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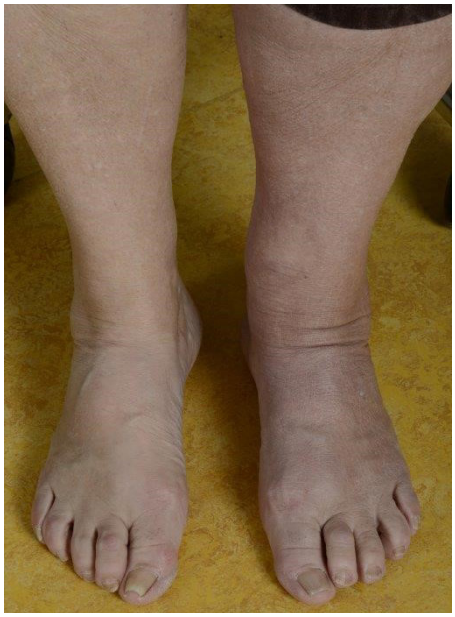

A unilateral blue leg; what is your diagnosis?

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EDITORIAL

Surviving HIV beyond prolonged viral suppression

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Unless patients present very late during the course of HIV infection, mortality as the consequence of AIDS has become exceptional. With the current antiretroviral armamentarium, viral replication can be halted and immune reconstitution achieved in almost all patients living with HIV. Unfortunately, prolonged control of viraemia, in many patients for more than a decade now, does not mean that the (very) long-term health outcome of HIV-infected patients will be comparable with individuals not infected by HIV. The virus already irreversibly damages the immune system shortly after transmission and only through very early diagnosis and treatment can this damage be partially avoided. Especially the gut mucosal barrier, corroded by HIV, is a pivotal contributing factor in the continuous low-grade systemic inflammation, which is thought to precipitate the development of non-AIDS-related diseases early in life.[1] On top of this, if the average age at HIV diagnosis is 35 years, antiretroviral therapy will have to be taken for perhaps half a century. This prolonged exposure puts patients at risk for significant therapy-related organ specific toxicities. Several antiretroviral drugs have been withdrawn from the market for reasons of toxicity. Some of these side effects only became apparent years or even 10 years after EMA approval. Although current antiretroviral drug development is now much more focused on short- and mid-term toxicity, it remains to be seen whether current regimens will withstand the test of time. These factors form a strong rationale for extended follow-up of patients in specialised HIV-treatment centres. The management of ageing during follow-up is developing as a complex new research field in HIV care. Its complexity is reflected by the various organ systems that can be prematurely affected. However, the optimal screening approach to diagnose premature ageing in the setting of HIV is unclear. And if ageing is prematurely present, how can these individuals be optimally treated? Do HIV-infected patients just need more pills earlier in life?

One affected organ system in HIV is the bone, where osteopenia seems to develop at a younger age. In this issue of the Netherlands Journal of Medicine, Krikke et al. focused on T-cell activation markers and this condition.[2] Despite the obvious limitations of this small cross-sectional pilot study on a selected group of 16 elderly males on long-term HIV therapy, they did not find an association between T-cell activation and this aspect of ageing. Another Dutch research group could not find an association either between other pro-inflammatory markers and lower bone mineral density in their cross-sectional analysis.[3] Other factors, including advanced HIV at diagnosis and exposure to tenofovir treatment, seem to be predominantly involved.[4] Nonetheless, the results are surprising for three reasons. First, in non-human primate models, disease progression is primarily related to ongoing massive immune activation and not uncontrolled viraemia.[5] Also, strong T-cell activity is pivotal for a subgroup of HIV patients, the elite controllers, who can control the virus without therapy but observational studies show that their massive immune activation comes at the cost of more non-AIDS-related morbidity and mortality.[6] Third, a link between immune activation markers with ageing-related conditions and survival in HIV has been described including ambiguous reports on the role of T cells in this field.[7,8] With this in mind, an interesting follow-up study would be to see whether changes in innate and adaptive immune activation are associated with bone mineral density over time, especially if younger patients were to be followed from the initiation of HIV therapy. These studies nonetheless underline the complexity of ageing in HIV and the lack of one single biomarker that can reliably predict all comorbidities.

There is a clear need for reliable screening methods to identify conditions associated with premature ageing, including osteoporosis, in the setting of treated HIV.

An interesting Dutch study in this field, the AGEhIV cohort, has untangled important issues regarding ageing with HIV.[9] In lifestyle-matched HIV infected and uninfected individuals aged over 45 years, it confirmed that ageing-related conditions are more prominent in HIV-suppressed patients. This is one example of ongoing studies that explore potential factors associated with premature cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, renal insufficiency, malignancies, and osteoporosis in HIV.

How to prevent premature ageing and frailty in a continuously growing HIV population is a critical question. The relevance of it is strikingly illustrated by the average age of the Dutch HIV population. Indeed, in 1996, only 9% of HIV patients in the Netherlands were over 50 years of age. In 2016, this percentage had increased to 45% and is likely to approach 50% within just a few years. Classical risk equations, such as the Framingham risk score, seem to incorrectly assess the risk in HIV-infected individuals.[10,11] Despite some additional modest biological effects at best of anti-inflammatory drugs, extra antivirals, statins, or drugs that restrict microbial translocation in HIV-infected patients, clinical outcome studies are still lacking. Importantly, these measures to decrease inflammation are obsolete without viral suppression by antiretroviral therapy or changes in unhealthy lifestyles.

Correctly identifying those at risk and finding additional means to prevent inflammation is one of the major challenges in HIV clinical science. Already over 90% of HIV-infected patients in resource-rich countries die of non-AIDS defining illnesses.[12] Due to premature ageing, these people are affected at a younger age. Preventing morbidity is a significant challenge in this vulnerable population. It is not unthinkable that even if HIV could be cured, this would not affect premature ageing due to irreparable damage to the immune system. Globally, the successful rollout of antiretroviral therapy to those most in need is a major achievement by the international community. Preventing complications of premature ageing should start to accompany these global HIV treatment strategies to prevent massive loss of quality of life. Ensuring survival does not end with achieving viraemic control: it starts there.

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ORIGINAL ARTICLE

Osteoporosis and osteopenia are not associated with T-cell activation in older cART-treated HIV-infected patients

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ABSTRACT

Background: A higher risk of developing osteopenia/osteoporosis has been seen in HIV-infected patients. We compared HIV-infected patients, all treated with combination antiretroviral therapy (cART), with a low bone mineral density (BMD) (T-score < -1) to those with a normal BMD (T-score > -1), examining the relation with T-cell activation and bone turnover markers (c-terminal telopeptide (CTX) and procollagen type 1 amino-terminal propeptide (P1NP)).

Methods: In this single visit pilot study, bone turnover markers, T-cell activation (CD38 + HLA – DR +) and senescence (CD57+) of T cells were measured in patients who had previously undergone dual energy X-ray absorptiometry scanning.

Results: All study participants (n = 16) were male, on cART, with a median age of 61 years (IQR 56-66). Nine patients had osteopenia/osteoporosis. When comparing the patients with osteopenia/osteoporosis with those with a normal BMD, no differences in activation and senescence were found. A relation was seen between higher bone formation (P1NP) and patients who were on cART for longer. The median length of cART use was 5.5 years (IQR 4.5-7.8), with all patients on nucleoside reverse transcriptase inhibitors, 88% on tenofovir, 63% on non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 38% on protease inhibitors. Osteopenia/osteoporosis was seen in 100% of the patients on protease inhibitors versus 30% of those on NNRTIs.

Conclusion: This study did not find an association between activated T cells and BMD, thus did not explain the higher prevalence of osteopenia/osteoporosis in HIV-infected patients. Interestingly, this small pilot showed that cART might influence BMD, with a possible negative effect for protease inhibitors and a possible protective effect for NNRTIs. These results warrant further investigation.

KEYWORDS

Bone turnover markers, combination antiretroviral therapy, HIV-infection, immune activation, osteoporosis, T-cell activation

INTRODUCTION

A higher risk of developing osteopenia (6.4 fold) and osteoporosis (3.6 fold) has been seen in HIV patients compared with uninfected controls,[1] with a prevalence of 52% and 15% respectively.[2] Not only the traditional risk factors that are more prevalent among HIV-infected patients, such as low body mass index, smoking, alcohol abuse and glucocorticoid therapy, but also HIV-related risk factors, such as HIV-viral load, nadir CD4+ T-cell count, combination antiretroviral therapy (cART) use and immune activation, contribute to this low bone mineral density (BMD).[3-5]

However, recently published contradictory data showed that after correction for traditional risk factors, no association with BMD was found in HIV-infected patients, compared with uninfected controls.[6]

HIV-induced immune activation is thought to promote osteoporosis through activated T cells.[7-9] In HIV-infected patients an increased T-cell activation (defined as CD38 and HLA-DR positivity) is seen, even in those suppressed (HIV-RNA viral load < 50 copies/ml) by cART.[10] Two

studies, however, found no significant relation between activated CD38+HLADR+ T cells or inflammatory markers and BMD in HIV-infected patients.[11,12] In these studies not all patients were on cART, making interpretation for the majority of suppressed HIV patients difficult.

Bone remodelling is a continuous coordinated process of osteoclast resorption and osteoblast formation.[13] The bone turnover marker c-terminal telopeptide (CTX) can be used to measure bone resorption and procollagen type 1 amino-terminal propeptide (P1NP) for bone formation.[14] Here we present a pilot study investigating the relation between BMD and either T-cell activation or bone turnover markers by comparing these in HIV-infected cART-treated male patients above 50 years of age with osteopenia/osteoporosis (T-score below -1) to those with a normal BMD (T-score above -1).

MATERIALS & METHODS

Patients

HIV-infected patients were recruited from the University Medical Centre Utrecht (UMCU) for participation in the OASIS-HIV study. All patients were previously enrolled in the 'A Phase 4 Cross-Sectional Study of BMD in HIV-1 Infected Subjects' study (ID:GS-US-104-0423; ClinicalTrials.gov:NCT01850212), where they underwent dual energy X-ray absorptiometry (DEXA) scanning to quantify bone loss and measure BMD. All patients provided written informed consent in accordance with the Declaration of Helsinki and the local Medical Ethics Committee approved the study.

Study design

The OASIS-HIV study was a cross-sectional single visit study where blood was drawn for further comprehensive in-depth immunological analysis. Data of the previous DEXA scan, performed approximately six months earlier, was used for classification of patients into two groups: osteopenia/osteoporosis (T-score below -1) and normal BMD (T-score above -1). The T-score was measured in the lumbar spine, hip and femoral neck using the DEXA scan. Osteoporosis and osteopenia was classified as a T-score below -2.5 and -1 respectively in one or more locations on the DEXA scan.

Laboratory measurements

Blood samples were obtained by venous puncture, with collection and isolation of plasma and peripheral blood mononuclear cells (PBMCs) performed within four hours at the immunology lab of the UMCU. Plasma was frozen and stored at -80 °C until all the samples were collected. They were then sent to the laboratory of Medicine and Bone Metabolism at the UMC Groningen for analysis of bone markers (CTX and P1NP), as described previously.[15] For these markers Z-scores were used, based on more than 350 gender- and age-matched uninfected controls.[15] These were volunteers working at the UMC Groningen, the Netherlands. To mathematically calculate net bone formation CTX was subtracted from P1NP. The HIV-RNA viral load was measured (COBAS® AmpliPrep/ COBAS® TaqMan®, Roche Diagnostics, Indianapolis, USA) with a lower limit of detection of 50 copies/ml.

PBMC processing, cell staining and flow cytometric analyses

PBMCs were isolated using Ficoll-Paque™ Plus (GE Healthcare) density gradient centrifugation and washed with RPMI 1640 culture media (Gibco®, life technologies™) containing 5% foetal calf serum (FCS) and penicillin-streptomycin before being cryopreserved with RPMI 20% FCS. Cryopreserved PBMCs were thawed with RPMI 20% FCS and subsequently used for flow cytometric analysis. Cells were washed using PBA (Sigma®, Life Science), stained with cocktails of monoclonal antibodies and left to incubate for 20 min at 4°C. Fluorescence minus one controls were used to define positive gates for the expression of different proteins. Lymphocytes and monocytes were gated based on forward and side scatter using a FACS LSR Fortessa (BD Biosciences, Franklin Lakes, USA) and FACS Diva software version 8.0 (BD Biosciences, Franklin Lakes, USA).

Data analyses

The primary outcome of this study was the relation between BMD (osteoporosis/osteopenia versus normal) and either T-cell activation or bone turnover markers. Secondary outcomes were the association of patient characteristics with BMD. A Mann-Whitney test was used to compare non-paired continuous variables. Data were presented as percentages for categorical variables and as median with interquartile ranges (IQR) for continuous variables. Differences were considered statistically significant when $p < 0.05$. Linear regression modelling was used to evaluate the relation between BMD and T-cell activation or bone turnover markers and the association with patient characteristics. Analyses were performed using SPSS version 21 (SPSS, Chicago, Illinois, USA).

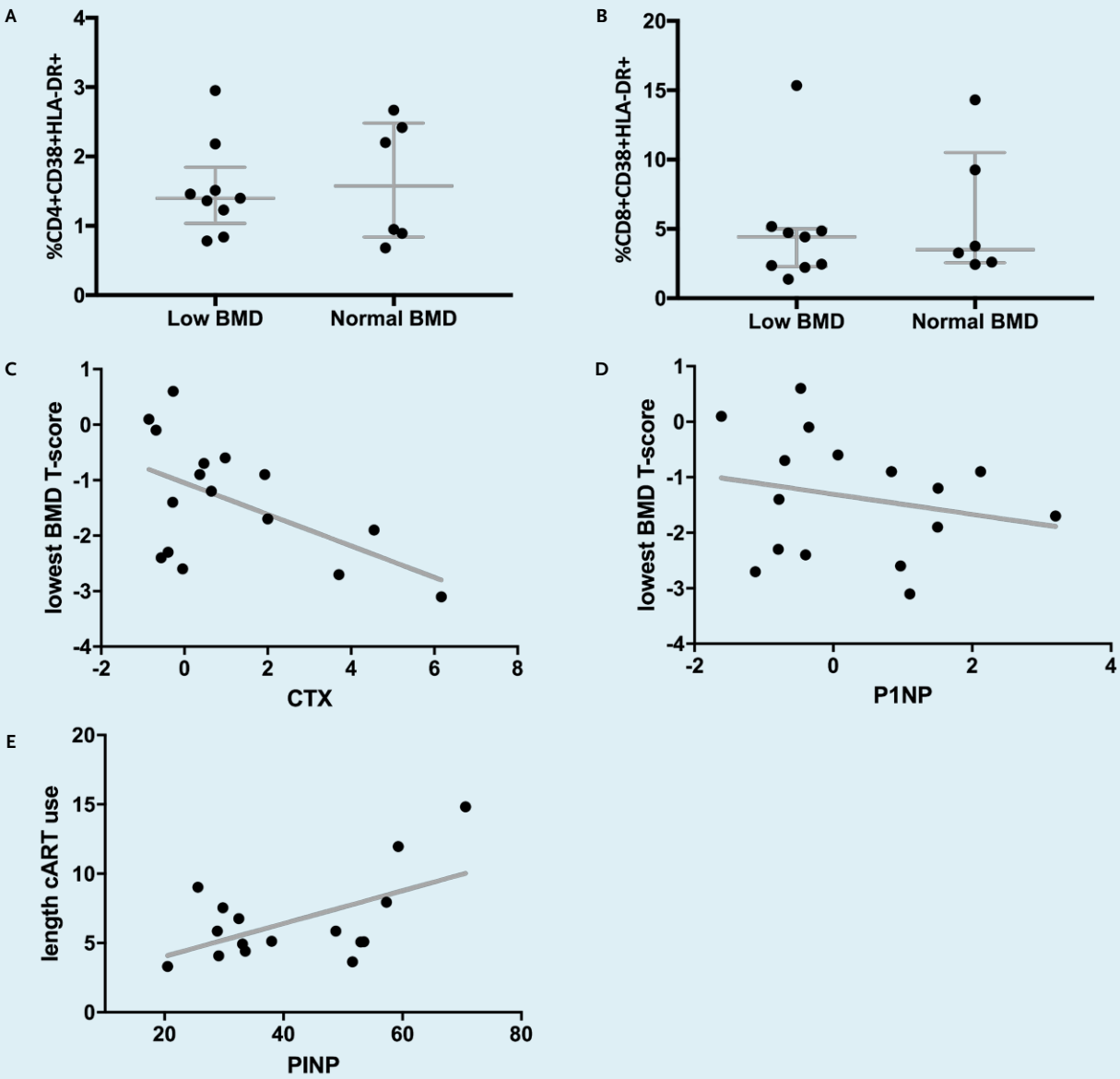
RESULTS

For this pilot study we enrolled 17 out of the 20 patients previously enrolled in GS-US-104-0423, as three patients objected to study participation. To increase the homogeneity of the group, one patient was excluded, as she was female, resulting in 16 patients, all male, with a median age of 61 years (IQR 56-66).

