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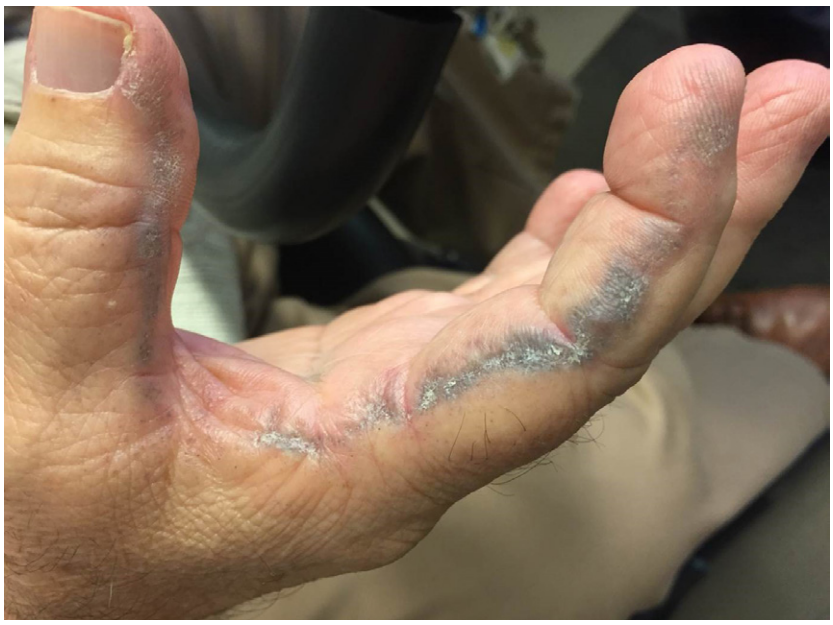

Bluish-black skin pigmentations on a right hand; what is your diagnosis?

Blood culture processing after office hours

•

Surveillance guidelines for colorectal adenomatous polyps

•

Variable workup in type 2A haemochromatosis

•

HCV in HIV-negative MSM

•

Urine discoloration with propofol

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The Netherlands Journal of Medicine

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EDITORIAL

When to stop colonoscopy surveillance in the elderly?

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In the article by Puylaert et al. in this issue of the *Netherlands Journal of Medicine*, adherence to surveillance guidelines for colorectal adenomatous polyps in the elderly was assessed. In this retrospective study the authors presented data on the adherence to surveillance guidelines after polypectomy and gastroenterologists' recommendations, and on the other hand the actual surveillance that was practised in elderly patients. They found that in 41% of the cases the recommendation of the gastroenterologist was not in accordance to the guideline, but reasons for deviation were foremost unknown.

As the authors stated, timely surveillance is important but timely cessation is just as relevant, especially in the elderly. Although age is associated with an increased risk of adenoma and colorectal cancer development, this risk may be lower when patients are already under surveillance and have undergone (repeated) colonoscopies, especially in the elderly,[1,2] while the risk of colonoscopy-related complications is increased in this group of patients.[3] The increasing age of patients and the start of the national colorectal screening program is resulting in a growing number of surveillance colonoscopies, making this a more common dilemma which we will increasingly encounter in daily practice. The fact that they found that in around 40% of the elderly patients recommendations were not in accordance to the guideline may be an indication that guidance in selecting the right older patients to undergo surveillance colonoscopy is lacking. However, this should be interpreted with caution because the data are from one single centre. The current Dutch guideline recommendations on surveillance after polypectomy are based on polyp characteristics, location of polyp, age and gender. However, clear recommendations on when to stop surveillance are still lacking. Also tools to assess the frailty / vitality of the patient in order to evaluate the clinical relevance of performing a surveillance colonoscopy versus the risk of complications in an individual elderly patient are absent. This lack in guidance and tools to assess a more individual benefit-harm risk profile for the elderly is probably reflected in the 40% non-adherence to guidelines. As advised by the authors, a decision algorithm to support cessation of surveillance colonoscopies for elderly patients but maybe for all ages will help to give guidance for daily practice and will result in a more uniform approach providing surveillance colonoscopies for those who benefit the most.

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ORIGINAL ARTICLE

The impact of laboratory closing times on delay of adequate therapy in blood stream infections

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ABSTRACT

Background: Patients with bloodstream infections need early adequate antimicrobial treatment to reduce mortality. This raises the question of timing and logistics. How important is the time of day when a culture is flagged positive to the processing of blood cultures and optimisation of antimicrobial therapy?

