morbidity. Obstruction, infection and deterioration of kidney function were not uncommon a few decades ago. Although new therapies have been introduced, an overall recurrence rate of 50-75% still puts patients at risk for these complications. When renal stones are the symptom of an underlying metabolic disorder there is a higher risk of recurrence.[1] Therefore we emphasise the importance of screening for secondary causes. In this case we demonstrate the need for metabolic screening, with a focus on hypocitraturia, a less well-known cause of nephrolithiasis.

CASE REPORT

A 37-year-old woman was referred by the urologist because of recurrent nephrolithiasis. She has suffered many episodes of nephrolithiasis. In total she had undergone 15 extracorporeal shock wave lithotripsy therapies and two ureterorenoscopies. However, a recent X-ray still showed the presence of four concrements. Because of persistent left-sided lumbar pain and impaired renal function of the left kidney, a nephrectomy was carried out. Urine saturation and stone analysis were not performed. She was on a normal diet and was not taking calcium or vitamin D supplements.

On physical examination she had a blood pressure of 120/80 mmHg. Further examination was unremarkable. Laboratory results revealed a serum creatinine of 66 μmol/l, serum potassium of 3.9 mmol/l, serum bicarbonate of 26 mmol/l and a normal level of serum calcium. The 24-hour urine sample showed a total urine volume of 1455 ml/24 hour, calcium of 3.0 ml/day and

normal levels of uric acid and oxalic acid. However, the 24-hour urine sample revealed a profound hypocitraturia of 0.9 mmol/24 hours (n = 2.2-4.4 mmol/24 hours). Repeated 24-hour urine collection showed similar results. As the other laboratory results were normal, no secondary cause was found for the hypocitraturia.

After treatment with potassium citrate, the urinary excretion of citrate normalised and serum potassium remained within normal values. Since the start of potassium citrate, no new episodes of nephrolithiasis have occurred.

DISCUSSION

Nephrolithiasis is a very common problem in which calcium stones are most frequently found. Recurrence rates among idiopathic stone formers is approximately 40-50% and even higher in the presence of an underlying metabolic disorder.[1] A thorough work-up will assess the aetiology of nephrolithiasis in up to 30-90% of patients.[2,3] Hypercalciuria, hyperoxaluria, hyperuricosuria and hypocitraturia are the metabolic disorders which are most commonly found (*table 1*). Hypocitraturia is estimated to be present in 20-60% if calcium stones are detected.[1,4] Several secondary causes concerning hypocitraturia are known: renal tubular acidosis, malabsorption, potassium deficiency, low intestinal alkaline absorption, low urinary calcium level and low urine volume (*table 1*). In about 50% of the cases, no obvious cause can be found.[5,6] A urine citrate excretion below 1.7 mmol/24 hour in men and 1.9 mmol/24 hour in women is considered to be diagnostic for hypocitraturia.

There are three ways in which citrate normally plays a role in the prevention of calcium stone formation. First, citrate complexes with calcium in the renal tubulus causing a reduction of ionic calcium concentrations in the urine. Second, these citrate-calcium complexes limit calcium supersaturation. Finally, citrate binds to the crystal surface and prevents calcium oxalate and calcium phosphate crystal agglomeration and growth.

When hypocitraturia is found, potassium citrate is the primary treatment with potassium bicarbonate as an alternative.[7] Potassium citrate increases urinary citrate concentration, decreases urinary calcium excretion and enhances the inhibitory function of Tamm-Horsfall proteins on urine crystal growth. Besides the increase of citrate, potassium citrate also induces bicarbonate regeneration, which results in an increase in potassium and urine alkalisation. The latter directly effects distal tubular calcium channels and reduces bone turnover. Together this will diminish urine calcium excretion.[8] The alkalising of the urine has a less beneficial effect for calcium phosphate stone formers, but a beneficial effect for cysteine and uric acid stone formers. Long-term results show an important reduction of the recurrence of nephrolithiasis with potassium citrate treatment.[9]

In case of nephrolithiasis, and when hypercalciuria is present, another option in the pharmacological treatment is thiazide diuretics. These have also shown to be successful in reducing the recurrence rate. Thiazide diuretics enhance the reabsorption of calcium in the proximal and the distal tubule. This effect is most likely caused in two ways. First by stimulation of proximal tubular calcium reabsorption through contraction of extracellular fluid volume, which induces an increase in proximal tubular reabsorption of sodium and hence an increase of reabsorption of calcium. Secondly it is thought to be caused by a direct increase in calcium reabsorption in the distal tubule.[1,10] What is essential is that, in contrast to loop diuretics, thiazide diuretics act distal to the medullary ascending limb so they can enhance secretion of sodium without also increasing that of calcium.