Osteoporosis was diagnosed in 3 patients (19%), osteopenia in 6 patients (38%) and a normal BMD was found in the remaining 7 patients (44%) (*table 1*). When looking at the specific measuring sites the prevalence of osteoporosis was 12%, 0% and 6% for the lumbar spine, hip and femoral neck respectively. For osteopenia this was 25%, 31% and 44% respectively. None of the patients were using osteoporosis medication at the time of the study.

First, we analysed the association of T-cell activation, defined by CD38+HLA-DR+ positivity, with bone pathology. The overall CD38+HLADR+ expression was 1.4% (IQR 0.9-2.2) for CD4+ and 3.8% (IQR 2.4-5.2) for CD8+ on T cells. No differences were seen between patients with osteoporosis/osteopenia (T-score below -1) and normal BMD (T-score above -1) for either CD4+ (1.4% versus 1.6%, p = 0.95) or CD8+ T cells (4.4% versus

Figure 1. *The association between T-cell activation, bone turnover markers and BMD*



Differences in expression of CD38+HLADR+ on A. CD4+ and B. CD8+ T cells between osteopenia/osteoporosis and normal BMD. T-cell activation was only measured in 15 out of the 16 patients due to a technical malfunction of the flow cytometric machine. Linear regression modelling analysing the relation between; C. CTX and the lowest BMD T-score (β -0,004, 95% CI -0,007;0,00, p = 0,03). D. P1NP and lowest BMD (β -0,02, 95% CI -0,06;0,02, p = 0,37). E. Length of cART use and P1NP (β 0,26, 95% CI 0,0;0,46, p = 0,01).

Table 1. *Baseline characteristics*

	All patients (n = 16)
General	
Age (years)	61 (56- 66)
Male sex	100%
Body mass index (kg/m²)	27 (24-28)
Smoking (yes)	37.5%
Alcohol (more than 3 units/day)	12.5%
Use of glucocorticoids	0%
HIV	
Length of known HIV (years)	8.2 (6.4-9.8)
Nadir CD4	239 (101-364)
Length of untreated HIV (years)	1.0 (0.1-3.2)
Length of cART use (years)	5.7 (5.1-8.2)
Current cART use (yes)	100%
Suppressed viral load (< 50 copies/ml)	100%

Variables presented as median (IQR) or percentages; cART = combination antiretroviral therapy.

3.5%, p = 0.69) (*figure 1A+B*). Similarly, no differences in senescence were seen between the groups (*table 2*). Furthermore, no relation for T-cell activation with BMD was found using linear regression (CD4+: β 0.03, 95% CI -0.84;0.90, p = 0.95 and CD8+: β -0.01, 95% CI -0.16;0,14, p = 0.87).

Next, we analysed T-cell activation in relation to the bone turnover marker CTX and bone resorption marker P1NP. No relation between T-cell activation and bone markers was found to depict the net bone formation (data not shown), even when subtracting CTX from the P1NP. However, CTX itself was inversely correlated with BMD (β -0,004, 95%CI -0.007;0.00, p = 0.03) (*figure 1C*). Furthermore, higher levels of P1NP (*figure 1D*), though not significant (β -0.02, 95% CI -0.06;0.02, p = 0.37), were found in patients with a lower BMD. No difference between patients with osteoporosis/osteopenia and those with a normal BMD was seen for CTX (0.65 versus 0.37, p = 0.30) and P1NP (0.97 versus -0.35, p = 0.61).

When analysing the effects of cART on bone formation and resorption, a relation was seen between higher bone formation (P1NP) and patients on cART for longer (β 0.26, 95% CI 0.0;0.46, p = 0.01) (*figure 1E*). The median length of cART use was 5.7 years (IQR 5.1-8.2), with all patients on nucleoside reverse transcriptase inhibitors (NRTIs), 88% on tenofovir, 63% on non-nucleoside reverse transcriptase inhibitors (NNRTIs) and 38% on protease inhibitors. On a similar note, osteopenia/osteoporosis was seen in 100%

Table 2. *T-cell activation and bone markers expression: T-cell activation and bone marker expression in the overall group and specified per BMD, those with osteoporosis/osteopenia or with a normal BMD*

	All patients	Osteopenia/osteoporosis	Normal BMD
T-cell activation	(n = 16)	(n = 9)	(n = 7)
T cells CD4+38+DR+(%)	1.4 (0.9-2.2)	1.4 (1.0-1.8)	1.6 (0.8-2.5)
T cells CD8+38+DR+(%)	3.8 (2.4-5.2)	4.4 (2.3-5.0)	3.5 (2.6-10.5)
T cells CD4+57+(%)	12.5 (6.0-19.5)	12.5 (7.1-20.6)	9.6 (3.5-18.6)
T cells CD8+57+(%)	46.8 (34.3-52.0)	46.8 (29.6-55.3)	44.4 (32.9-55.0)
T cells CD4+95+(%)	36.8 (28.0-46.4)	36.8 (27.6-41.9)	34.4 (29.2-50.7)
T cells CD8+95+(%)	19.4 (17.6-26.0)	19.3 (17.2-25.7)	20.0 (17.7-26.1)
Bone markers			
P1NP	35.80 (29.3-53.4)	48.8 (29.0-55.4)	33.6 (29.8-51.6)
Z-score P1NP	-0.1 (-0.8-1.4)	1.0 (-0.8-1.5)	-0.4 (-0.7-0.8)
CTX	223.0 (167.5-374.4)	241.6 (168.1-518.5)	219.2 (135.5-267.7)
Z-score CTX	0.4 (-0.4. 2.0)	0.7 (-0.3-4.1)	0.4 (-0.7-1.0)

Variables presented as median (IQR) or percentages; P1NP = procollagen type 1 amino-terminal propeptide; CTX = C-terminal telopeptide of type 1 collagen.

of the patients on protease inhibitors versus 30% of those on NNRTIs. A positive relation for current NNRTI use with BMD was seen (β 1.26, 95% CI 0.24;2.28, p = 0.02), whereas current use of protease inhibitors was related to a lower BMD (β -1.26, 95% CI -2.28;-0.24, p = 0.02). A relation between BMD and tenofovir could not be analysed, as only two patients were not on tenofovir.

Table 3 not only shows the linear regression results for the lowest T-score, it also shows the association of the above-mentioned variables per DEXA measured region, femoral, lumbar spine and hip respectively. No differences were seen per region or as a whole (*table 3*).

DISCUSSION

The postulated mechanism of how HIV-induced immune activation promotes osteoporosis is via the replication of the viral protein gp120, which is present on the HIV envelope or possibly through the increased production of receptor activator of nuclear factor kappa-B ligand (RANKL) by activated T cells.[7-9] Although suggested in previous publications, our pilot study could not confirm a possible role for activated T cells in the pathogenesis of osteoporosis, as no association was found.[16,17] This could possibly be explained by the relatively low level of T-cell activation, 1.4% and 3.8% respectively for CD4+ and CD8+ T cells as found in our study of cART-treated patients with a supressed viral load. Previously published studies showed a more than 2.5 fold higher prevalence for activated CD4+ and CD8+ T cells in similarly cART treated HIV-infected patients with a supressed viral load.[10,18,19] Our prevalence is similar to that of HIV-negative patients, which is 1-2.2% and 1-5.1% for CD4+ and CD8+ T cells respectively.[10,18]

In this study we did, however, find an inverse correlation for the bone turnover marker CTX, a bone resorption marker, and BMD. In untreated HIV-infected patients, higher levels of CTX correlated with advanced HIV disease, which returned to levels of uninfected controls upon initiation of cART.[20-22] In addition, increased levels of bone turnover markers predicted BMD decreases in HIV.[23] However, CTX has also been shown to increase upon initiation of cART, reaching a stable, but higher plateau compared with cART-naïve patients with high CD4 T cells.[24] We also found a correlation for P1NP, a bone formation marker, and BMD. This observation is expected as the bone formation increases due to increased resorption and its subsequent production of growth factors.[25] Despite its small sample size, several of our study outcomes strengthen our observation. First, the prevalence of both osteoporosis and osteopenia in our study patients was representative for other published HIV populations.[2,26-29] Second, an even distribution of our data was seen upon linear regression, meaning the data were not skewed to an outlier (*figure 1*). Finally, we also increased our homogeneity by only including male patients over 50 years of age. Therefore, we think that a larger sample size would not influence the conclusions of our study.

The question then remains how to explain the increased prevalence of osteoporosis in HIV, as activated T cells do not seem to influence its pathogenesis. It is well known

Table 3. *Coefficients (β) with 95% confidence intervals based on linear regression modelling estimating the relation for T-cell activation, bone turnover markers and HIV-specific variables with T-score of BMD*

	Lowest T-score		T-score femoral neck		T-score lumbar spine		T-score hip	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
CD4+38+HLA-DR+	0.03(-0.84;0.90)	0.95	-0.11(-0.84;0.62)	0.75	-0.07(-1.52;1.38)	0.92	-0.03(-0.73;0.67)	0.94
CD8+38+HLA-DR+	-0.01(-0.16;0.14)	0.87	-0.02(-0.15;0.10)	0.71	-0.05(-0.29;0.20)	0.70	-0.02(-0.14;0.10)	0.73
CTX	-0.004(-0.007;0.00)	0.03	-0.003(-0.01;-0.001)	0.02	-0.004(-0.01;0.002)	0.16	-0.004(-0.01;-0.001)	0.01
CTX (Z-score)	-0.28(-0.54;-0.03)	0.03	-0.28(-0.5;-0.05)	0.02	-0.31(-0.76;0.14)	0.16	-0.29(-0.49;-0.10)	0.01
P1NP	-0.02(-0.06;0.02)	0.37	-0.02(0.06;0.02)	0.33	-0.04(-0.10;0.03)	0.21	-0.01(-0.05;0.03)	0.57
P1NP (Z-score)	-0.18(-0.64;0.28)	0.41	-0.18(-0.6;0.23)	0.36	-0.40(-1.11;0.32)	0.25	-0.10(-0.49;0.30)	0.62
Current PI use	-1.26(-2.28;-0.24)	0.02	-1.16(-2.09;-0.23)	0.02	-1.24(-3.10;0.62)	0.17	-1.19(-2.01;-0.37)	0.01
Current NNRTI use	1.26(0.24;2.28)	0.02	1.16(0.23;2.09)	0.02	1.24(-0.62;3.10)	0.17	1.19(0.37;0.01)	0.01

A p-value < 0.05 was considered statistically significant. β = coefficient; CI = confidence interval; p = p-value < 0.05. CTX = c-terminal telopeptide; P1NP = procollagen type 1 amino-terminal propeptide; PI = protease inhibitors; NNRTI = non-nucleoside reverse transcriptase inhibitors.

that HIV-infected patients have higher incidences of classic osteoporosis risk factors, such as alcohol abuse and smoking compared with the general population possibly contributing to this higher prevalence.[3,5,30] Furthermore, the increase could be explained by the activation induced at the start of the HIV infection. In a study performed in primary HIV infection, an increased prevalence was already seen in their population with a mean age of 38 years, compared with HIV-negative controls. Thus, possibly alluding to a role for viral load, as a high viral load has been known to correlate to lower BMD.[31] Most likely it is a multifactorial problem not caused by just one but several HIV-related components.

Another known component is cART; upon starting cART the BMD is known to decrease.[24] In this small pilot an effect of cART on BMD was also seen, with a possible negative effect for protease inhibitors and a possible protective effect for NNRTIs. Previous studies have reported on the possible negative effect of protease inhibitors, especially correlated to time on therapy containing protease inhibitors.[28,29] For NNRTIs, negative effects have also been reported, especially in the lumbar spine in patients of an older age with a low body mass index,[26,27] contrary to what we have found. Therefore, the effect of NNRTIs on BMD warrants further investigation.

In conclusion, this pilot study could not confirm a possible role for activated T cells in the pathogenesis of osteoporosis. However, cART seems to influence BMD, with a possible negative effect for protease inhibitors and a possible protective effect for NNRTIs.

DISCLOSURES

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Conflicts of interest: None.

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ORIGINAL ARTICLE

Current practice of closed-loop mechanical ventilation modes on intensive care units – a nationwide survey in the Netherlands

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ABSTRACT

Background: The most recent modes for mechanical ventilation are closed-loop modes, which are able to automatically adjust certain respiratory settings. Although closed-loop modes have been investigated in various clinical trials, it is unclear to what extent these modes are actually used in clinical practice. The aim of this study was to determine closed-loop ventilation practice on intensive care units (ICUs) in the Netherlands, and to explore reasons for not applying closed-loop ventilation. Our hypothesis was that closed-loop ventilation is increasingly used.

Methods: A short survey was conducted among all non-paediatric ICUs in the Netherlands. Use of closed-loop modes was classified as frequently, occasionally or never, if respondents stated they had used these modes in the last week, in the last month/year, or never, respectively.

Results: The response rate of the survey was 82% (72 of 88). Respondents had access to a closed-loop ventilation mode in 58% of the ICUs (42 of 72). Of these ICUs, 43% (18 of 42) frequently applied a closed-loop ventilation mode, while 57% (24 of 42) never or occasionally used it. Reasons for not using these modes were lack of knowledge (40%), insufficient evidence reporting a beneficial effect (35%) and lack of confidence (25%).

Conclusion: This study does not support our hypothesis that closed-loop ventilation is increasingly used in the Dutch ICU setting. While industry continues to develop new closed-loop modes, implementation of these modes in clinical practice seems to encounter difficulties. Various barriers could play a role, and these all need attention in future investigations.

KEYWORDS

Respiration, artificial; Ventilators, mechanical; Critical care; closed-loop; survey

INTRODUCTION

Mechanical ventilation in intensive care unit (ICU) patients is a rapidly evolving field. Closed-loop ventilation modes are increasingly available, but it is uncertain to what extent they are used. Closed-loop ventilation modes automatically adjust certain respiratory settings based on digital algorithms and physiological inputs of the patient (e.g. pulse oximetry results, end-tidal CO_2 levels, and respiratory system resistance and compliance). Typical examples of closed-loop ventilation modes include Adaptive Support Ventilation (ASV®), INTELLiVENT®–ASV, SmartCare®/PS, Proportional Assist™ Ventilation (PAV™+), Neurally Adjusted Ventilatory Assistance (NAVA), Automode® and Mandatory Minute Ventilation (MMV).[1,2] An international survey, published in 2011, reported that a majority of ICUs do not commonly use these modes, which was recently confirmed by a Ukrainian single-country study.[3,4] Now, several years later, we hypothesise that closed-loop ventilation is increasingly applied. We performed a nationwide survey to determine closed-loop ventilation practice in ICUs in the Netherlands.