Methods: We performed a retrospective study assessing the time delay of a positive blood culture result during and after office hours and its impact on adequate antimicrobial therapy. Process duration from the moment of culture positivity to Gram stain completion was compared at different timepoints during the day in a medium-sized hospital with an offsite microbiological laboratory.

Results: Ninety-four patients with positive, non-contaminated blood cultures were included. Sixty-six patients (70%) received adequate empirical therapy; this increased to 76 cases (82%) and to 88 cases (95%) after analysis of Gram stain results and complete determination, respectively ($p < 0.05$ for all comparisons). Median duration from culture positivity to Gram stain completion (including offsite culture transport) increased from a median of four to 12 hours if time of cultures turned positive after office hours ($p < 0.05$), irrespective of the adequacy of empirical coverage. This also resulted in a median 12-hour delay for the complete process from time of culture positivity to administration of the antimicrobial drug ($p < 0.05$).

Conclusion: Processing blood cultures after office hours is often deferred, which can lead to a delay in adequate antimicrobial therapy for patients with bloodstream infections.

KEYWORDS

Antibiotics, bloodstream infections, blood cultures, laboratory staffing, microbiology, delayed treatment, after office hours

INTRODUCTION

Timely adequate treatment of bloodstream infections is important to reduce mortality and morbidity,[1-5] so in most suspected cases patients immediately receive empirical broad-spectrum antibiotics. Nevertheless, because these empirical antibiotic regimens can still be inadequate, delayed reporting of blood culture results is associated with increased infection-related mortality.[6,7] Delayed culture reporting may also impede important antimicrobial stewardship goals such as streamlining and de-escalating of antimicrobial therapy.[8,9] It is therefore important to identify unnecessary delays in the process, from blood culture collection to administration of a culture-based antimicrobial agent.

Previous studies have shown that patient care delivered during hospital office hours is associated with a shorter length of stay and lower mortality in comparison to care delivered after hospital office hours.[10-13] In the United Kingdom, this has even led to a call for equal standards of performing care, seven days a week.[14] With regard to blood culture processing, a study by Morton et al. demonstrated that culture yield can be lower during the weekend,[15] possibly due to lower staff presence or delayed incubation or processing.[16] Immediately incubating collected blood samples has been shown to reduce these delays.[17] However, most microbiological laboratories do not process blood cultures after office hours, leaving room for potential

delays. Furthermore, there is an increasing number of onsite hospital microbiological laboratories being moved offsite to save costs and to increase performance.[18,19] As culture specimen transport is generally only performed during office hours, cultures identified as positive after the last transport of the day are not processed until the next morning. Having the laboratory and clinical ward at different sites has been shown to increase time between culture collection and start of incubation.[16,20] Unfortunately, it is unknown whether the time to administration of an adequate antibiotic regimen is influenced by what time of day a culture is flagged as positive. We performed a retrospective study to determine the duration of each step of the process, from culture positivity to antimicrobial administration, in a hospital with an offsite microbiological laboratory. We compared the duration of each step during and outside of laboratory office hours.

MATERIALS AND METHODS

Setting: the hospital

The retrospective study was performed in a 550-bed general teaching hospital in Amsterdam, the Netherlands. It has no onsite microbiological laboratory, except for a small facility where blood culture bottles can be immediately incubated, using the BacT/ALERT incubation system (BioMérieux, Marcyl'Etoile, France). Three times a day, clinical samples including blood culture bottles that were flagged positive were transported to the offsite microbiological laboratory by transport van, with a travel time of 15-25 minutes, depending on traffic. Van departure times were 9:30 hrs, 12:00 hrs, and 16:00 hrs on weekdays. Departure time during the weekend was at 10:00 hrs; for cases with positive blood cultures, an additional transport occurred in the afternoon, and cultures were then always processed at the offsite laboratory that same day. There were no transports at other times. At least one of the two regular microbiologists were present in the hospital for consultation and communication of results during office hours (08:00-17.30) on weekdays but not during the weekend. Outside of these hours, microbiological consultation was performed by telephone by one of nine microbiologists who were affiliated with the hospital and the offsite laboratory. When present on weekdays, the microbiologist telephoned results of all positive blood cultures to the treating physician. During the weekend, only relevant positive cultures (as determined by the microbiologist) were reported to the physician. Microbiological results and therapeutic advice were also communicated to clinicians via the electronic health record system Epic (Epic Systems Corporation, USA). Microbiologists based their advice on the hospital's local antibiotic guidelines.