Table 1. *Common metabolic disorders of nephrolithiasis*[1,4]

Hypercalciuria[A]	**Hyperoxaluria**[B]	**Hyperuricosuria**[C]	**Hypocitraturia**[D]
Hyperparathyroidism	High oxalate intake	Myeloproliferative disorders	Renal tubular acidosis
Immobilisation	Bowel pathology	High purine intake	Malabsorption
Metastatic tumours	Increased production of endogenous oxalate	Enzymatic defects	Metabolic acidosis
High dietary salt		Renal leakage	Potassium deficiency
High protein intake		Uricosuric drugs	Hypomagnesaemia
Range of monogenic disorders			Low urine volume
Absorptive hypercalciuria			Low urinary calcium level

[A,B,C,D] **Metabolic disorders and their known aetiologies**

A common side effect of thiazide diuretics is potassium loss. Low serum potassium is known to cause intracellular acidosis, which in turn will lead to hypocitraturia. To preserve the beneficial effect of thiazide it is advised to prescribe potassium supplements.[I]

Next to pharmacological therapy there have been suggestions for dietary adjustments depending on the causative metabolic disorder. Fluid intake over 2 litres/24 hours, limited sodium intake, less than 2 g a day and a moderate protein intake are the most common dietary recommendations.[I,II]

When therapy is only directed at the relief of symptoms, patients with recurrent nephrolithiasis are at risk of kidney function decline. As stated above, a substantial number of the patients have an underlying metabolic disturbance, which in most cases is relatively easy to treat. Unfortunately, nephrectomy had to be performed in our patient. This emphasises the need for early referral to clarify the cause of recurrent nephrolithiasis.

CONCLUSION

This case underlines the importance of early metabolic screening for patients with recurrent nephrolithiasis, and awareness of the diagnosis of hypocitraturia. This approach will help to predict recurrent stone formation and further complications.

DISCLOSURES

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

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CASE REPORT

Acute sinusitis and blindness as the first presentation of chronic lymphocytic leukaemia

K.H. Lim[1,2],*, G. Thomas[2], E.J. van Beers[3], A.E. Hosman[2], M.P. Mourits[4], C.J.M. van Noesel[5], A.P. Kater[3], S.M. Reinartz[2]

[1]Department of Otorhinolaryngology, Tan Tock Seng Hospital, Singapore, Departments of [2]Otorhinolaryngology, [3]Haematology, [4]Ophthalmology, [5]Pathology, Academic Medical Centre, Amsterdam, the Netherlands, *corresponding author: tel.: +31(0)20-5663789, fax: +31(0)20-5669662, email: lim_kenghua@yahoo.com.sg

ABSTRACT

Chronic lymphocytic leukaemia (CLL) is the most frequent form of leukaemia among adults in the Western world, presenting at a median age of 65 years. The diagnosis is usually made incidentally during routine blood examination while the disease is still in its early phase. We report a case of blindness of 24 hours due to acute sinusitis based on CLL localisation in a patient with undiagnosed CLL. Emergency endoscopic sinus surgery and intra- and extra-ocular orbital decompression were performed. The sinusitis resolved after surgery and intravenous antibiotics. Her vision improved within 24 hours and eventually recovered completely after six months. Her CLL remained in an indolent state, needing no active treatment. This case illustrates that blindness from a lymphoproliferative disorder may be treated with emergency endoscopic sinus surgery instead of conventional chemotherapy in order to salvage the vision first, even if the vision is lost for more than 24 hours.

KEYWORDS

Chronic lymphocytic leukaemia, blindness, sinusitis

What was known on this topic?
Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in adults. Ocular symptoms are uncommon in CLL. CLL is treated with immunochemotherapy. Blindness from orbital cellulitis usually originates from bacterial sinusitis. Urgent surgical decompression within 24 hours is needed to salvage the vision.

What does this case add?
Patients with undiagnosed early CLL can present with acute blindness due to orbital infiltration and orbital CLL involvement. This has not been reported in the literature. Visual loss in early CLL can benefit from surgical decompression instead of conventional chemoradiotherapy in order to achieve quick decompression of the orbit and hence salvage vision, even when blindness is present for more than 24 hours.