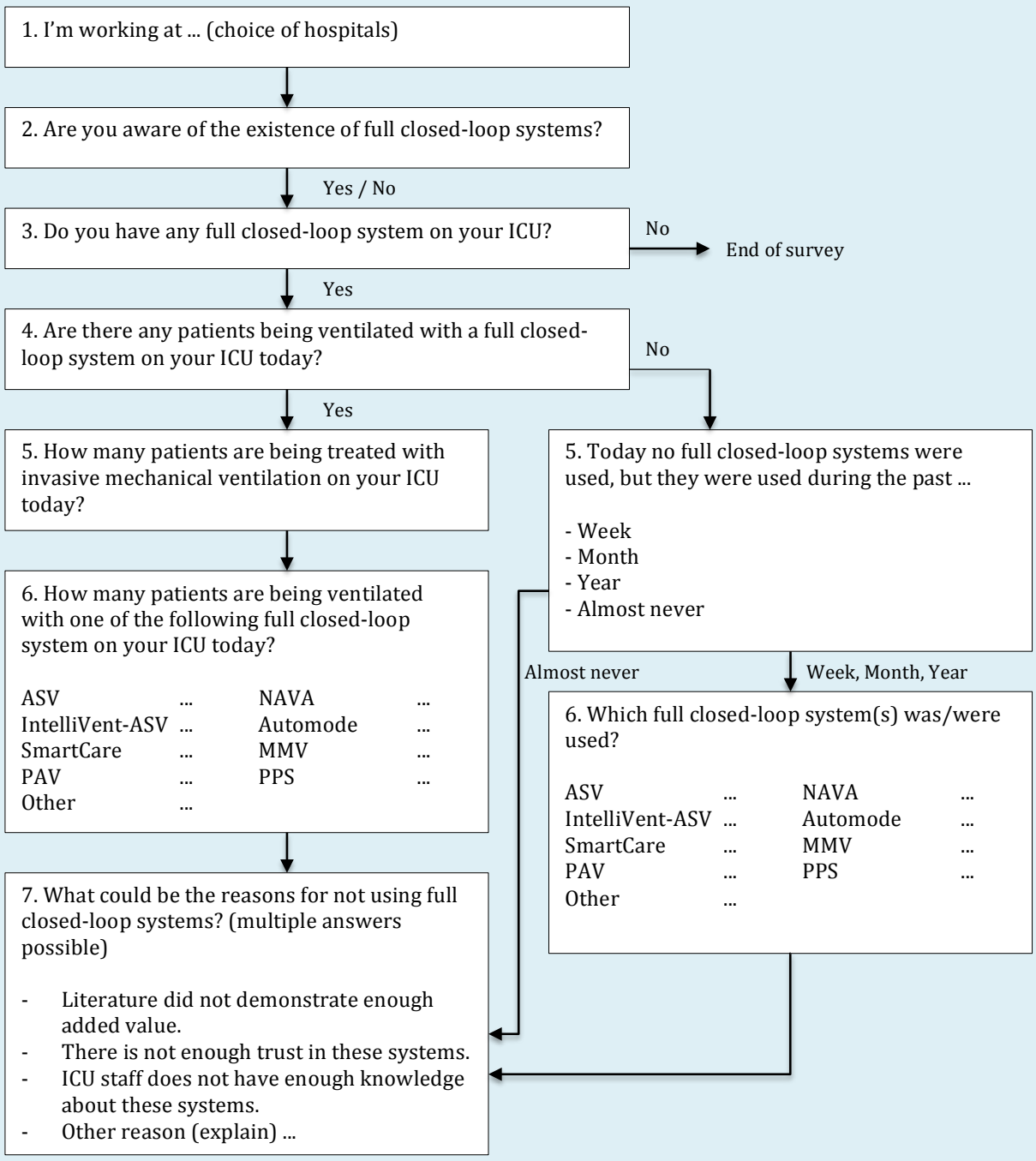
METHODS

A survey was conducted among all non-paediatric ICUs in the Netherlands. The study was registered at the Local Institutional Review Board of the Catharina Hospital, Eindhoven, the Netherlands. In September 2016, a representative ICU physician or nurse was identified for each ICU, and was then asked to participate in the survey. Participants could either answer the survey questions immediately by phone, or receive the survey by e-mail to be completed at a later stage. Two reminders were sent, one week and two weeks after the initial invitation. Non-responders were contacted again once more in November 2016.

The survey

The survey consisted of seven questions regarding the application of closed-loop modes (*figure 1*). The use was classified as frequently, occasionally or never if respondents with a closed-loop ventilation mode had

Figure 1. *Flowchart-like survey for representatives of each non-paediatric ICU*



applied this mode at least once in the preceding week, month to year, or never, respectively. Reasons for not using closed-loop modes could be scored as 'lack of knowledge', 'insufficient evidence reporting a beneficial effect', or 'lack of confidence in the mode '. Respondents were also able to suggest additional reasons using an open field. An independent medical epidemiologist verified the methodological quality of the survey.

Analysis

The availability of a closed-loop mode and the frequency of use was analysed per ICU level. In the Netherlands, all Dutch ICUs have been classified from level 1, low level ICUs, to 3, high level ICUs, based on the ICU size, patient volume, ventilation days, and staffing.[5] Data were collected and entered into Microsoft® Excel® version 14 (©2010 Microsoft Corporation). Categorical responses of questions were described as the proportion (percentage) of respondents selecting each response.

RESULTS

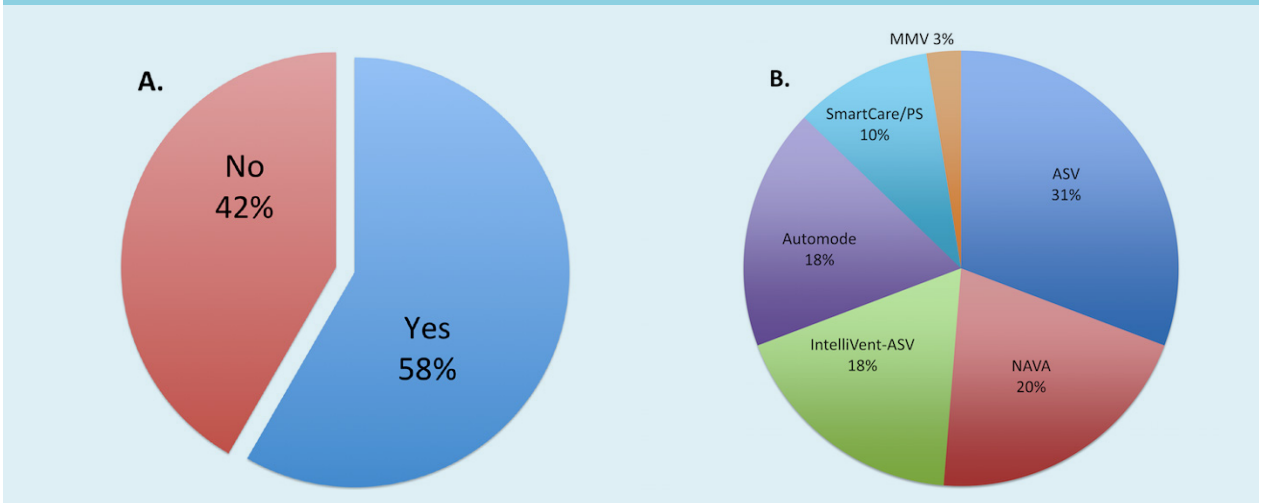
The response rate of the survey was 82% (72 of 88). Respondents had access to a closed-loop ventilation mode in 58% of the ICUs (42 of 72) (*figure 2*). Of these ICUs, 43% (18 of 42) frequently used a closed-loop ventilation mode, while 57% (24 of 42) occasionally or never used it (*figure 3*). The majority of the frequent users were level 3 ICUs (50% vs. 11% and 39% level 1 and 2, respectively), whereas the majority of the occasional users consisted of level 1 ICUs (54% vs. 16% and 29% level 2 and 3, respectively) (*table 1 and 2*). The ICUs with INTELLiVENT®–ASV never classified the frequency of use as occasional or never. No other noticeable differences were observed between the frequent users and the occasional users with regard to the types of modes. On the day of the survey, 24% of the ICUs (10 of 42) reported having at least one patient on a closed-loop ventilation mode. These ICUs averagely ventilated 51% of their ventilated patients with a closed-loop mode.

Respectively 17, 14 and 11 ICUs with access to a closed-loop ventilation mode stated that reasons for not using this mode were lack of knowledge (41%), insufficient evidence reporting a beneficial effect (33%) and lack of confidence in the mode (26%) (*figure 4*). Another 10% of these respondents mentioned that a perceived lack of control with the use of these modes might also play a role. With regard to (INTELLiVENT®–)ASV, 17% of the respondents expressed the concern that this mode selects higher tidal volumes than desired. Concerning NAVA, 7% of the respondents stated that the costs of the necessary disposables were a barrier for its use.

DISCUSSION

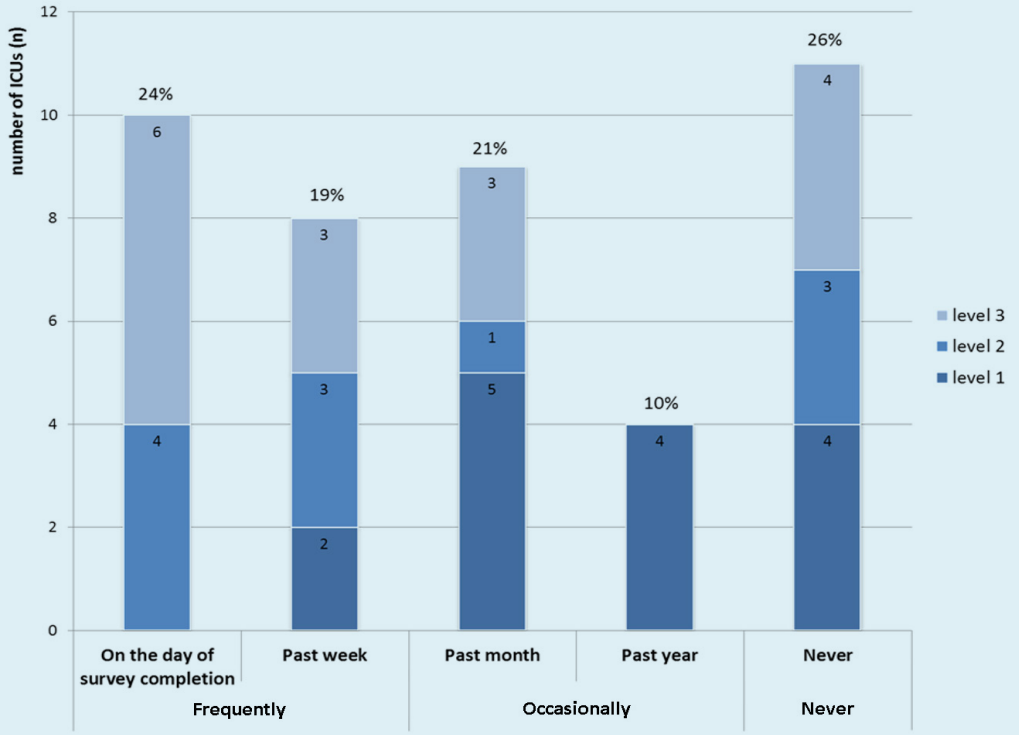
The results of this survey echo those from the international European survey in 2011 and the Ukrainian survey in 2013,[3,4] but do not support our hypothesis that closed-loop ventilation is increasingly used in the Dutch ICU setting. The most reported reason for resistance in our survey was 'lack of knowledge', which might be explained, at least in part, by a lack of experience and insufficient

Figure 2. *The percentage of ICUs that had access to a closed-loop ventilation mode (A), and the kinds of closed-loop ventilation modes that were used on these ICUs (B)*



ASV®= adaptive support ventilation; NAVA = neurally adjusted ventilatory assistance and MMV = mandatory minute ventilation.

Figure 3. *Frequency of use of closed-loop ventilation modes*



The last time a closed-loop ventilation mode was used on the different levels of ICUs with access to a closed-loop ventilation mode (n = 42). The use was classified as frequently, occasionally or never if respondents with a closed-loop ventilation mode had applied this mode at least once in the preceding week, month to year, or never, respectively.

Table 1. *The availability of a closed-loop mechanical ventilation mode (yes/no) per ICU level (level 1, 2 or 3)**

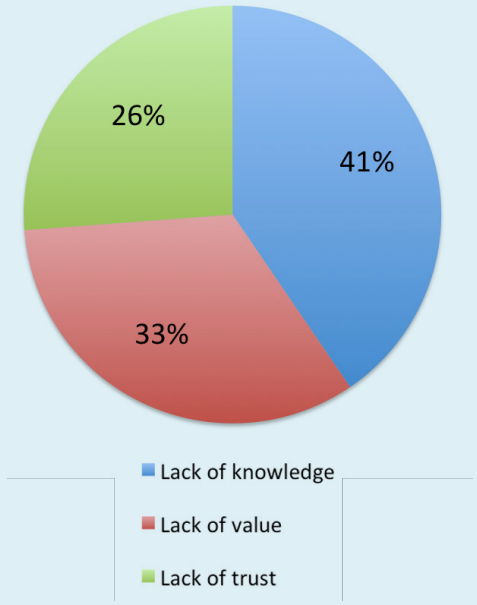
	Availability of a closed-loop mechanical ventilation mode	
	Yes	**No**
Level 1	15 (35.71%)	15 (50.00%)
Level 2	11 (26.19%)	7 (23.33%)
Level 3	16 (38.10%)	8 (26.67%)
Total	**42 (100%)**	**30 (100%)**

Table 2. *The frequency of use (frequently or occasionally/never) per ICU level (level 1, 2 or 3)**

	Frequency of use	
	Frequently	**Occasionally or Never**
Level 1	2 (11.11%)	13 (54.17%)
Level 2	7 (38.89%)	4 (16.67%)
Level 3	9 (50%)	7 (29.17%)
Total	**18 (100%)**	**24 (100%)**

*Data on ICU levels was extracted from http://www.ziekenhuizentransparant.nl.

Figure 4. *Reported reasons for not using closed-loop ventilation modes*



The percentage of participants, of ICUs with access to a closed-loop ventilation mode, that agreed with possible reasons for not using closed-loop ventilation modes.

education, which are needed for acquiring knowledge and for successful implementation.[6] Both explanations depend on local manpower and on the case mix dependent culture of the ICU. Interestingly, this study shows that frequent users mainly consisted of high level ICUs, while occasional users were mostly lower level ICUs. One explanation could be that lower level ICUs have less staff and less time and means available for the introduction of new modes of ventilation, all leading to a more conservative culture.

The second-most mentioned reason for not using closed-loop ventilation modes was 'insufficient evidence reporting a beneficial effect'. While various studies have been performed, among which three recent meta-analyses, results are still not conclusive.[7-9] Additionally, in research, closed-loop mechanical ventilation modes are often grouped together, while these modes operate according to different techniques in order to achieve different goals for various indications. These considerations make the translation of research outcomes into clinical practice challenging, since it is uncertain to what extent this evidence can help the clinicians to choose for a specific closed-loop ventilation mode which best suits their specific case mix and local culture of the ICU.

The third reason for not using closed-loop modes was 'lack of confidence in the mode'. In highly controlled environments such as the ICU, where the staff attempt to control each parameter as much as possible, it can be difficult to entrust this process to a machine, also known as the 'black box effect'.[10] This could explain why 'lack of control' was added as an additional reason for not using closed-loop modes.

Our study has certain limitations. First, although we reached a high response rate, the design of the study potentially introduces selection bias as clinicians who use closed-loop ventilation modes may be more inclined to respond. This means that the implementation rate might be even lower in reality. Secondly, this survey did not register the version of the modes used, and some comments may be related to older versions of the ventilation modes. For instance, ASV, INTELLiVENT®-ASV and NAVA have had several updates, which improved safety (e.g., lower tidal volumes in the first two modes) and ease of use (e.g., less alarms in the last mode).

Finally, this study does not provide a complete overview of all possible reasons that can influence the implementation of closed-loop modes. Many other possible contributing factors were not asked about in the survey, such as economic factors and long-term contracts with specific manufacturers.

In conclusion, while industry continues to develop new closed-loop modes, implementation of these modes in clinical practice seems to encounter difficulties. Various barriers could play a role, and these all need attention in future investigations.