Setting: the offsite laboratory

Gram stains were performed on every positive blood culture. Determination of blood culture pathogen and susceptibility testing were performed using MALDI-TOF (VITEK®MS; bioMérieux, Marcyl'Etoile, France) and disk diffusion testing was conducted according to EUCAST methodology. Laboratory opening hours were from 08:00-19.30 on weekdays and varied depending on work demands during the weekend. Gram stains and pathogen determination were not performed outside of these hours. At least one microbiologist was present at this site during these hours. Both the laboratory and the hospital used the GLIMS Microbiology Laboratory System (CliniSys Group, UK) to document all logistics steps and therapeutic recommendations. Microbiologists were immediately notified of any culture positivity via this system.

Data collection

We performed retrospective case reviews of hospital inpatients with positive blood cultures during two pre-selected non-consecutive weeks per month between 1 December 2011 and 31 October 2012. There was one culture with multiple pathogens; it was treated as a single culture. Subsequently drawn cultures were only included as a new case if separated by eight or more days. The microbiologist on duty excluded cultures with pathogens determined as contaminants after complete pathogen determination and in consultation with the treating physician. We retrieved all information on blood cultures, starting time of each logistics step, and antimicrobial advice from the GLIMS laboratory system. Time of culture collection could not be retrieved from this system. We lacked data to separate culture transportation from the Gram staining process so this was analysed as a single step. For each case, we assembled information on the empirical antimicrobial regimen and all changes in this regimen until 48 hours after the final pathogen determination report became available. Time of prescribing and nurse-reported time of administration were retrieved from the electronic pharmacy system Pharma ('Apotheek Informatie Systeem Pharma', VCD Healthcare, the Netherlands). The responsible medical ethical board approved the study.

Office hours variables

We created a dichotomous variable called 'regular office hours' if a culture was flagged positive by the incubator between the hours of 08:00-17:00 in order to differentiate it from blood culture positivity that occurred between 17:00-08:00. Because the variable depended on the moment of culture positivity in the hospital and not on time of arrival at the offsite laboratory, we used the 17:00 time point rather than the actual laboratory closing time of 19.30 to demarcate the end of regular office hours. To

further analyse the effect of laboratory closing times in our data, we constructed a second variable called 'optimal office hours' that divided the 24-hour day into most optimal (between 02:00-14:00) and least optimal (14:00-02:00) periods with regard to timely culture processing.

Primary and secondary outcomes

Primary outcome for the study was the duration of each culture-processing step between incubation completion and administration of the first dose of the adjusted antimicrobial regimen. We assessed the influence of time of day of culture positivity on culture transport, Gram stain duration, and all subsequent culture processing steps using the two office hours variables introduced above. To check whether severity of the infection affected culture-processing time, we also focused specifically on the processing of patients with a *Staphylococcus aureus* bloodstream infection admitted to the intensive care unit (ICU).[21]

Additionally, an infectious disease specialist from a neighbouring hospital determined if each administered antimicrobial regimen provided adequate coverage of the microorganisms in the blood culture. Adequate coverage after Gram staining was defined as therapy with high expectation of clinical activity against the pathogen. Adequate coverage after full determination of the pathogen was defined as therapy for which the pathogen was susceptible *in vitro*. Naturally, treatments with antimicrobial drugs with insufficient pharmacokinetic characteristics were always determined inadequate, e.g. nitrofurantoin for *Escherichia coli* bacteraemia. Our definition of adequate therapy is independent of certain circumstances such as guideline adherence. For example, treating amoxicillin-susceptible *Escherichia coli* with ceftriaxone constitutes adequate coverage, despite the fact that the lack of streamlining may be seen as inappropriate from the viewpoint of antimicrobial stewardship.[22] Decisions by this specific infectious disease specialist on the related concept of appropriateness of antimicrobial therapy have been shown valid and reliable when compared to colleagues.[23] Finally, we also report data on treatment advice adherence.