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is the most common form of leukaemia in adults, in Western countries, representing about 25-30% of all leukaemias.[1] Most patients are diagnosed incidentally during a routine blood investigation when they are still in the early phase of CLL.[2] Frequent presenting symptoms include lymphadenopathy, hepatosplenomegaly and/or cytopenias, and some cases present with unintentional weight loss fever, night sweats, or extreme fatigue.[3]

Ocular symptoms are uncommon and, to our knowledge, especially acute blindness due to fulminant orbital cellulitis has not previously been reported as the presenting symptom in CLL. Moreover, it is unusual to recover vision if there is a delay in intervention. Our patient's vision completely recovered after endoscopic sinus surgery and surgical decompression of the orbital content.

CASE REPORT

A 59-year-old female with left periorbital swelling and blindness for 24 hours was referred to us. She had a three-week history of flu-like symptoms with cervical lymphadenopathy, and four days of left periorbital pain with progressive periorbital swelling. The vision in her left eye rapidly deteriorated to complete blindness during the evening before she sought help. She had bilateral rhinorrhoea, hyposmia and nasal congestion. She had no history of diabetes or any other immunocompromising disease. She was febrile but had no signs and symptoms of sepsis. She had severe left periorbital oedema, proptosis with absent light and red reflex, and no extraocular eye movement. Nasendoscopy showed bilateral purulent discharges and inflamed nasal mucosa. Computed tomography (CT) showed left-sided pansinusitis, left proptosis and diffuse infiltrates within the orbit but no obvious orbital abscess (*figure 1*). She was started on intravenous amoxicillin/clavulanate.

Her laboratory results were as follows: leucocyte count 61.2 x 10^9/l (neutrophils 44%, lymphocytes 48% and monocytes 8%), haemoglobin 12.0 g/l, thrombocyte count 365 x 10^9/l, C-reactive protein 276 mg/l, lactate dehydrogenase 169 U/l, serum glucose 7.5 mmol/l, and the plasma levels of immunoglobulins were normal (IgA 1.75 g/l, IgG 6.9 g/l, IgM 0.47 g/l).

Our primary working diagnosis was a left sinogenic, likely bacterial, orbital cellulitis with blindness. Leukaemia was suspected in view of her leucocytosis. The chance of her recovering her vision was low since it had been more than 24 hours. Nevertheless, surgical drainage to decompress the orbit was the only option to save the patient's vision. Hence, after informed consent, an emergency external lateral orbital decompression and complete fronto-maxillo-ethmoidectomy with medial orbital decompression was performed. Purulent fluid was drained from the sinuses. Unexpectedly, no pus was found during the orbital decompression. No bacteria were cultured. Tissue biopsies from the paranasal sinus and lamina papyracea showed dense infiltrates dominated by a monotonous population of small lymphocytic cells that expressed CD20, CD23 and CD5 (*figure 2*). Molecular analyses showed immunoglobulin heavy chain gene rearrangement. Immunophenotyping of peripheral blood showed expansion of CD5 and CD23 positive B cells. Total number of circulating leukaemia cells (CD5/CD19/CD23 triple positive) was 29.4 cells/ul. Immunoglobulin levels were normal. A diagnosis of CLL was made, clinically staged Rai 0 or Binet A. She improved clinically after surgical decompression. Hence, anti-leukaemic therapy was not started.[3,4]

Figure 1. *Contrast-enhanced CT scan of orbit/sinuses illustrating left ethmoiditis and left proptosis secondary to intra-orbital infiltrates*