DISCLOSURES

All authors have disclosed that they do not have any conflicts of interest.

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ORIGINAL ARTICLE

How to determine an impaired health status in COPD: Results from a population-based study

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is associated with a significantly impaired health status and lost work productivity across all degrees of airflow limitation. The current study investigated whether an impaired health status is better represented by the recommended COPD Assessment Test (CAT) cut-point of 10 points, or the 95th percentile of the CAT score in a non-COPD population. Additionally, the impact of COPD on health status in a Dutch population, after stratification for work status, was measured.

Methods: Demographics, clinical characteristics, post-bronchodilator spirometry, and CAT were assessed in subjects from the Longitudinal Aging Study Amsterdam (LASA), a large Dutch population-based study. Normative values for the CAT score were described by percentiles using the mean, standard deviation, median and range.

Results: In total, 810 COPD and non-COPD subjects (50.4% male, mean age 60.5 ± 2.9 years) were analysed. Significant differences were observed in CAT scores between non-COPD and COPD subjects (6.7 ± 5.2 vs. 9.5 ± 5.9, $p < 0.001$ respectively). The proportion of COPD subjects with an impaired health status differed between applying the CAT ≥ 10 cut-point (50.0%) and applying the 95th percentile of CAT in non-COPD subjects (> 18 cut-point; 7.6%). Higher CAT scores were seen in working COPD patients compared with working non-COPD subjects (9.3 ± 5.2 vs. 6.0 ± 4.6, $p < 0.001$).

Conclusion: We suggest a CAT cut-point of > 18 points to indicate an impaired health status in COPD. This would imply an adaptation of the current GOLD classification of the disease.

KEYWORDS

Health status, Chronic Obstructive Pulmonary Disease (COPD), COPD assessment test, normative values

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a lung disease characterised by persistent respiratory symptoms and chronic airflow limitation.[1] In addition to the pulmonary manifestations, COPD often provokes symptoms of anxiety and depression and causes limitations in daily life. In addition, a recent international patient survey revealed that 6% to 52% of working age patients are completely prevented from working due to their COPD.[2] The impact of these restrictions is often underestimated.[3,4] The COPD Assessment Test (CAT) is a simple patient-completed questionnaire developed to quantify the impact of COPD on health status, focusing on daily symptoms and activities.[5] Patients with an impaired health status experience a high burden of symptoms.[6]

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy describes an impaired health status as a CAT total score of ≥ 10 points, which is derived from the St. George Respiratory Questionnaire (SGRQ) cut-point of ≥ 25 points.[1] Previous studies determined normative values for other health-related measurements, e.g. echocardiographic measurements, according to the 95th percentile in a reference population.[7,8] Defining abnormalities based on percentile values will account for an asymmetric distribution and the range of abnormality

present within a population.[9] With the current GOLD CAT cut-point of ≥ 10 points, only a minority of primary care COPD patients have a normal health status.[6,10] Moreover, previous research showed that an impaired health status, derived from the current GOLD CAT cut-point, already occurs in half of the current or former smokers without airway obstruction.[11] This highlights the importance of understanding what an abnormal CAT value is, as it consequently also influences treatment choices in these patients. The large proportion of patients with an impaired health status resulting from the current GOLD CAT cut-point raises the question as to whether deriving abnormal values from the 95th percentile of CAT in a non-COPD population would give a better representation of reality, as suggested for several countries.[12-14]

While the societal burden of COPD is considerable, the impact of sociodemographic characteristics, such as work status on health status, is largely unknown. Less ability to participate in society and not being able to work are important concepts in an impaired health status.[15] Previous research showed comparable CAT scores between a working COPD population and a working non-COPD population.[12]

The primary aim of this study was to investigate which cut-off value represents an abnormal CAT score for non-COPD subjects in a Dutch population. The secondary aim was to measure the impact of COPD on health status in a Dutch population, after stratification for work status.

METHODS

Current data are collected from a subsample of the Longitudinal Aging Study Amsterdam (LASA), a large population-based study.[16] The study was initiated by the Dutch Ministry of Welfare, Health and Culture to determine consequences and predictors of ageing, focusing on physical, emotional, cognitive and social functioning in late life. Ethical approval for the LASA study was given by the Medical Ethics Committee of the VU University Medical Center Amsterdam (METC number 2012/361).

Population

Subjects aged between 55-65 years were randomly sampled from 11 municipalities of three culturally different geographic regions (Amsterdam, Zwolle and Oss) in the Netherlands. Subjects were drawn from the population registers and subsequently interviewed by trained persons in their homes. To make up the original sample, no inclusion or exclusion criteria were outlined in the LASA study. However, individuals who did not complete the medical interview and/or did not perform a spirometry were not included in the analyses.

Measurements

Between November 2012 and November 2013, subjects received a baseline interview assessing demographics, smoking history, work status, self-reported diseases and post-bronchodilator spirometry (forced expiratory volume in the first second, FEV1, and forced vital capacity, FVC). The spirometry was conducted with a Vmax Vyntus SPIRO – USB PC Spirometer from CareFusion (Höchberg, Germany), 15 minutes after inhalation of 200 µg salbutamol (Airomir autohaler, Teva). Global Lung Function Initiative (GLI) reference values were applied. Instead of managing the GOLD suggested fixed cut-off point for obstruction (FEV_1/FVC ratio < 0.7), an FEV_1/FVC ratio after bronchodilator lower than the 5th percentile (from reference values) was applied to define airway obstruction.[17] Patients with COPD were divided into four groups: spirometric grade 1 (FEV_1 ≥ 80%), spirometric grade 2 (FEV_1 50-79%), spirometric grade 3 (FEV_1 30-49%), and spirometric grade 4 (FEV_1 <30%), based on the GOLD strategy 2017.[1] Additionally, health status was assessed with the CAT. The CAT is an eight-item patient-completed questionnaire, designed to measure health status in patients with COPD. Item scores range from 0 to 5 points, whereby the total score varies between 0 (best health status) and 40 points (worst health status).[5] An impaired health status was defined with the CAT ≥ 10 cut-point and 95th percentile of the non-COPD population.

Statistics

Descriptive statistics, including means (standard deviation, SD) and medians (interquartile range, IQR), were applied. Categorical variables were described as frequencies. CAT normative values were described by percentiles using mean (SD), median and range. First, the calculation of normative values was performed in the whole non-COPD population. All variables were tested for normality with the Kolmogorov-Smirnov test. Differences between non-COPD subjects and COPD patients were assessed by performing an independent Student's t-test, when normally distributed. Otherwise, a Mann-Whitney U test and two-independent-samples tests were done to compare the two groups. When appropriate, a post hoc least significance difference multiple comparison was performed. A Kruskal-Wallis test was assessed for not normally distributed variables and a Chi-square test was applied for categorical variables. Similar analyses were performed to compare working and non-working groups. A p-value of less than 0.05 was considered statistically significant. All statistics were done using SPSS V.20.0.

RESULTS

In total, 810 subjects (50.4% male, mean age 60.5 (2.9) years) were included, 68 (8.4%) of which had a chronic airflow limitation: 18 GOLD spirometric grade 1, 43 GOLD spirometric grade 2, 5 GOLD spirometric grade 3, and 2 GOLD spirometric grade 4 (see *figure 1* for flowchart). Twenty-seven of the COPD subjects (40%) were previously diagnosed with a respiratory disease; 10 (37%) received COPD treatment from a general practitioner, 7 (26%) received COPD treatment from a specialist and 10 (37%) received no treatment. The non-COPD and COPD groups were similar regarding age, gender, BMI and comorbidities. Non-COPD subjects were less often current smokers and had a higher FEV1% predicted than subjects with COPD (*table 1*).

COPD versus non-COPD

CAT total scores were significantly lower in non-COPD subjects than in COPD subjects. COPD subjects had significantly higher scores on CAT questions related to cough, phlegm and breathlessness during activities (*table 1*). CAT values of non-COPD subjects ranged from 0 to 29 points, with the 95th percentile at 18 points (*table 2*). When applying the CAT ≥ 10 cut-point, 50.0% of COPD subjects had an impaired health status and when using a CAT > 18 cut-point 7.6% of COPD subjects had an impaired health status (*table 2*).

Work status

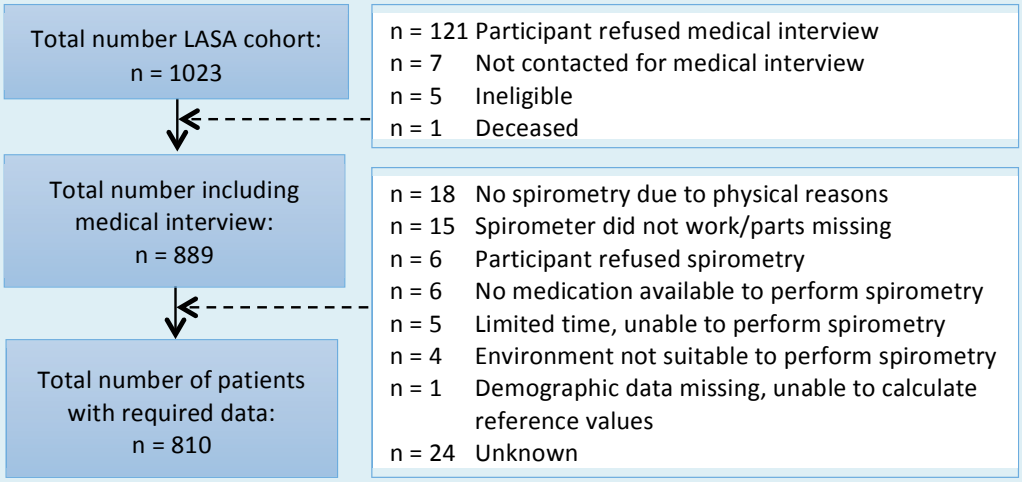
As shown in *figure 2*, CAT total scores were significantly lower in non-COPD subjects with a job, compared with non-COPD subjects without a job, $p < 0.001$. No significant differences were observed between a working and non-working COPD population, $p < 0.741$. Moreover, significantly higher CAT scores were observed in a working population with COPD in comparison with a working population without COPD, $p < 0.001$.

DISCUSSION

This is the first study examining normative values for CAT performed in a Dutch population. It shows that approximately 20% of the non-COPD subjects had an impaired health status according to the current cut-point suggested by GOLD (CAT ≥ 10 points). Based on the 95th percentile of the CAT in a non-COPD population, a new CAT cut-point of > 18 points was suggested to indicate an impaired health status. No significant differences in CAT score were found between a working and non-working COPD population. Normative values should be taken into account when applying the refined GOLD assessment to Dutch COPD patients in clinical practice.

In accordance with previous research,[18] the current study showed that patients with COPD had significantly

Figure 1. *Flow diagram of subject inclusion*



LASA = Longitudinal Aging Study Amsterdam.

Table 1. *Baseline characteristics of study participants*

	Non-COPD subjects (n = 742)	COPD subjects (n = 68)	p-value
Men, n (%)	372 (50.1)	36 (52.9)	0.658
Age, years	60.4 (2.9)	60.9 (2.8)	0.227
BMI, kg/m²	26.6 (23.8-29.5)[a]	26.0 (23.3-28.9)[h]	0.407
Current smoker, n (%)	120 (16.2)	28 (41.2)	< 0.001*
Pack-years, n	11.7 (16.9)	28.5 (25.5)	< 0.001*
FEV_1, %predicted	99.5 (90.4-109.6)[b]	67.6 (60.4-80.4)	< 0.001*
FEV_1/FVC, %	79.9 (75.7-83.2)	61.6 (54.2-64.3)	< 0.001*
Self-reported diseases			
Heart disease, n (%)	77 (10.4)	12 (17.6)	0.067
Artery disease or abnormalities, n (%)	22 (3.0)	4 (5.9)	0.191
Diabetes, n (%)	57 (7.7)	5 (7.4)	0.922
Cerebrovascular accident, n (%)	16 (2.2)	2 (2.9)	0.674
Osteoarthritis, n (%)	307 (41.4)	24 (35.3)	0.329
Rheumatoid arthritis, n (%)	59 (8.0)	5 (7.4)	0.861
Cancer, n (%)	67 (9.0)	7 (10.3)	0.729
Other chronic disease, n (%)	248 (33.4)	17 (25.0)	0.156
CAT total score, points	6.7 (5.2)[c]	9.5 (5.9)[i]	< 0.001*
CAT cough, points	1.2 (1.0)	1.7 (1.2)	< 0.001*
CAT phlegm, points	0.0 (0.0-1.0)	1.0 (0.0-2.0)	< 0.001*
CAT chest tightness, points	0.0 (0.0-1.0)	0.0 (0.0-1.0)	0.214
CAT breathlessness during activities, points	1.2 (1.2)[d]	2.1 (1.6)	< 0.001*
CAT activity at home, points	0.0 (0.0- 0.0)	0.0 (0.0-1.0)	0.195
CAT confidence in leaving home, points	0.0 (0.0-0.0)[e]	0.0 (0.0-0.0)[j]	0.099
CAT sleep, points	1.4 (1.4)[f]	1.4 (1.5)[k]	0.995
CAT energy, points	1.3 (1.2)[g]	1.3 (1.2)[l]	0.974
Subjects with CAT ≥ 10 points, n (%)	165 (22.8)[c]	33 (50.0)[i]	< 0.001*
Subjects with CAT > 18 points, n (%)	28 (3.9)[c]	5 (7.6)[i]	0.150
SR-physician's respiratory diagnosis, yes (n)	46 (6.2)	27 (39.7)	< 0.001*
Treatment for respiratory diagnosis, yes (n)	30 (4.0)	17 (25.0)	< 0.001*

Values expressed as mean (SD), median (IQR), number of patients (n) or proportion (%). *= p ≤ 0.05. [a]= 2 participants missing, [b]=1 participant missing, [c]=19 participants missing, [d]= 3 participants missing, [e]= 15 participants missing, [f]= 10 participants missing, [g]= 2 participants missing, [h]= 1 participant missing, [i]= 2 participants missing, [j]= 1 participant missing, [k]=2 participants missing, [l]= 1 participant missing. BMI = body mass index; FEV_1, = forced expiratory volume in the first second; FVC = forced vital capacity; SR = self-reported; COPD = chronic obstructive pulmonary disease; CAT = COPD Assessment Test.