Statistical analysis

Linear regression was used to compare the duration of culture processing steps between office hours. All time variables were logistically transformed prior to the analysis. Additionally, a variable denoting antimicrobial coverage of the cultured microorganism(s) was added to each crude model to assess potential confounding. Confounding was considered relevant if the regression coefficient from the univariable analysis differed from the coefficient in the multivariable model (containing the potential confounder) by more than 10%. We compared antimicrobial coverage percentages of the cultured microorganism(s) between the empirical phase, after Gram stain completion, and after final determination/susceptibility using logistic generalised estimating equations to adjust for clustering within patient cases. All analyses were performed using Stata 13 (StataCorp, USA). $P < 0.05$ was considered significant for all analyses.

RESULTS

Patients and cultures

We included positive blood cultures drawn from 136 patients. Culture results from 37 patients were determined to be a result of contamination and were excluded. Five patients were excluded because they were discharged or died before complete microorganism determination. See *table 1* for baseline characteristics.

Impact of time of day of incubation completion

Median durations of each post-incubation culture-processing step during or outside of office hours are shown in *figure 1*. The difference in processing times between culture positivity during and outside of office hours was largest for the transport and Gram stain step, which also resulted in significant differences when all post-incubation steps were added together. *Figure 2* demonstrates how duration of transport and Gram stain step varied for time of day of culture positivity. Cultures positive for *Staphylococcus aureus* were highlighted to illustrate that processing times for cultures with this specific pathogen, which indicate a serious infection with high mortality,[21] were similar to those of cultures with other microorganisms. The same applied to patients admitted to the ICU. Based on the pattern of *figure 2*, we constructed an optimal office hours variable denoting 02:00 -14:00 as the optimal period for speedy culture processing, results of which are also shown in *figure 1*. Adding a variable denoting adequacy of empirical treatment to the model did not change the above findings substantially.

DISCUSSION

Our findings suggest that blood culture-processing time is influenced by the time of day a culture is flagged positive, in a medium-sized teaching hospital with an offsite microbiological laboratory. We showed that median time from incubation completion to Gram stain completion increased from four to 12 hours or even 16 hours, depending on the definition of office hours, irrespective of the adequacy of the empirical antimicrobial regimen. This translated to a similar increase in the cumulative time from culture positivity to administration of the adjusted