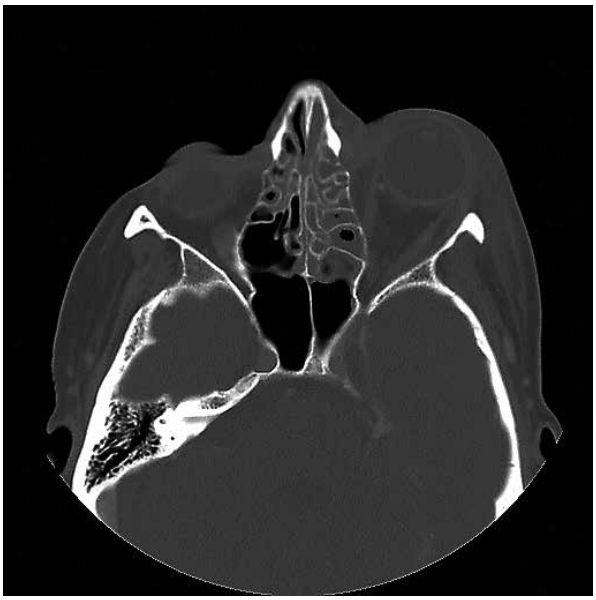
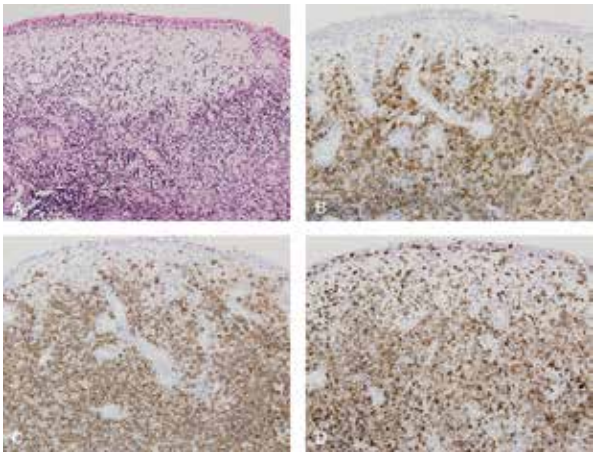


Figure 2. *Mucosal biopsy from the paranasal sinus infiltrated by CLL cells, stained with (A) haematoxylin&eosin and with antibodies specific for (B) CD20, (C) CD23 and (D) CD5. The CLL cells demonstrate moderate expression of CD5 as opposed to the strongly CD5-expressing T cells (100x magnification)*



After surgery, the patient's perception to light improved within 24 hours. Her vision was better two weeks later, and had completely recovered within six months. She had residual diplopia for three months. Besides the first episode, her CLL remains asymptomatic with stable peripheral blood counts and no other signs of disease progression. Therefore, she is currently being monitored without therapy.

DISCUSSION

The clinical presentation of our patient was caused by the infiltration of sinus mucosa and orbital content by malignant B cells displaying a CLL phenotype. This resulted in sinusitis, orbital cellulitis and subsequently blindness.

Sinusitis in early CLL is not common. Sinusitis in advanced stages of CLL is usually due to increased susceptibility of bacterial infections due to acquired hypogammaglobulinaemia or (following T cell depleting treatment) due to an opportunistic infection.[5] The spread of infection from the sinus to the eye is through the lamina papyracea or by venous spread. Complications of sinusitis, if not treated promptly, can lead to visual loss, meningitis, septicaemia and death.[6] Visual loss can occur because of central retinal artery occlusion, optic neuritis, corneal ulceration, pan-ophthalmitis and orbital apex syndrome.[7]

Unlike most sinusitis, our patient's sinusitis and blindness was due to infiltration of CLL in both the sinus mucosa and orbital content. We hypothesised that mucosal infiltration by clonal lymphocytes obstructed the sinus drainage pathway and augmented local inflammation and promoted its spread.

Progressive loss of visual acuity is very rare as an early clinical manifestation of CLL.[8] There were case reports of progressive visual loss in patients who were either in remission or with known CLL in early stage who had not started treatment.[9,10] Gonsalves et al. reported a case of progressive visual loss that was caused by optic neuritis in a previously untreated patient with early stage CLL, in whom a complete resolution of visual defects was achieved with chemoimmunotherapy using fludarabine and rituximab.[10]

Sinogenic orbital cellulitis requires aggressive treatment with intravenous antibiotics, early imaging to exclude abscess and close monitoring.[11] Surgery is required if symptoms do not improve after 24 hours of appropriate antibiotic treatment, or sooner in case of visual loss or orbital abscess. In our patient, contrast-enhanced CT showed diffuse infiltrates in the left orbit without signs of subperiosteal or orbital abscess. Indeed, during surgery no abscesses were found. However, always keep in mind that CT and even MRI have a false-negative rate of 10-15% for subperiosteal abscess.[12,13] Therefore, a reduced visual acuity alone is a good indication for imminent endoscopic sinus surgery and medial orbital decompression.[11,13] External orbital exploration and drainage is required in case of lateral orbital abscesses. Longer duration of blindness predicts poorer visual outcome.[7] Magnetic resonance imaging delineates soft tissue infiltrates better, but there was no time for that since her left vision was lost for more than 24 hours. All fluid and tissue sampled showed features of CLL.