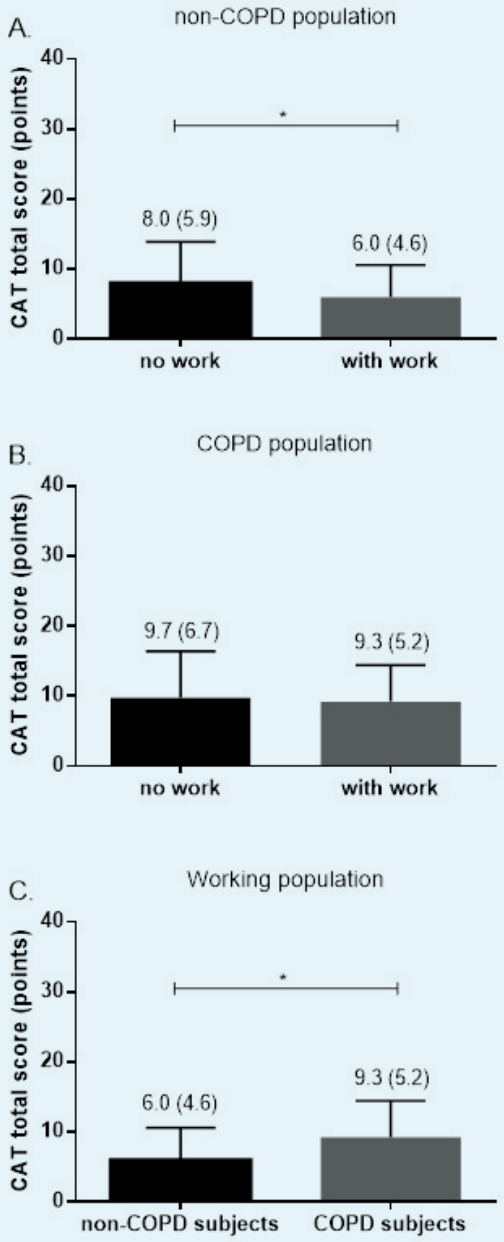
more symptoms of cough, phlegm and breathlessness during activities. Reported mean CAT total scores in COPD subjects vary between 7.3 points (± 5.2 [n = 67, Japan]), 16.6 points (95% CI = 15.5-16.8 [n = 806, Arabic countries]) and 20.9 points (95% CI = 16.9-22.2 [n = 229, Turkey]).[12-14] A mean CAT of 9.5 points was observed in the current study. Moreover, a value of 18 points was found as the 95th percentile of CAT total scores in non-COPD subjects, resulting in 7.6% of the COPD patients with an impaired health status. Previous studies showed that the 95th percentile of CAT total scores in non-COPD subjects varies from 14 points (n = 1266, Japan), 16 points (n = 500, Canada), 21 points (n = 2863, Arabic countries) and 28 points (n = 872, Turkey).[12-14] Differences in disease severity, demographics, comorbidities, care setting, religion, culture and socio-economic factors may account for the observed variation.[19-22] When comparing disease severity between studies, airflow obstruction was equivalent.[12-14] Furthermore, not all previously performed studies measured comorbidities, and if they did, often other diseases were assessed, making a comparison difficult. Indeed, Nishimura and colleagues showed that a working population is often more active during the day,[12] resulting in a better health status and less symptoms.[23] Moreover, the use of a CAT cut-point of > 18 points is supported by the study of Casanova and colleagues, stating that a CAT cut-point of > 18 points more comprehensively categorises patients with COPD according to the GOLD classification and more adequately predicts all-cause mortality.[24]

Table 2. *CAT normative values for COPD and non-COPD subjects*

	N	Non-COPD subjects[a+b]
Mean CAT (SD)	723	6.7 (5.2)
Median	723	6.00
Range CAT (min-max)	723	0-29
CAT 5th percentile	723	0.00
CAT 10th percentile	723	1.00
CAT 25th percentile	723	3.00
CAT 75th percentile	723	9.00
CAT 90th percentile	723	14.00
CAT 95th percentile	723	18.00
	N	**COPD subjects[c]**
Mean CAT (SD)	66	9.5 (5.9)
Median	66	9.50
Range CAT (min-max)	66	0-27
CAT 5th percentile	66	0.00
CAT 10th percentile	66	2.70
CAT 25th percentile	66	4.75
CAT 75th percentile	66	13.25
CAT 90th percentile	66	17.30
CAT 95th percentile	66	19.65

[a]= 11 participants missing, [b]= 8 participants missing, [c]=2 participants missing.
COPD = chronic obstructive pulmonary disease; CAT = COPD Assessment Test; SD = standard deviation.

Figure 2. *CAT stratified by work status. A) Working population versus non-working population in non-COPD subjects; B) Working population versus non-working population in patients with COPD and C) non-COPD subjects versus patients with COPD in a working population*



CAT = COPD Assessment Test; COPD = chronic obstructive pulmonary disease.

While the current study found differences in CAT scores between a working and non-working non-COPD population, no significant differences were found between a working and non-working COPD population. In addition, COPD patients with a job still had significantly higher CAT scores than non-COPD subjects with a job. So, it is possible that solely having a job improves heath status (to a certain extent) in a general population, but this does not apply for COPD patients. Previous research indicates that variation between multiple populations can also be explained by differences in quality of life between countries.[25] Defining influences of the other variables (demographics, care setting, religion, culture and socio-economic factors) goes beyond the scope of this study.

Another important factor to consider when describing normative values is the impact of comorbidities. In the current study, approximately 10% of the non-COPD subjects reported heart disease, presumably leading to a higher CAT total score. However, there are several reasons for not excluding comorbidities in the non-COPD group. First, a study by Gupta and colleagues indicated that only depression, myocardial infarction, angina and/or pneumonia influence CAT score.[26] Another study

showed that solely gastro-oesophageal reflux disease and depression have an impact on CAT.[27] However, not all these comorbidities were assessed. Therefore, we were not able to determine these influences. Also, comorbidities in the current study were self-reported. The study by Triest and colleagues showed a poor agreement between objectively identified and chart-based comorbidities in patients with COPD,[28] resulting in unreliable outcomes. Finally, as patients with COPD also experience many comorbidities,[29] it would be unrealistic to compare patients with completely healthy individuals. Subsequently, the current results showed no differences in comorbidities between subjects with or without COPD. This indicates that comorbidities are not specifically related to COPD, making them valuable for normative values of the CAT.

Limitations

There are some limitations to this study. First, selection bias could have occurred, indicating that people who lack motivation or with a worse health condition are less willing to participate. These people may also be less willing to perform a lung function test, leading to more favourable outcomes. However, we tried to minimise selection bias by randomly selecting the participants. Second, participants were between the age of 55-65 years. Despite the limited variance in age, results are in accordance with previous research in other countries. Therefore, it is expected that the results are representative, though one should be careful in generalising to other age groups. Third, comorbidities and a former diagnosis of COPD were self-reported. Also, COPD was defined as a self-reported diagnosis of chronic bronchitis, asthma, emphysema, or COPD. Initially, it is a disadvantage that the diagnosis is self-reported as it is less accurate than the original diagnosis of the doctor. Besides that, no distinction was made between the various respiratory diseases. This makes it impossible to specify whether the participant was diagnosed with chronic bronchitis, asthma, emphysema or COPD.

CONCLUSION

A new CAT cut-point of > 18 points is suggested to indicate an impaired health status in patients with COPD, as approximately one in five non-COPD subjects have abnormal CAT scores according to current international standards. These normative values should be taken into account when applying the updated GOLD assessment to Dutch COPD patients in clinical practice.

ACKNOWLEDGEMENTS

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DISCLOSURES

The authors have nothing to disclose.

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CASE REPORT

Severe neurological symptoms following synthetic cannabinoid intoxication

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ABSTRACT

Synthetic cannabinoids are becoming increasingly popular as substances of abuse. However, in the Netherlands synthetic cannabinoid intoxications are rare. We report a 16-year-old male who became deeply comatose and was admitted to the intensive care unit for invasive mechanical ventilation after abuse of an initially unknown drug. Routine toxicology screening with an immunoassay only detected tetrahydrocannabinol, but additional tests with liquid chromatography mass spectrometry revealed synthetic cannabinoid use. This case underlines the challenging diagnosis of synthetic cannabinoid intoxications and the severe complications they can produce.

KEYWORDS

Synthetic cannabinoids, intoxication, coma, drugs of abuse, LC-MS/MS

What was known on this topic?
Although on the rise globally, synthetic cannabinoid intoxications are extremely rare in the Netherlands. Symptoms can be similar to cannabis. However, depending on the exact substance more serious neurological and cardiovascular complications may occur.

What does this add?
Neurological symptoms of synthetic cannabinoid intoxications can be as serious as a comatose state with absence of some brain stem reflexes. Because of their low prevalence and the fact that synthetic cannabinoid intoxications are not detected in most urine toxicology screenings, their diagnosis in a clinical setting can be challenging.

INTRODUCTION

Synthetic cannabinoids are becoming increasingly popular as drugs of abuse, mainly in Europe and the United States.[1] As a result, patients are presenting to emergency departments with unwanted effects of these drugs. Synthetic cannabinoids are sold in colourful packages under various names, including 'spice', 'K2', 'crazy monkey' and 'chill out'. Synthetic cannabinoids are a chemically very diverse class of drugs and structurally different from tetrahydrocannabinol in natural cannabis, but all substances were designed to act as agonists of cannabinoid receptors (CB1 and CB2). This explains their psychoactive effects.[1] Although intoxications with synthetic cannabinoids are globally on the rise, they are still a very rare phenomenon in the Netherlands.[2] This case report describes a patient with very severe neurological symptoms after synthetic cannabinoid intoxication and explains why these intoxications can be very difficult to diagnose.

CASE REPORT

A 16-year-old male with no medical history presented to our emergency department. The patient had lost consciousness after smoking cannabis and subsequently a drug called 'Bonsai'. On arrival to the emergency department physical examination revealed a Glasgow Coma Score of E1M1V1, dilated pupils, unresponsive to light and absence of some of the other brainstem reflexes.

Vital signs on presentation were normal except for mild tachycardia (100 beats/minute). ECG and laboratory examination showed no abnormalities. Because of persisting lack of consciousness the patient was intubated and invasive mechanical ventilation was started. A CT scan and CT angiography of the brain showed no pathological abnormalities.

A urine sample, which was obtained at admission, was positive for tetrahydrocannabinol but negative for amphetamines, barbiturates, benzodiazepines, cocaine, methadone, opioids and tricyclic antidepressants in our immunoassay testing kit. Ethanol and gamma-hydroxybutyric acid were measured in the serum, but could not be detected.

On the ICU we attempted to stop sedation. However, the patient became very agitated, so he was kept sedated overnight. The next morning sedation was successfully stopped and the patient was extubated and discharged home in good clinical condition.

After discharge, a toxicology screening of the patient's serum was obtained by liquid chromatography mass spectrometry (LC-MS/MS). The target screening method was based on the method described by Jaenicke et al.[3] and consisted of 32 synthetic cannabinoids. Based on the retention time and mass spectra, the presence of O-2545, a synthetic cannabinoid, was confirmed.

DISCUSSION

Synthetic cannabinoids were originally developed as potential therapeutics.[4] However, since the early 2000s several synthetic cannabinoids are sold online and in smart shops as legal alternatives for cannabis. In American surveys amongst selective groups (students, club visitors) both life-time and past year intake of synthetic cannabinoid was reported to be around 6-8%.[5-7] In the Netherlands, synthetic cannabinoid use appears to be very rare.[2] This may be related to the availability of high-quality cannabis without legal consequences for possession. Because of the low prevalence of synthetic cannabinoids in the Netherlands, hospital admissions due to intoxications are very rare.

In this case, testing urine for the most common drugs of abuse only revealed tetrahydrocannabinol, which was consistent with the anamnesis as the patient had smoked cannabis. However, it was unlikely that tetrahydrocannabinol produced these severe neurological symptoms, so this was not helpful for the diagnosis. A quick online search on 'Bonsai'[8] pointed to a possible intoxication with the benzodiazepine phenazepam. However, neither the negative benzodiazepine screening in urine nor the clinical state of the patient seemed to support this hypothesis. Furthermore, phenazepam is usually taken orally instead of being smoked. Only after additional searching of the literature did we define the working diagnosis as synthetic cannabinoid intoxication, which was confirmed after discharge by LC-MS/MS. This underlines the diagnostic difficulties that arise because synthetic cannabinoids are not detected by the commonly used kits for screening on drugs of abuse. Fortunately, definite confirmation of synthetic cannabinoids by LC-MS/MS is usually not necessary because their use is clear from the anamnesis and treatment is aspecific and mostly supportive.

After acute synthetic cannabinoid intoxication psychoactive symptoms, such as agitation, restlessness, confusion, anxiety and psychosis, are common.[9] Patients usually display physical signs that also occur with cannabis intoxication, including dilated pupils, red conjunctivae, nausea, slurred speech and sweating. Cardiovascular symptoms, such as hypertension, tachycardia and chest pain, may also be present and rarely synthetic cannabinoid intoxications can lead to myocardial infarction, kidney injury or death. Laboratory tests and electrocardiogram are generally normal and in many cases urine toxicology screens are negative for drugs other than tetrahydrocannabinol, which is often used on the same occasion. Importantly, synthetic cannabinoid use is not ruled out by negative drug screening for tetrahydrocannabinol nor confirmed by a positive tetrahydrocannabinol result.

Treatment of synthetic cannabinoid intoxication is usually supportive and determined by the order and magnitude of symptoms.[10] Agitation and confusion can often be managed with reassurance and avoidance of stimulation, while benzodiazepines can be administered for more serious symptoms such as psychosis. Rarely, prolonged sedation or intubation can be necessary to prevent end-organ damage or rhabdomyolysis. Mild intoxications typically last less than eight hours.[11] However, clinical effects and the duration and degree of toxicity depend on the specific compound used.

In conclusion, synthetic cannabinoid intoxications are on the rise in Europe and the United States. Although they are still very rare in the Netherlands, it is quite possible that Dutch emergency departments will be increasingly confronted with synthetic cannabinoid intoxications. Symptoms can be of greater magnitude and duration compared with cannabis intoxication. This patient presented with severe neurological symptoms including absence of some brain stem reflexes. Mechanical ventilation was necessary. Diagnosis of a synthetic cannabinoid intoxication can be challenging because it is not revealed by most urine drugs of abuse kits. Confirmation is possible with LC-MS/MS, but this technique is costly and is not readily available in most cases.

DISCLOSURES

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CASE REPORT

Acenocoumarol as a risk factor for calciphylaxis: a feature clinicians should be aware of

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ABSTRACT

In contrast with uraemic calciphylaxis in end-stage renal disease, causes of and risk factors for non-uraemic calciphylaxis are relatively unknown to clinicians and have yet to become fully established. This report describes a case of non-uraemic calciphylaxis, in which the use of acenocoumarol might have been a risk factor. It is important to raise awareness about this association among clinicians, as vitamin K antagonists have to be stopped for an optimal treatment of this severe condition.

KEYWORDS

Calciphylaxis, acenocoumarol, warfarin, calcaemic uraemic arteriolopathy, vitamin K antagonist, non-uraemic

What was known on this topic?
Calciphylaxis is associated with end-stage renal disease. However, it also occurs in patients with normal renal function, known as non-uraemic calciphylaxis. The different causes of and risk factors for this disease have yet to become fully established.

What does this add?
This case report raises the awareness that vitamin K antagonists interfere with calcium metabolism and may play a role in calciphylaxis. Therefore, vitamin K antagonists should be stopped in order to optimise treatment.

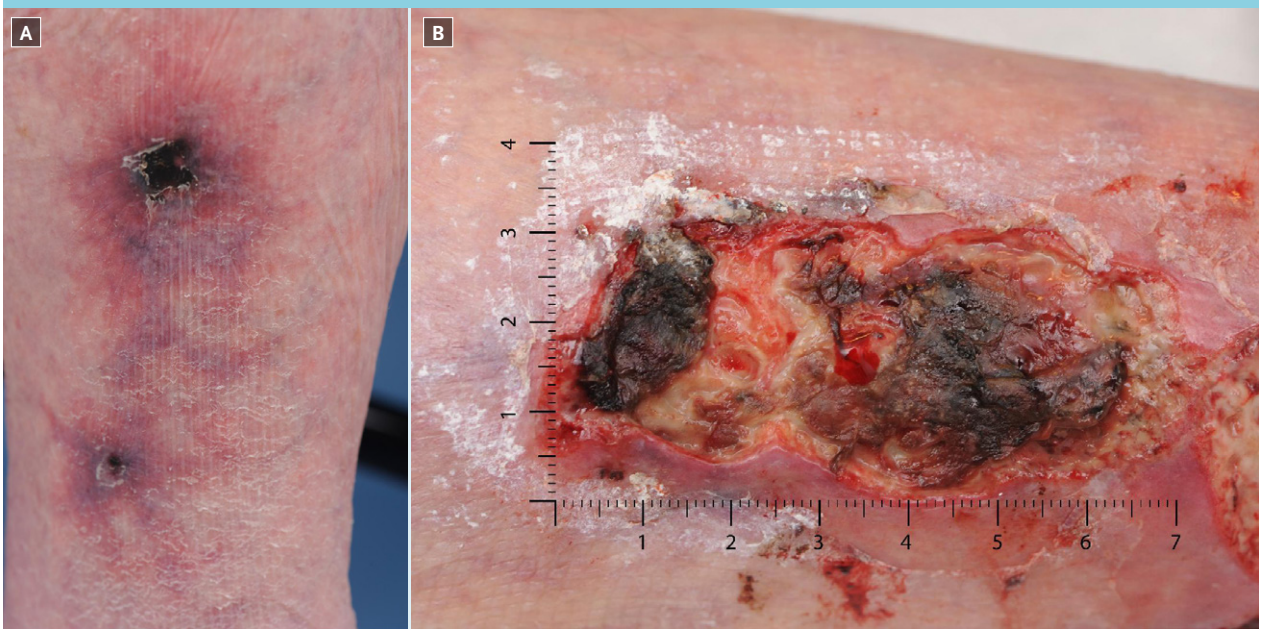
INTRODUCTION

Calciphylaxis is a rare disease that consists of calcifications in the blood vessels, which lead to secondary ischaemia and painful necrotic lesions of the skin. It is known to be associated with dialysis treated end-stage renal disease in combination with secondary hyperparathyroidism. This is called uraemic calciphylaxis, or a more recent term: calcaemic uraemic arteriolopathy. If calciphylaxis occurs in earlier stages of renal disease or in patients with a normal kidney function, the term non-uraemic calciphylaxis is used. It is crucial that calciphylaxis and its risk factors are recognised, since both uraemic and non-uraemic forms have a severe prognosis with a one-year mortality of 45-80%.[1] In contrast with uraemic calciphylaxis, causes of and risk factors for non-uraemic calciphylaxis are relatively unknown to clinicians and have yet to become fully established. A relatively new insight is that vitamin K antagonists may play a role. This report describes a patient with non-uraemic calciphylaxis in which this might have been the case.

CASE REPORT

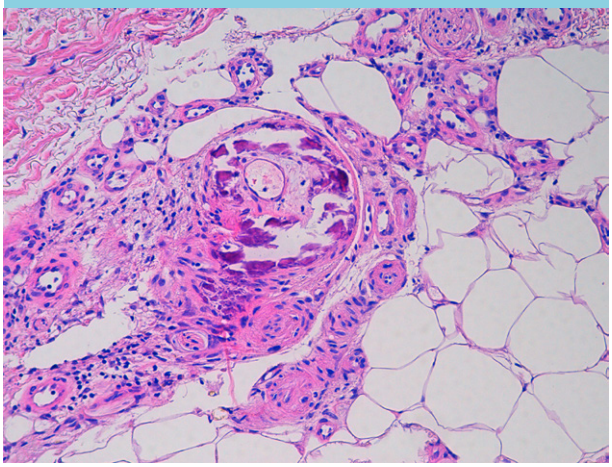
A 82-year-old female presented with a painful lower right extremity after a fall five weeks before. There were two necrotic lesions on her right leg, surrounded by livedoid reticularis (*figure 1a*). The medical history of this patient included obesity, diabetes mellitus, hypertension, mechanic heart valve implantation, chronic obstructive pulmonary disease, and chronic kidney disease (modification of diet in renal disease (MDRD) of around 30 ml/min). The patient had been using acenocoumarol for 5 years, and additional medication included metoprolol, simvastatin, furosemide, formoterol/ beclomethasone, tiotropium, and oxazepam. Anti-phospholipid syndrome, vasculitis, systemic lupus erythematosus, and cryoglobulinaemia were excluded. Calcium and phosphate levels were normal, and parathyroid hormone was slightly elevated. An ankle-brachial index test showed no

Figure 1. *A. Two necrotic lesions surrounded by livedoid reticularis on the lower right leg. B. Large ulcerated lesion on the right lower leg a few weeks after initial presentation*



abnormalities, and a biopsy was taken. Differential diagnoses included polyarteritis nodosa, cholesterol embolisation, and calciphylaxis. Topical corticosteroid therapy and pain management were pragmatically started. Unfortunately, the biopsy was inconclusive. Meanwhile, the symptoms worsened, but the patient did not agree to a new biopsy. Oral prednisone was started, with vasculitis/polyarteritis nodosa as the main working diagnosis. A wound culture did not reveal any non-physiological flora. The symptoms continued to worsen (*figure 1b*) and a few months later the patient agreed to a new biopsy. This time the biopsy demonstrated a clear image of calciphylaxis (*figure 2*). Further laboratory evaluation showed a normal serum calcium of 2.32 mmol/l (2.10-2.55 mmol/l), a normal phosphate level of 1.04 mmol/l (0.80-1.50), a mildly increased parathyroid hormone of 10.0 pmol/l (1.6-6.9 pmol/l), and decreased vitamin D (25-OH) of 17 nmol/l (> 50 nmol/l). Creatinine level was 119 µmol/l and the MDRD was 38 ml/min. Subsequently, suppletion of vitamin D was started, and the hyperparathyroidism was corrected by prescribing cinacalcet (mimpara). Simultaneously, acenocoumarol was replaced by a low-molecular-weight heparin (LMWH). Unfortunately, two weeks later our patient died, with cardiac failure being the most presumable cause in a multifactorial situation. No autopsy was allowed.

Figure 2. *The skin biopsy revealed the characteristic calcifications of several small- to medium-sized arteries and arterioles. In addition to mural calcification, this particular vessel also showed intimal proliferation*



DISCUSSION

A systematic review assessing the different causes of non-uraemic calciphylaxis, indicated primary hyperparathyroidism as the most common cause (28%).[2] However, serum parathyroid hormone in our case was only mildly elevated compared with the levels in the included cases.[2] Therefore, we examined whether other relevant factors might have played a role. The suggested risk factors for non-uraemic calciphylaxis are numerous, including white race, female sex, obesity, diabetes mellitus, use of a vitamin K antagonist, liver disease, malignancy, systemic corticosteroid use, and protein C and S deficiency.[2,3] The first five risk factors were present in our case. Of these, we found the use of a vitamin K antagonist of

particular interest, as it is the only one of the mentioned risk factors that can immediately be adjusted. Several case reports state that the use of a vitamin K antagonist was the main cause of calciphylaxis in their patient.[4-6] Of note, larger studies are not that unambiguous. A retrospective analysis concluded that vitamin K antagonist use was not statistically associated with calciphylaxis.[7] However, this analysis only made a comparison between *uraemic* calciphylaxis patients and a dialysis control group without calciphylaxis. While vitamin K antagonist use was present in 60% of the *non-uraemic* patients, this group was not compared with a control group.[7] In the earlier mentioned systematic review about non-uraemic calciphylaxis, vitamin K antagonist use was present in 25% of the cases.[2] Unfortunately, existing literature either consists of retrospective studies or analyses of case reports. Therefore, selection bias and confounding by indication cannot be ruled out, and a definite conclusion cannot be drawn. Although epidemiological studies have thus not yet elucidated the role of vitamin K antagonists in calciphylaxis, this association is increasingly receiving attention in pathophysiological studies.

Pathophysiology

In contrast to atherosclerotic disease, in which the intima is the site of calcification, calciphylaxis involves calcification of the tunica media. The intima is not left untouched, as a process of fibrosis takes place there. Progressive calcification and endothelial dysfunction lead to thrombotic occlusion and ischaemia, which causes tissue necrosis of the skin. The process of calcification starts with the transformation of vascular smooth muscle cells (VSMCs) into osteoblast-like phenotypes. VSMCs normally produce matrix gla protein (MGP), a protein that binds calcium phosphate and thus has a strong inhibitory effect on tissue calcification. Vitamin K antagonists are thought to reduce functional MGP, as they interfere in the vitamin K carboxylation by which MGP is normally activated.[8] Vitamin K consists of vitamin K1 and K2, and vitamin K antagonists are not selective for either one of these. Vitamin K2 is involved in the inhibition of calcium deposition in blood vessels, while vitamin K1 leads to the contemplated anti-thrombotic effect. Currently, there are different ongoing trials that examine the role of vitamin K in vascular calcification to a greater extent.[9]

Differential diagnosis

Calciphylaxis in the presence of vitamin K antagonist use should be distinguished from warfarin skin necrosis, a condition that is clinically similar. However, warfarin skin necrosis typically occurs a few days after the start of warfarin therapy, while calciphylaxis is associated with prolonged use.[10] Histological findings are able to differentiate between the two, which is important since it determines the treatment of choice. Laboratory evaluation needs to be done as well and serves two major goals: to detect potential associated risk factors and to exclude other differential diagnoses. These diagnoses include vasculitis, atherosclerotic disease, cholesterol embolisation, nephrogenic systemic fibrosis, oxalate vasculopathy, and purpura fulminans.[2,11] Laboratory work-up is extensively described elsewhere but mainly includes parameters for infection, hypercoagulability, and autoimmune diseases.[11]

Treatment

Due to its rarity, there are no evidence-based guidelines for the treatment of both uraemic and non-uraemic calciphylaxis and a combined approach is often implemented. If vitamin K antagonists are used, they should be stopped. In warfarin skin necrosis, this is the only necessary step. However, in calciphylaxis, it is also essential to restore calcium, phosphate, and parathyroid hormone homeostasis.[10,11] If this treatment fails, intravenous administration of sodium thiosulfate is another option. In a similar case to ours, in which vitamin D suppletion and replacement of acenocoumarol by LMWH did not induce any improvement, sodium thiosulfate was successfully administered.[12] In 2011, a review of 41 case reports described a success rate for sodium thiosulfate of more than 90%, although publication bias has to be considered.[13] Another option that seems to be successful is hyperbaric oxygen therapy, as more than half of the patients benefited from this treatment.[14]

With this case report, the authors want to point out that calciphylaxis is an important differential diagnosis in patients without end-stage renal disease as well, since this disease has a severe prognosis with a high mortality. Whether vitamin K antagonists are an independent risk factor for non-uraemic calciphylaxis has yet to be determined. Pathophysiological studies provide interesting new links, but epidemiological studies lack adequate designs to rule out selection bias and confounding by indication. Nevertheless, awareness of a possible association is essential, and internists need to know they should stop these drugs for an optimal treatment.

DISCLOSURES

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CASE REPORT

Purpura fulminans mimicking toxic epidermal necrolysis – additional value of 16S rRNA sequencing and skin biopsy

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ABSTRACT

Both purpura fulminans and toxic epidermal necrolysis (TEN) are rare and life-threatening disorders with a high mortality. We present a case of suspected rapidly progressive, severe pneumococcal sepsis-induced purpura fulminans complicated by multiple organ failure, severe epidermolysis and cutaneous necrosis. We show the diagnostic challenge to differentiate between purpura fulminans and TEN, as the extensive epidermolysis in purpura fulminans may mimic TEN and we highlight the additional value of repeated skin biopsies and 16S rRNA gene sequencing.

KEYWORDS

Purpura fulminans, toxic epidermal necrolysis, disseminated intravascular coagulation, sepsis

What is known on this topic?
Both purpura fulminans and toxic epidermal necrolysis are rare and life-threatening disorders with a high mortality. Purpura fulminans is rapidly progressive and characterised by intravascular thrombosis and haemorrhagic infarction of the skin, accompanied by vascular collapse and disseminated intravascular coagulation. TEN manifests with mucocutaneous lesions leading to necrosis, sloughing of the epidermis and is usually drug induced.

What does this add?
Extensive epidermolysis and cutaneous necrosis in purpura fulminans may mimic toxic epidermal necrolysis. Although blood cultures remain the gold standard for diagnosing sepsis, additional histopathological analysis via skin biopsy and 16S rRNA gene sequencing may be helpful in confirming the diagnosis when blood cultures remain sterile.

INTRODUCTION

Acute purpura fulminans is a rare and life-threatening disorder, frequently associated with acute disseminated intravascular coagulation secondary to sepsis. The features of purpura fulminans include tissue necrosis and small vessel thrombosis.[1] Toxic epidermal necrolysis (TEN) is characterised by extensive apoptosis and detachment of the epithelium of the skin and mucous membranes and is mostly attributable to adverse drug reactions.[2,3] Both diseases have a high morbidity with mortality rates up to 50%.[3,4] Early recognition as well as identification and treatment of the aetiology may halt disease progression and prevent further complications. We present a case of severe sepsis-induced purpura fulminans resulting in severe epidermolysis, mimicking TEN.

CASE REPORT

A 56-year-old woman presented to the emergency department as self-referring patient with acute fever, chills and severe pain in both legs. Her medical history revealed depression, breast augmentation and alcohol abuse. Her current medication consisted of ibuprofen, citric acid and sodium chlorite 25%. The last two are for dermal application only, but the patient used these orally, unaware of the details of the prescription. Apart from hay fever, she had no known allergies. On physical examination she was confused and severely ill, with tachypnoea and a pulse oxygen saturation of 98% without supplemental oxygen. Capillary refill time was delayed

with cold, white extremities and cutis marmorata, a pulse of 90 beats/minute, and blood pressure 130/95 mmHg. Her body temperature was 36.8° Celsius and neurological, pulmonary, cardiac and abdominal examination showed no abnormalities. Because of suspected aortic thrombosis, CT abdomen was performed which showed no vascular or other abnormalities. Urine samples and chest X-ray showed no signs of infection. The presumed diagnosis of septic shock with unknown focus was made and blood cultures were drawn after the first bolus of antibiotics was administered. She was treated with cefuroxime, clindamycin and tobramycin.

Despite volume resuscitation, vasopressor therapy and initiation of mechanical ventilation in the ICU, her clinical condition deteriorated into multiple organ failure (ARDS, acute renal failure and disseminated intravasal coagulation, see *table 1*). Furthermore, she rapidly developed a sharply demarcated retiform purpuric skin rash with a positive Nikolsky's sign, resulting in haemorrhagic bullae and epidermolysis in the next two days (*figure 1*). Eventually, this resulted in bilateral symmetrical gangrene of four extremities, parts of her torso and face, without involvement of the mucosa (*figure 2*). Skin biopsies on day 9 and 10 showed a largely necrotic epidermis with subepidermal blistering, intravascular thrombi and extravasation of erythrocytes (*figure 3*). Although blood cultures remained negative, blood samples drawn in the emergency department eventually showed a sequence 100% identical to the 16S rRNA gene sequence of *Streptococcus pneumoniae*.

Because of the severe epidermolysis with 67% of the body surface area involved, she was transferred to the ICU of a burn centre when her condition had stabilised two weeks after admission. There, she underwent an amputation of the right lower limb and necrotectomy of her left leg, abdomen and right mamma. Despite maximal intensive care support, the patient deteriorated progressively due to nosocomial infection, with positive cultures with *Candida albicans, Enterococcus faecium* and *Aspergillus fumigatus*. For reasons of futility, taking into account this patient's goals and her extensive mutilation and persisting multi-organ failure, treatment was discontinued and she died on day 29.

Table 1. *Laboratory results 3-12 hours after presentation in the emergency room*

Haemoglobin (mmol/l)	5.8
Thrombocytes (x10⁹/l)	21
Leukocytes (x10⁹/l)	19.3
Activated partial prothrombin time (APTT) (sec)	72
Prothrombin time (sec)	35
Fibrinogen (g/l)	0.6
D-dimer (mg/l)	>4
pH	7.15
Serum lactate (mmol/l)	11.8
Creatinine (µmol/l)	201
Procalcitonin (ng/ml)	24

Table 2. *Review of features of purpura fulminans and toxic epidermal necrolysis*

	Purpura fulminans	**Toxic epidermal necrolysis**
Clinic	Flu-like prodrome Haemorrhagic purpura and bullae DIC	Flu-like prodrome Exfoliative mucocutaneous disease Epidermal necrolysis of > 30% TBSA
Aetiology	Inherited or acquired protein C/S deficiencies Acute infectious purpura fulminans Idiopathic	Mostly drug induced Infectious (HIV, CMV, etc) Other
PA	Microthrombi, leading to necrosis dermis and epidermis	Apoptosis keratinocytes Separation epidermis at dermal-epidermal junction
Treatment	Treat cause (e.g. administration antibiotics) Supportive	Cessation of causative agent Supportive
Mortality	± 50% in purpura fulminans secondary to sepsis	± 30%

PA = pathological anatomy; TBSA = total body surface area; HIV = human immunodeficiency virus; CMV = cytomegalovirus.