In summary, undiagnosed CLL can present with acute blindness from orbital infiltration which may be best treated with surgical decompression instead of immunochemotherapy in order to save the vision.

DISCLOSURES

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

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CASE REPORT

Peritoneal dialysis-associated peritonitis of zoonotic origin, when minor gets major

H.F. Dresselaars[1]*, B. Zwart[3], A.M. Pettersson[2], M.C. Rijnsburger[2], M.M. Ho-dac-Pannekeet[4]

Departments of [1]Internal Medicine, [2]Medical Microbiology, VU University Medical Centre, Amsterdam, the Netherlands, Departments of [3]Medical Microbiology, [4]Internal Medicine, Zaans Medical Centre, Zaandam, the Netherlands,*corresponding author: tel.: +31-(0)6-47677373, email: lenadresselaars@gmail.com

ABSTRACT

A 62-year-old patient with peritoneal dialysis (PD)-associated peritonitis is described. Identical strains of *Pasteurella multocida* and *Streptococcus minor* were cultured from the dialysate, and from the saliva of her recently adopted stray cat. *Pasteurella* is not often encountered as pathogen in PD-associated peritonitis, *Streptococcus minor* has never been cultured in human infection before. We emphasise the importance of hygiene in peritoneal dialysis and the need for testing pets when zoonotic pathogens are cultured.

KEYWORDS

Continuous ambulant peritoneal dialysis, pasteurella, PD peritonitis, *Streptococcus minor*, zoonotic infection

What was known on this topic?
PD peritonitis is a potentially dangerous complication of peritoneal dialysis and hygiene is of crucial importance. Skin bacteria such as staphylococci are most frequently encountered in PD-peritonitis cultures.

What does this case add?
PD peritonitis with *Pasteurella multocida* is rare, as is confirmation of the bacterial strain deriving from a pet. *Streptococcus minor* has never before been cultured in human bodily fluid/material. Amplified fragment length polymorphism is a highly reliable technique in the confirmation of bacterial strains. To identify the cause of disease, the domestic situation should carefully be analysed.

CASE REPORT

A 62-year-old female patient treated with continuous ambulatory peritoneal dialysis (PD) for 37 months presented with mild abdominal discomfort and cloudy effluent. Her medical history consisted of hypertensive nephropathy and two exit-site infections (with *Candida* and *Difteroides* species cultured, respectively) as well as an episode of PD-associated peritonitis with *Streptococcus mitis* cultured in peritoneal effluent. At that time, the patient was reinstructed on maintaining good hygiene. The patient had been using mupirocine cream around the exit site daily ever since.

Physical examination on admission showed a slightly tender abdomen on palpation; there were no signs of tunnel or exit-site infection or damage to the catheter. Vital signs including temperature were normal.

According to the local protocol (based on the national guidelines (SWAB) and the relatively few cases of Gram-negative peritonitis encountered in our population), cefalexin 500 mg three times daily, orally, and cefalotin 250 mg four times daily, intraperitoneally in each bag, were started immediately. The patient refused to be admitted and recovered initially at home, but two days later she deteriorated and was admitted to the hospital where laboratory findings revealed a leukocyte count of 11.2 x 10^9/l (4-10 x 10^9/l) of which 8.15 x 10^9/l granulocytes (1.5-7.5 x 10^9/l) and C-reactive protein of 317 mg/l (< 10 mg/l). Analysis of the effluent showed a white blood cell count of 5.37 x 10^9/l (< 0.10 x 10^9/l), of which 4.67 x 10^9/l granulocytes. Effluent cultures showed

the presence of *Pasteurella multocida* and a *Streptococcus* species. After sequence analysis of the 16S rRNA gene the definitive determination was *Streptococcus minor*.[1] Both bacteria were pan-sensitive to regularly used antibiotics.

Since the patient was allergic to penicillin (she had developed a mild rash in the past) she started on ciproxin upon admission to ensure coverage of Gram-negative bacteria. Soon she developed tendon pain so she was switched to cotrimoxazole intravenously 960 mg twice daily for 14 days, according to the antibiogram of cultured microorganisms. Intraperitoneal cefalotin was continued (for 14 days in total). The abdominal tenderness and cloudy peritoneal effluent soon cleared. After 25 days the white blood cell count in the effluent was < 0.1 x 10^9/l.