DISCUSSION

Both purpura fulminans and TEN are rare and life-threatening disorders with a high mortality (up to 50% and 30% respectively).[5] Epidermal necrolysis secondary to the thrombotic and haemorrhagic cutaneous infarction in purpura fulminans during sepsis might be hard to differentiate from other life-threatening skin disorders, such as TEN (see *table 2* for a review of features of purpura fulminans and toxic epidermal necrolysis).
Moreover, both syndromes can be present simultaneously or successively, as has previously been described.[3] Early

recognition and treatment of the underlying cause of both diseases is vital to prevent further disease progression and reduce the incidence of complications.

Purpura fulminans is a rapidly progressive disorder, accompanied by vascular collapse and disseminated intravascular coagulation,[6] which is characterised by the formation of microthrombi through activation of the inflammatory cascade and consumption of protein C, S, and antithrombin III, leading to widespread intravascular thrombosis.[4] The consumption of coagulation factors and platelets in turn led to bleeding and haemorrhagic infarction of the skin.[7] Multiple organ failure is common, since the haemorrhagic infarction is not limited to the skin, but also affects lungs, kidneys, central nervous system and adrenal glands.[8] Acute infectious purpura fulminans usually presents during severe sepsis, particularly following exposure to endotoxin producing bacteria.[1] Although it is common in meningococcal infection, *Streptococcus pneumoniae* is also a well-known cause.[8] Previous literature shows that 6% of patients with pneumococcal sepsis developed tissue necrosis resulting in symmetrical peripheral gangrene.[9] Treatment is supportive and includes treatment of the underlying cause, most importantly the administration of a broad-spectrum antimicrobial agent.[3,8] Complications of purpura fulminans are scarring, secondary infection, digital or limb necrosis and amputation.

TEN is defined as extensive skin sloughing with the involvement of mucosa, with epidermal necrolysis in > 30% of the body surface area.[3,10] Most cases of TEN are drug induced (80-95%) and immune mediated, combined with a genetic susceptibility for drug hypersensitivity. Several immune mediators, including the key mediator granulysin, a cytotoxic protein produced by natural killer cells and cytotoxic T lymphocytes, are suggested to be responsible for extensive apoptosis in keratinocytes.[11] Multiple organs may be involved, including the respiratory, renal and hepatic system. Its primary treatment is instant withdrawal/cessation of the causative agent.[10] Further treatment is supportive with special care for the skin lesions and prevention of secondary infections.

In the case we present here, we suspected a sepsis on day 1, rapidly evolving into sepsis-induced purpura fulminans and this diagnosis was eventually histologically supported by the presence of fibrin thrombi on skin biopsy. However, because of the impressive epidermolysis on day 2-4, the positive Nikolsky's sign of the skin and absence of bacterial growth in blood cultures, we questioned this diagnosis. We considered these skin lesions to be TEN provoked by the use of ibuprofen, with secondary disseminated intravascular coagulation. Initial biopsy showed an epidermal necrosis and low epidermal or subepidermal detachment of the skin, both indicative of TEN, but not specific for this disease. However, the second skin biopsy showed necrosis due to embolism with microthrombi, extravasation of erythrocytes and detachment of the epidermis. Retrospectively, the first skin biopsy was also compatible with purpura fulminans with thrombosis of a larger vessel more proximal to the biopsy site. Epidermolysis is a well-known phenomenon in purpura fulminans. Furthermore, in this stage, the presence of 16S rRNA gene sequence of *S. pneumoniae* was confirmed which contributed to the diagnosis of purpura fulminans.

Sequence analysis of the 16S rRNA gene is useful for bacterial detection and identification and remains the standard approach in investigating microbial diversity.[12,13] Its main advantage is the processing time to detect and identify bacteria, which is significantly shorter than a culture-based approach.[14] Also, it is not dependent on the presence of replicating organisms and can be detected from intact dead or living bacteria. In the clinical setting,

Figure 1. *Haemorrhagic bullae and epidermolysis with Nikolsky's sign*

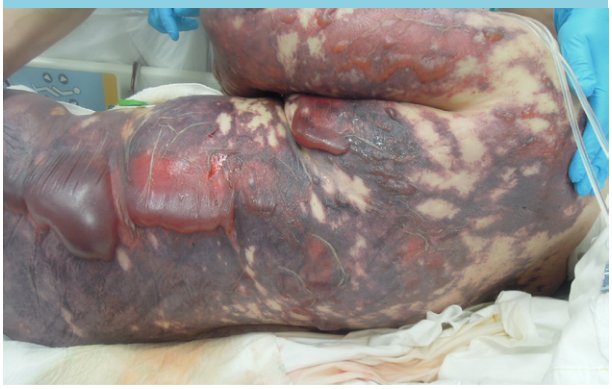


Figure 2. *Symmetrical gangrene of four extremities and parts of her torso*

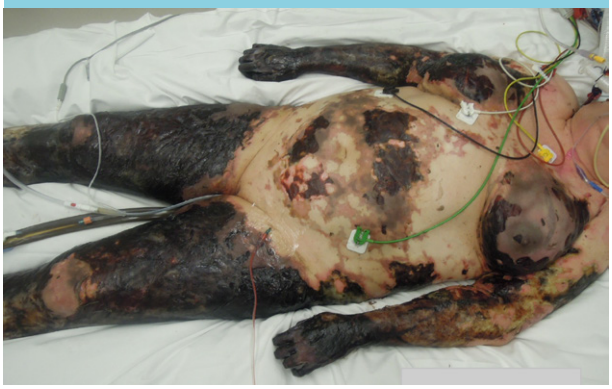
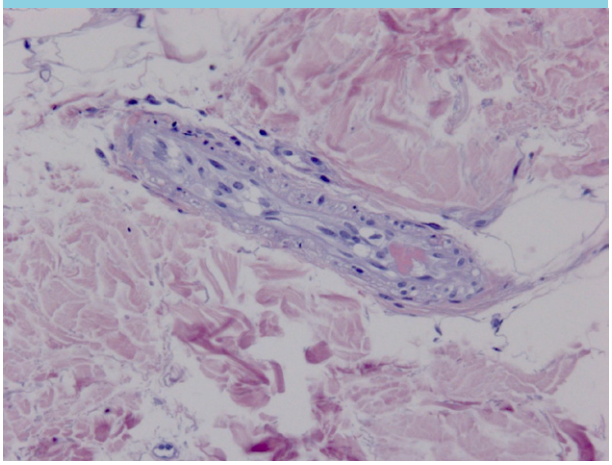


Figure 3. *Skin biopsy on day 9. Intravascular thrombus*



using 16S RNA sequencing accurately detects bacteria in over 90% of all cases of culture-confirmed blood stream infections and can detect up to 71% additional blood stream infections compared with blood culture alone.[14,15] 16S rRNA gene PCR for detection of bacterial DNA applied directly to peripheral whole blood of patients suspected of bacteraemia has been documented to have a sensitivity and specificity of 66.7-77.8% and 94.4-99.3% respectively, excluding contaminants.[14,16]

In the case presented here, we sequenced the variable regions 1, 2 and 3 using standard BLAST for the comparison. The sequence showed 446 base pairs identical to *Streptococcus pneumoniae* forward and reverse and resulted in a 100% query cover and 100% match with exclusively *S. pneumoniae* (23 strains). As for the reliability of species identification, it is important to discriminate between *S. pneumoniae* and other closely related species such as *S. oralis* and *S. mitis*, which can have overlapping 16S RNA sequences. If results show less than 100% homology, additional testing with a *S. pneumoniae* specific PCR, such as the lytA gene, is recommended to confirm the 16S sequence diagnosis.[17] In the case presented here, additional testing was not performed as 100% homology was found. However, the possibility of a contamination of the blood sample should be taken into account.

In conclusion, we present a case with a suspected rapidly progressive, severe pneumococcal sepsis induced purpura fulminans complicated by multiple organ failure, severe epidermolysis and cutaneous necrosis. We show the diagnostic challenge to differentiate between purpura fulminans and TEN, as the extensive epidermolysis in purpura fulminans may mimic TEN. Through additional diagnostic testing with repeated skin biopsies and 16S rRNA gene sequencing, diagnosis of purpura fulminans was eventually thought most suitable.

DISCLOSURES

The authors declare no conflicts of interest.

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CASE REPORT

A case of rivaroxaban-associated acute tubulointerstitial nephritis

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ABSTRACT

Rivaroxaban is a direct oral anticoagulant that is prescribed for the prevention and treatment of thromboembolisms. Rivaroxaban is cleared renally and a common side effect (1-10%) is renal impairment of unknown pathophysiology. We are the first to describe a case of biopsy-proven acute tubulointerstitial nephritis, most likely caused by rivaroxaban.

KEYWORDS

Direct oral anticoagulants, rivaroxaban, tubulointerstitial nephritis

What was known on this topic?
It is known that rivaroxaban can reduce renal function, although the pathophysiology remains unclear.

What does this add?
This is the first description in the literature of tubulointerstitial nephritis caused by rivaroxaban.

INTRODUCTION

Acute tubulointerstitial nephritis accounts for approximately 10-15% of all cases of acute renal failure.[1-3] More than two-thirds of these cases are drug-induced.[4] The incidence of tubulointerstitial nephritis has increased due to the increasing number of prescribed drugs and easy availability of over-the-counter medication. Drugs commonly associated with tubulointerstitial nephritis are non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics, such as penicillins. However, many other drugs can induce tubulointerstitial nephritis as well. In this report, we describe a case of tubulointerstitial nephritis caused by a direct oral anticoagulant (DOAC), rivaroxaban. Rivaroxaban is prescribed for several indications, including the prevention of systemic embolic events in patients with non-valvular atrial fibrillation. Although rivaroxaban is prescribed frequently and has been registered since 2008, we are the first to report a case of rivaroxaban-associated tubulointerstitial nephritis.

CASE REPORT

An 82-year-old male presented to his general practitioner because of fatigue, weakness and weight loss for three weeks. The patient's history revealed hypertension, a DDDR pacemaker for a third-degree atrioventricular block and decreased renal function of unknown cause (estimated glomerular filtration rate (eGFR) 39 ml/min/1.73 m^2). His daily medication comprised amlodipine 5 mg, enalapril 20 mg, hydrochlorothiazide 12.5 mg and omeprazole 40 mg. Three weeks before presentation he was diagnosed with atrial fibrillation and started on rivaroxaban 15 mg. Several days before presentation the patient had stopped taking rivaroxaban because of his symptoms. Other medication had not been changed and the patient was not taking any over-the-counter medication or supplements. Laboratory assessment revealed an eGFR of 9 ml/min/1.73 m^2 and the patient was referred to the hospital. At physical examination the patient's blood pressure was 148/81 mmHg, pulse rate 62 beats/min and temperature of 35.8 °C. The patient appeared euvolaemic and further physical examination was unremarkable. Results of laboratory analysis including urinalysis are provided in *table 1*. Renal ultrasound showed normal sized kidneys of 12.2 and 13.6 cm and no postrenal obstruction.

Table 1. *Laboratory results of patient*

Laboratory analysis	
C-reactive protein	73 mg/l
Potassium	6.3 mmol/l
Urea	27.8 mmol/l
Creatinine*	573 µmol/l
eGFR	8 ml/min/1.73 m²
pH	7.39
Sodium bicarbonate	18.8 mmol/l
Urine analysis	
Urine sediment	
Leukocytes	> 50
Bacteria	> 50
Erythrocytes	0-5
Nitrite	Positive
24-hour urine	
Protein (total)	0.3 g

*Creatinine was calculated using the modification of diet in renal disease equation (MDRD)

Resonium and sodium bicarbonate were started, as well as ciproxin 500 mg to treat a urinary tract infection until urinary cultures proved negative after five days. The patient was also started on dialysis because of refractory hyperkalaemia. Renal biopsy was performed and showed extensive tubulointerstitial nephritis, as depicted in *figure 1*. As renal function had declined after rivaroxaban had been started and no other drugs were altered, rivaroxaban was considered the most likely causative agent. The patient was started on 40 mg prednisone for two weeks followed by a taper schedule of 5 mg per week. The patient continued dialysis for six weeks until renal function improved sufficiently. Eight weeks after presentation to the hospital the eGFR had recovered to 34 ml/min/1.73 m².

DISCUSSION

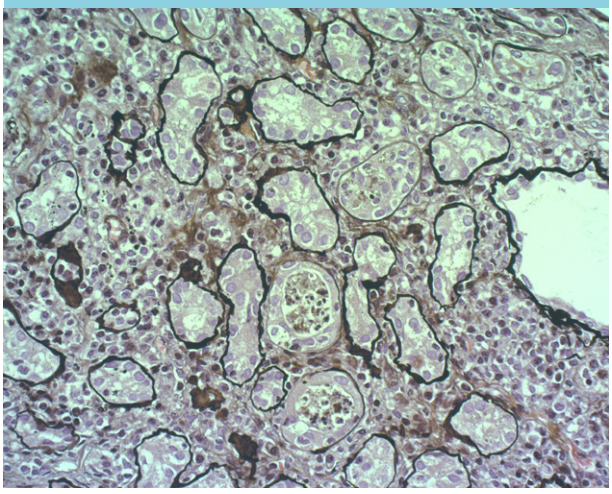
DOACs such as rivaroxaban are prescribed frequently because of advantages over vitamin K antagonists, including a quicker onset and offset of effect and eliminating the requirement for regularly monitoring coagulation.[5,6] Recent publications have shown that rivaroxaban has a similar efficacy and safety to vitamin K antagonists in terms of risk and outcome of bleeding.[7,8] However, less information exists about long-term safety and side effects. Unlike vitamin K antagonists, a commonly reported side effect (1-10%) of rivaroxaban is renal impairment. The pathophysiology remains unknown. This case report is the first to show rivaroxaban-associated tubulointerstitial nephritis. A literature search on DOAC-associated tubulointerstitial nephritis revealed one case of dabigatran toxicity associated with allergic interstitial nephritis, presented at a clinical meeting. However, no renal biopsy was performed to affirm the suspicion.[9] Lareb, the Dutch centre for the registration of drug side effects, received two earlier reports of tubulointerstitial nephritis attributed to rivaroxaban.[10] To date, the World Health Organisation (WHO) has reported 20 incidents of rivaroxaban-associated tubulointerstitial nephritis.[11] It is unclear whether the cases reported by Lareb and WHO are biopsy proven.

The pathophysiology of drug-induced tubulointerstitial nephritis is most likely a secondary immune reaction. This hypothesis is supported by the lack of a dose-dependent relationship, coinciding presence of extrarenal manifestations of hypersensitivity in many cases, and recurrence after accidental re-exposure to the same or closely related agent.[12] Risk factors for drug-induced tubulointerstitial nephritis include pre-existing chronic kidney disease, age, diabetes, hypoperfusion, and duration of therapy. In our case, it was an elderly patient with pre-existing impaired kidney function.

Clinical suspicion of drug-induced tubulointerstitial nephritis should arise when an unexplained increase in the serum creatinine level or an abnormal urinalysis

Figure 1. *Renal biopsy, AG-staining, 200x. This biopsy shows extensive intersitial inflammatory infiltrates (including mononuclear cells, eosinophils and plasmacells) that extend into the tubuli. Debris is visible in the lumina. A minimal amount of tubulus atrophy is present, but the biopsy predominantly shows an active tubulointerstitial nephritis.*

Special thanks to dr. I. Bajema, nephropathologist at the Leiden University Medical Centre



is present, when fever, rash and/or eosinophilia occur and when malaise occurs shortly after starting a new medicine.[13] Mild to moderate proteinuria is often present and sediment usually shows white blood cells, including white blood cell casts. The presence of eosinophilia has a low predictive value, and the absence does not exclude the possibility of drug-induced tubulointerstitial nephritis.[14] A relatively normal urinalysis should not exclude the diagnosis. Renal biopsy is the only definitive method of establishing the diagnosis of tubulointerstitial nephritis. Irrefutable proof of the cause of tubulointerstitial nephritis is hard to acquire, however, in this case rivaroxaban seems the most likely causative agent. Reintroduction of rivaroxaban could confirm the causative relationship, but was considered unethical because of the severity of the disease.