Because a zoonotic origin was suspected, saliva of the patient's two cats and dog was cultured. No pathogens were cultured in the dog, but one of her cats carried *P. multocida* and the stray cat she had recently adopted carried both pathogens. The bacteria found in the stray cat were identical to the ones cultured from our patient, as confirmed by amplified fragment length polymorphism (AFLP) (*figure 1*).[2]

DISCUSSION

The most common and potentially fatal complication of PD is peritonitis, with 0.24-1.66 episodes/patient-year. In most cases of PD-peritonitis bacteria originating from the skin track through and along the catheter. Infections with Gram-positive cocci such as *Staphylococcus epidermidis* and *S. aureus* are most frequently observed.[3] Transvisceral migration of intestinal bacteria due to intra-abdominal pathology is another pathophysiological mechanism.[4]

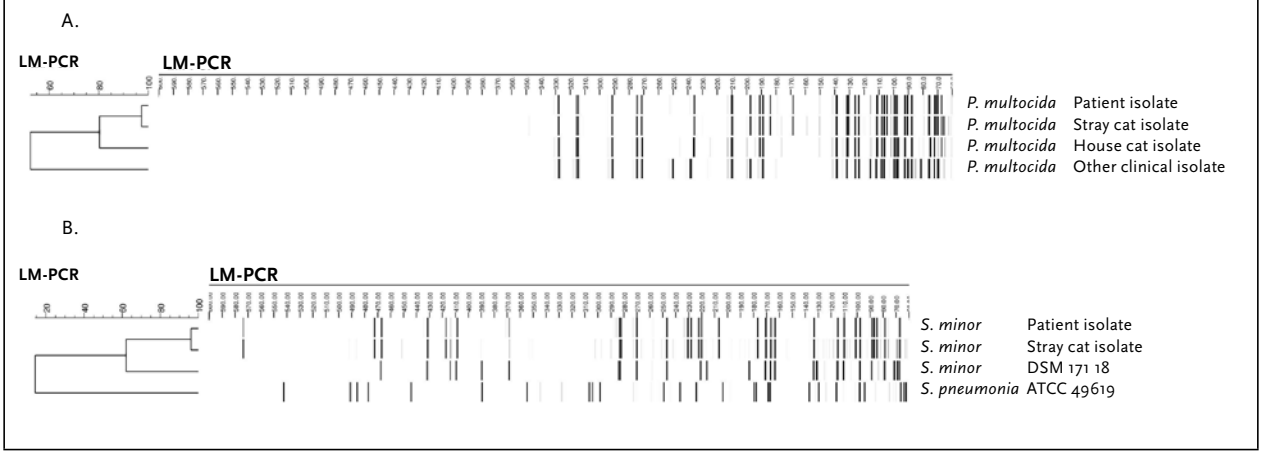
Streptococcus minor is a small, Gram-positive bacterium present in the throat and intestinal tract of cats and dogs, but has never before been shown in human disease.

This microorganism is typically susceptible to penicillin, as are all streptococci.[5] *Pasteurella multocida* is an aerobic, Gram-negative, coccoid rod named after Louis Pasteur, who first described the organism in 1880.[6] It is found in the oral cavity of many mammals, including cats (70-90%) and dogs (66%).[7] In humans, it generally causes cellulitis at the infection site. In more severe cases pneumonia, meningitis and osteomyelitis can develop.

Pasteurella is an uncommon cause of peritonitis in patients on peritoneal dialysis. We found only 23 cases to date in literature. In all but one case, close contact with pet cats was present and in most of the cases cats were in contact with the equipment or even damaged the bags or tubing by scratches or bites. Only one author confirms the strain of *P. multocida* found in the pet cat and peritoneal effluent to be identical. This was done by pulse field gel electrophoresis (PFGE).[8] In our case, we confirmed the strains to be identical by AFLP. AFLP is a DNA fingerprinting technique that has shown to be valuable in studying the molecular relationship of bacterial strains.[2] This technique is easy to use, relatively cheap and more reliable than PFGE.[9]

Usually beta-lactams can resolve infections with *Pasteurella*.[10] In this case, the explanation for the initial ambulant recovery could be the effective treatment of