Treatment of tubulointerstitial nephritis consists of discontinuation of the potential causative drug. The effectiveness of glucocorticoids remains unclear, as research shows inconsistent findings.[13,15] In general, the outcome of drug-induced tubulointerstitial nephritis is good; upon cessation of the causative agent, (partial) recovery of renal function occurs in 85-90% after several weeks to months.[2] In our patient, rivaroxaban had already been discontinued shortly before presentation and renal function had almost completely recovered to pre-existing level in eight weeks.

In conclusion, we present a case of biopsy-proven tubulointerstitial nephritis associated with rivaroxaban, which to our knowledge has not been described previously. As DOACs are increasingly being prescribed, this severe side effect might be encountered more often in the future.

DISCLOSURES

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

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PHOTO QUIZ

Intracardiac masses: utility of contrast-enhanced echocardiography

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CASE PRESENTATION

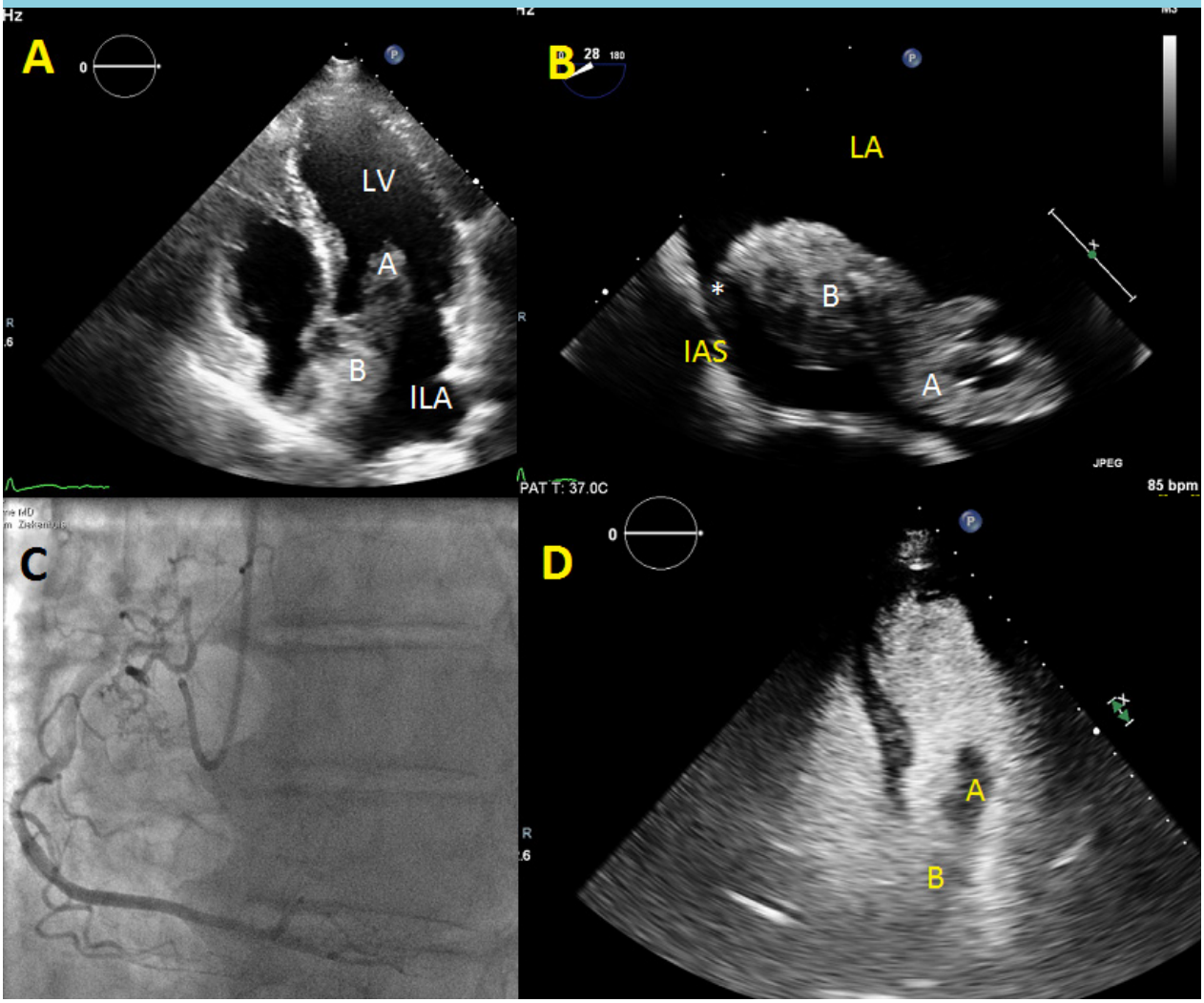
A 68-year-old patient, without relevant medical history, suffered from a syncope without prodromal signs. There were no constitutional symptoms such as fever or loss of weight.
The ECG demonstrated sinus rhythm with a first-degree atrioventricular block. The transthoracic echocardiographic study (TTE) showed preserved left and right systolic function. Attached to the interatrial septum, a giant left atrial mass, oscillating through the mitral valve in the left ventricle, was seen. However, the echo Doppler transmitral flow pattern could not document left ventricular inflow obstruction. The TTE images showed different echocardiographic tissue characteristics of the basal and apical segments of the mass (*figure 1, panel A*).
Transoesophageal echocardiography (TEE) confirmed the attachment of the heterogeneous mass to left atrial septum by a thin stalk (*figure 1, panel B*). Coronary angiography excluded severe coronary atherosclerosis. The vascular supply of the mass originated from the right coronary artery (*figure 1, panel C*).
Contrast-enhanced (Sonoview®, Braco Milan) TTE demonstrated a different density in contrast uptake, where the septal part of the mass was better vascularised (higher contrast uptake) compared with the apical segments of the mass (*figure 1, panel D*).
The patient was referred for prompt resection of the mass to avoid embolic events.

Given the above-mentioned information, what would be your single best answer, if you were asked to make a guess regarding the nature of this mass before the histological confirmations became available?

WHAT IS YOUR DIAGNOSIS?

See page 174 for the answer to this photo quiz.

Figure 1. *Panel A: Apical 4-chamber view. There is a giant mass in the left atrium (LA). The mass, attached to the interatrial septum, oscillates through the mitral valve in the left ventricle (LV). Note the different echocardiographic tissue characteristics of the basal part (B) and the apical part (A). Panel B: TEE image at 28° demonstrates the thin stalk (*) that attaches the tumour to the interatrial septum (IAS). Note the heterogeneity of the mass and difference in echocardiographic tissue characteristics of the basal (B) and the distal part (A). Panel C: Coronary angiography demonstrates that the vascular supply of the tumour originates from the right coronary artery (RCA). Panel D: Apical 4-chamber view after intravenous contrast administration (Sonoview®, Braco Milan) demonstrates a different density in contrast colouring of the tumour. The basal part (B) of the mass was better vascularised (higher contrast uptake) compared to the apical segments (A) of the tumour*



ANSWER TO PHOTO QUIZ (PAGE 172)

INTRACARDIAC MASSES: UTILITY OF CONTRAST-ENHANCED ECHOCARDIOGRAPHY

DIAGNOSIS

According to their aetiology, intracardiac masses are divided into three main classes: thrombi, tumours and vegetations.

In the workup of intracardiac masses, TTE examination provides the first diagnostic clues. The location and echocardiographic tissue characteristics of the mass, presence of wall motion abnormalities and pericardial effusion may contribute to the determination of the nature of the mass. Severe wall motion abnormalities will promote haemostasis with subsequent cloth formation. Thus, an apical mass in a patient with an apical aneurysm due to transmural infarction has a very high likelihood of being a thrombus.

The location of the intracardiac mass is another important clue. Myxoma, the most common benign tumour of the heart, has the atria, especially the left atrium, as its predilection place. Myxomas typically originate from the interatrial septum. Papillary fibroelastoma, the second most common benign cardiac tumour in adults, usually affects the left-sided valves.

Presence of pericardial effusion and heterogeneity of the mass are considered to be supportive arguments for a malignant aetiology of the mass.

In this case, TEE nicely demonstrated the attachment of the left atrial mass to the interatrial septum by a thin stalk. Due to the information obtained from TTE and TEE, left atrial myxoma is the most likely aetiology of the mass.

Over the last decade, contrast-enhanced ultrasound has made a major contribution to the determination of the nature of intracardiac masses in daily clinical practice. Especially, in the clinical setting where a thrombus has to be differentiated from a tumorous mass, contrast-enhanced ultrasound has been proven to be an elegant technique.[1,2] The avascular thrombus will not colour, while the vascularisation of tumours will result in contrast uptake.

In our case, the TTE images suggested different tissue characteristics of the basal and distal segments of the mass. During the discussion in the heart team, presence of thrombus attached to a tumour was postulated. Contrast-enhanced TTE demonstrated a different density in contrast uptake between the basal and the apical segment of the mass. Nonetheless, the presence of echocardiographic contrast in the distal segments rejected the diagnosis of a fresh cloth attached to tumour.

Histopathology confirmed the diagnosis of atrial myxoma and excluded a giant thrombus attached to the myxoma. The histology described the inhomogeneity of mass with several necrotic areas within the tumour. Probably, this heterogeneity contributed to the differences in contrast uptake.

In conclusion, this case is an example of the utility of contrast-enhanced ultrasound for the differentiation between thrombus and tumour.

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PHOTO QUIZ

Unilateral blue and oedematous leg with atrophic skin

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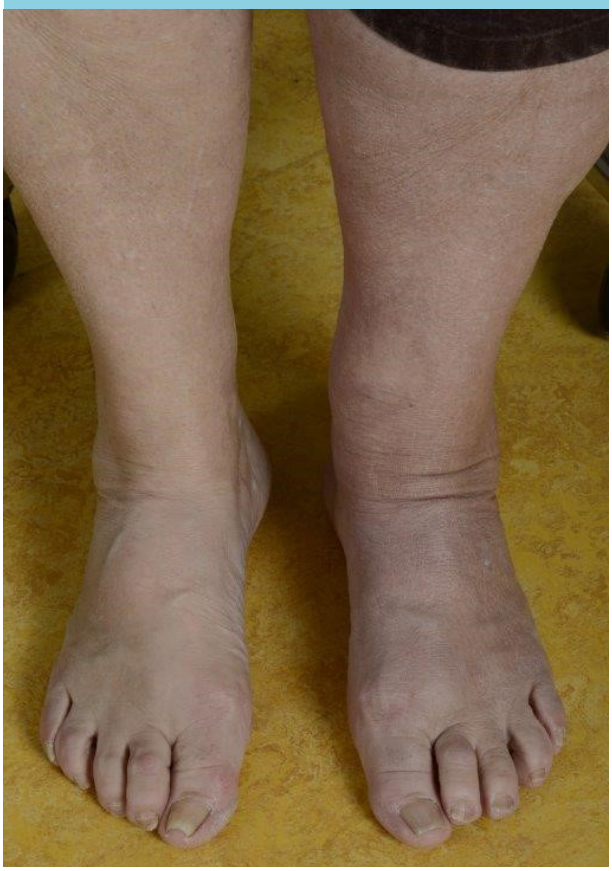
A 72-year-old woman was referred to our out-of-hospital clinic because of a painful and oedematous left leg in the last few months. Her medical history was unremarkable and she was not on any medication. The dermatologist who recently assessed our patient confirmed that there was no venous insufficiency and the ultrasound of the iliac region did not show any venous obstruction. The pain presented during walking and she had no complaints in the other leg. On examination, the lower left leg and foot were diffusely red and bluish, and the middle of the leg was 4 cm larger in circumference (*figure 1*).

WHAT IS YOUR DIAGNOSIS?

See page 176 for the answer to this photo quiz.

Figure 1. *Oedematous and bluish left leg*



ANSWER TO PHOTO QUIZ (PAGE 175)

UNILATERAL BLUE AND OEDEMATOUS LEG WITH ATROPHIC SKIN

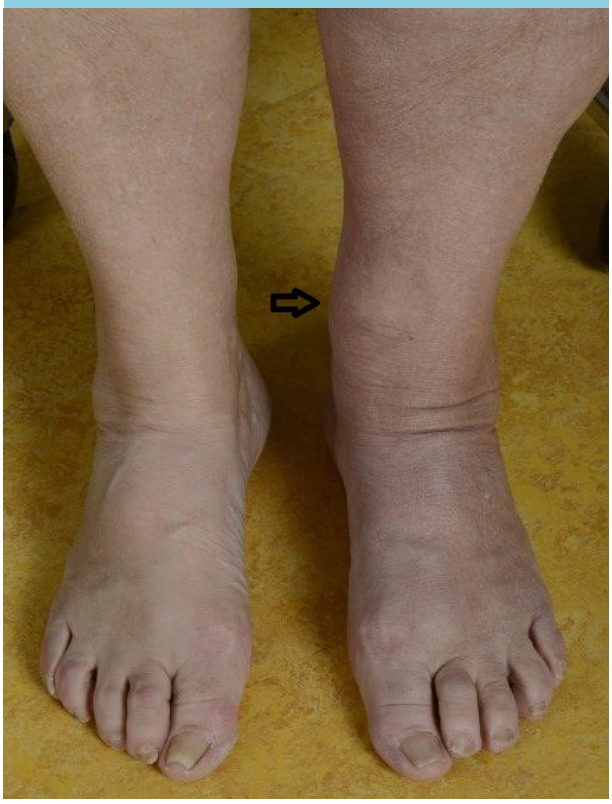
DIAGNOSIS

Besides the colour and swelling of the left leg, the skin also seemed different. It was more atrophic and there was probably an annulated skin lesion as a remnant of erythema migrans (*figure 2*). The patient did not recall any tick bites while she lived in the countryside. The biochemical results demonstrated no elevated inflammation parameters. ELISA and Western blot for *Borrelia burgdorferi* IgG were positive. The diagnosis was acrodermatitis chronica atrophicans (ACA), a chronic manifestation of Lyme disease. The patient was prescribed doxycycline for 28 days. The colour and the swelling of the leg disappeared, but the atrophy persisted. The pain was not a symptom of ACA and probably had a neurological origin. She was referred to a neurologist where spinal disc herniation was found to be an explanation for the pain.

It is known that five genospecies of *B. burgdorferi sensu lato complex* can be pathogenic to humans. The manifestations can be cutaneous, neurological or articular in nature. *B. afzelii*, one of these genospecies, is predominantly involved in skin manifestations, such as ACA.[1,2] *B. afzelii* causes human diseases in Europe and Asia and has not yet been identified in the United States.[2,3] Following the primary infection, this chronic skin condition may take years before it appears. It is most common in women older than 40 years of age.[4] The typical location is the extensor surfaces of the hands and feet. It usually begins as a unilateral lesion, but may become bilateral. In its early stage, also called the inflammation stage, ACA may present as a local or diffuse erythema with a bluish-red discoloration with associated swelling. At this stage, it may be misdiagnosed as venous insufficiency;[4,5] the symptoms are aching pain, tingling and fatigue in the affected foot. The erythema may slowly increase over months and years, and oedema often resolves as atrophy develops. The skin loses its elasticity and therefore has a tissue-paper-like appearance. This atrophy is irreversible.[4-6] Our patient had both swelling of the foot and atrophic skin, perhaps a symptom of transition from the early to the chronic stage. Biopsy of the skin, performed in case of non-conclusive clinical presentation or negative serology, shows an inflammation with lymphocytes and plasma cells and sometimes spirochetes. Seventy percent of the patients, such as our patient, do not remember having had any tick bites or erythema migrans, which leads to a delay in diagnosis.[4] ACA can be treated with a four-week course of one of the following antibiotics: penicillin G, doxycycline, ceftriaxone or phenoxymethylpenicillin.[7,8]

This case demonstrates again that an accurate clinical examination and knowledge of the late complications of Lyme disease can prevent development of the irreversible atrophic stage of ACA and also avoids additional tests.

Figure 2. *Acrodermatitis chronica atrophicans with an annulated skin lesion (arrow), probably utterance of erythema migrans*



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