Figure 1. *AFLP analysis of strains A) P. multocida strains isolated from the patient, the stray cat, the house cat and an independent clinical isolate. Strains clustering with a similarity of above 90% were identified as identical isolates. B) S. minor strains isolated from the patient and the stray cat plus control S. minor and S. pneumoniae strains. Strains clustering with a similarity of above 90% were identified as identical isolates. Strains clustering with a similarity below 35% were of different species.*



Streptococcus minor with first-generation cephalosporins, but since *Pasteurella* can be relatively insensitive it could have been left untreated causing the deterioration.[11] Since no damage to the catheter or fluid leakage was noted, the way of transmission was not definitively clarified, but hygiene measures may have been flawed in this patient.

CONCLUSION

We describe a patient with PD peritonitis with zoonotic bacteria that originated from an adopted stray cat, as confirmed by AFLP. This is the first observation of *Streptococcus minor* in human infection. This case again illustrates the importance of hygiene in peritoneal dialysis. When uncommon micro-organisms are cultured in PD peritonitis, their origin should be investigated thoroughly. When typical zoonotic pathogens are encountered, the pets should be considered as the source.

DISCLOSURES

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

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PHOTO QUIZ

Deep vein thrombosis: beware what lies beneath

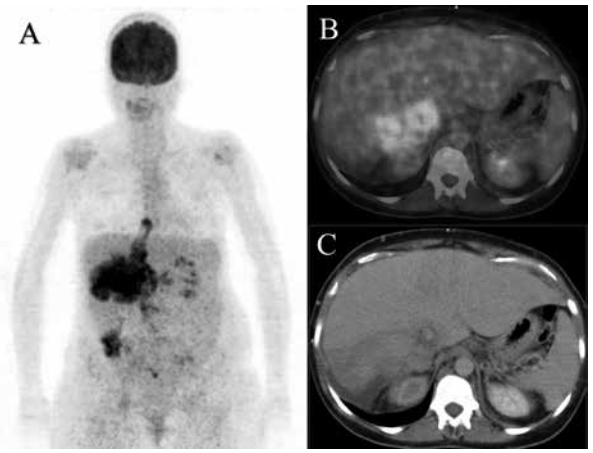
W. Holvoet[1]*, W.M.J. Schreurs[2], R. Clarijs[3], B. Weijs[1], G.J.M. Mostard[1]

Departments of [1]Internal Medicine, [2]Nuclear Medicine, [3]Clinical Pathology, Atrium Medical Center, Heerlen, the Netherlands, *corresponding author: tel.: +31(0)45-5766666, email: wouter_holvoet@hotmail.com

CASE REPORT

A 38-year-old Caucasian woman was admitted to our hospital with complaints of a swollen right leg. Her medical history comprised ductolobular breast cancer, which was treated 14 years earlier by lumpectomy, axillary lymph node dissection, and adjuvant radiotherapy, chemotherapy, and hormonal therapy. Last follow-up visit a year before presentation did not show any sign of relapse. On admission the swollen leg was accompanied by shortness of breath. Physical examination showed a swollen, red and painful leg. The liver area was painful on palpation. Subsequent investigation with duplex scanning demonstrated a deep venous thrombosis until the common femoral vein with the possibility of further extension proximally. Low-molecular-weight heparin and vitamin K antagonists were initiated. Because of shortness of breath and abnormal liver tests a computed tomography (CT) scan of the lungs and liver was performed, followed by positron emission tomography (PET) (*figure 1*).

Figure 1. *Positron emission shows FDG uptake in the internal vena cava with extraluminal expansion in surrounding structures and intraluminal expansion into the right atrium. (A) Maximum intensity projection (MIP) image from PET/CT; (B) Axial fused PET/CT image; (C) Contrast-enhancement pattern during portal venous phase of CT*



WHAT IS YOUR DIAGNOSIS?

See page 555 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 554)

DEEP VEIN THROMBOSIS: BEWARE WHAT LIES BENEATH

DIAGNOSIS

The CT scan revealed a 12 cm mass in the liver, which was associated with a tumour thrombus in the IVC extending into the right atrium, equilateral hydronephrosis, and intrapulmonary multiple small noduli.

Positron emission tomography (*figure 1*) showed pathological 18F-fluorodeoxyglucose (FDG) uptake in the right liver with expansion into surrounding structures as well as in the entire IVC thrombus mass. This finding corroborated the idea of encasement of the IVC by tumour with the presence of malignant cells in the caval thrombus extending into the right atrium. The noduli in the lung did not show FDG captation, these were probably too small to be PET-positive; nevertheless, these noduli were considered to be metastases. Transthoracic echocardiography revealed a non-mobile mass in the right atrium suggestive of extension of the tumour mass into the right atrium without haemodynamic consequences (*figure 2*).

Initially a recurrence of the earlier diagnosed breast cancer was suspected, but invasion of the IVC and right atrium did not fit with this diagnosis. Following the PET-CT an ultrasound-guided biopsy of the liver was obtained showing the histological and immunohistochemical characteristics of a leiomyosarcoma (*figure 3*).

Leiomyosarcoma is a relatively rare and aggressive form of cancer which can arise from virtually any site, including the gastrointestinal tract, nasal sinus, heart, tunica media of blood vessels, uterus, retroperitoneum, skin, and musculoskeletal system. Of these, leiomyosarcoma of the inferior vena cava (IVC) is barely reported in the literature. In most cases concerning primary involvement of blood vessels, the bulk of the tumour is located extraluminal.

Figure 2. *Two-dimensional echocardiography (apical four-chamber view) of right atrial extension of tumour (arrow). LA = left atrium; LV = left ventricle; RA = right atrium*

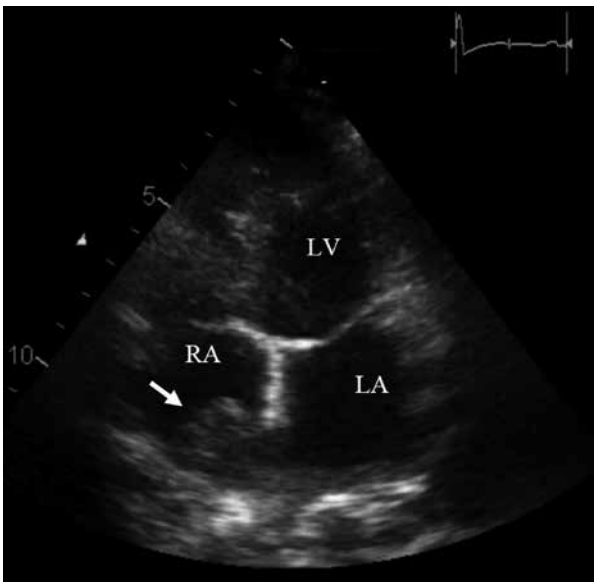
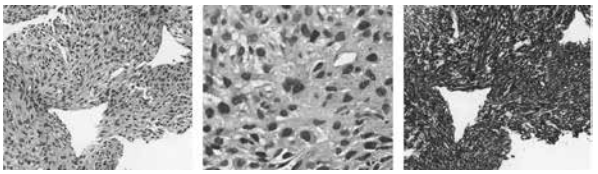


Figure 3. *Histological examination of the liver biopsy (haemotoxylin and eosin staining) showed a fascicular proliferation (left panel) of highly atypical cells (middle panel). The diagnosis of a leiomyosarcoma was confirmed by an intense immunohistochemical staining of several myogenic markers (right panel, smooth muscle actin)*



The treatment of choice for leiomyosarcoma remains surgical resection. Up to now, there is no proven benefit with chemotherapy or radiation therapy only.[1] From 1992-1994, The International Registry of Inferior Vena Cava Leiomyosarcomas enrolled 218 patients to support the most rational treatment. A radical tumour resection was undertaken in 134 (61.5%) patients, 26 (11.9%) had a palliative resection and 58 (26.6%) were inoperable. Radical tumour resection was associated with better 5- and 10-year survival rates (49.4% and 29.5%).[2]

In our case a curative resection was not feasible because of extensive invasion of the surrounding tissue, with high suspicion of pulmonary metastasis. The patient was treated with chemotherapy comprising ifosfamide with the cytoprotective drug sodium-2-mercaptoethane sulfonate. Unfortunately, the patient developed encephalopathy after the first cycle of ifosfamide. Methylene blue was started and ifosfamide discontinued with resolution of the neurological state after three days. Despite the neurological improvement, her condition further deteriorated until no further therapeutic options were possible and after a month of hospitalisation the patient left the hospital in a palliative setting.

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INFORMATION FOR AUTHORS

Aims and scope

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

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

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

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