Table 1. *Baseline characteristics*

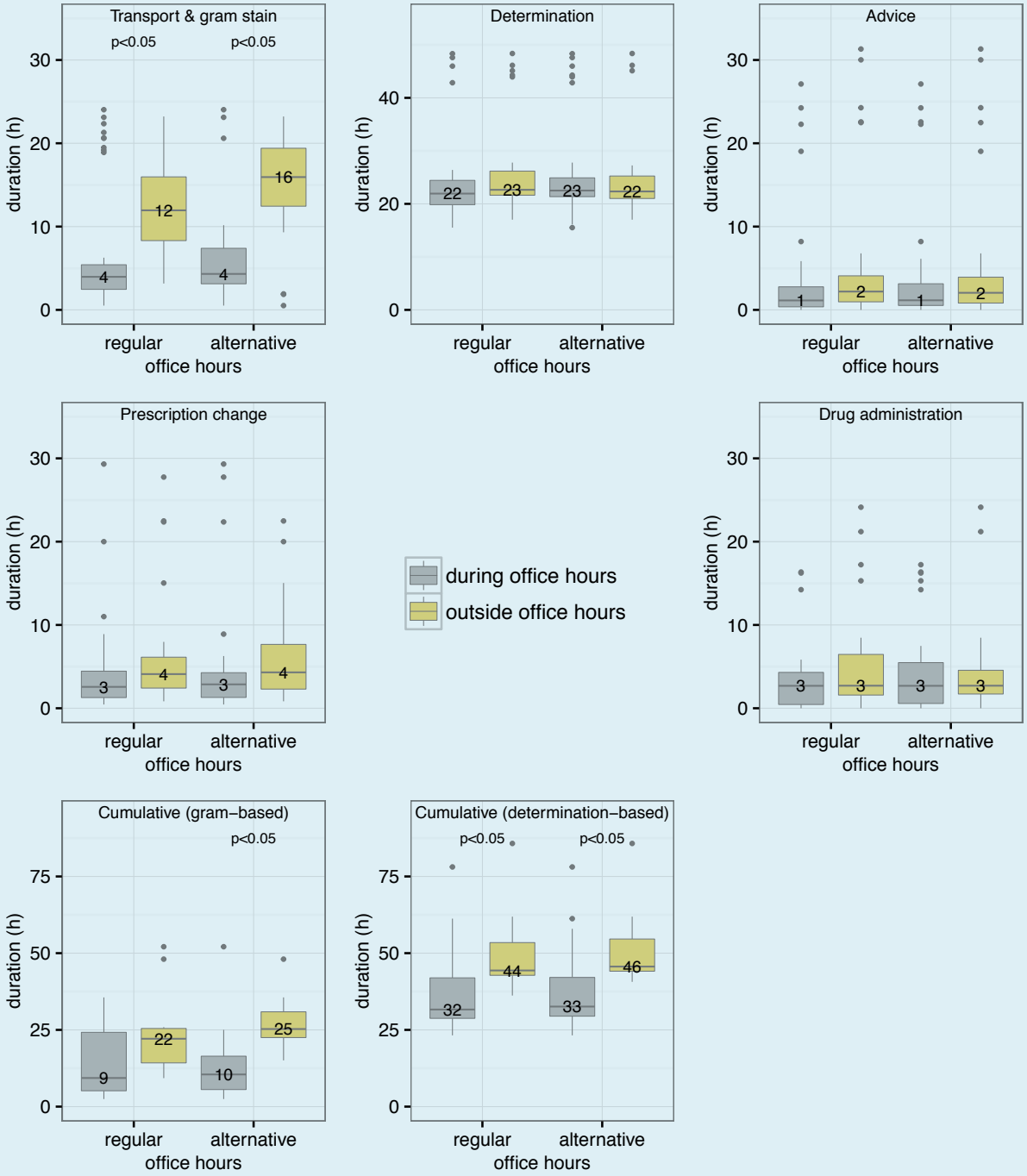
Patient characteristics				
Age in years: median (range)	69 (0-96)			
Admitted to ward	n (%)	Adequate empirical coverage (%)	Adequate coverage after Gram stain (%)	Adequate coverage after determination (%)
- Internal medicine	42 (45)	79	90	98
- ICU	13 (14)	54	62	100
- Cardiology	12 (13)	75	75	100
- Other (including surgery)	27 (29)	67	81	85
Site of suspected infection at time of culture collection: n (%)				
- Urinary tract	25 (27)	80	92	96
- Abdominal	18 (19)	67	67	88
- Sepsis without known site	17 (18)	65	88	100
- Lung	13 (14)	85	85	100
- Other	14 (15)	93	93	93
- No suspected infection	7 (7)	0	43	86
Total cultures/patients	94 (100)	70	82*	95**
Other characteristics			**After Gram stain**	**After determination**
Incubation in hours, median (IQR)	21 (17-34)			
Therapeutic advice given (%)			88	72
Advice comprised antimicrobial change (%)			33	54
Advice compliance (%)			95	93
Drugs & microorganisms				
Most cultured microorganisms (%)	*Escherichia coli* (21, ESBL 4)	*Staphylococcus aureus* (18, all methicillin-susceptible)	*Enterococcus faecium (11)*	
Most prescribed antibiotics empirically (%)	Ceftriaxone (47)	Amoxicillin-clavulanate (17)	Meropenem (11)	
Most prescribed antibiotics after Gram stain (%)	Ceftriaxone (35)	Amoxicillin-clavulanate (16)	Flucloxacillin (11)	
Most prescribed antibiotics after determination (%)	Ceftriaxone (22)	Flucloxacillin (17)	Amoxicillin-clavulanate (12)	

ESBL = extended spectrum beta-lactamase; IQR = interquartile range; *p = 0.03 compared to empirical treatment; **p < 0.05 compared to either previous phase.

antibiotic regimen. Previous studies showed that the offsite location of the laboratory is associated with increased time to start of culture incubation,[16,20] but the influence of offsite location and time of day of culture positivity on the whole culture process from culture positivity to antibiotic administration has not been reported. Interestingly, our data demonstrated that the delay already showed for cultures completing incubation as early as 14:00, long before the end of the working day. In the context of literature supporting early adequate treatment of bloodstream infections to reduce mortality,[1-7] this delay potentially undermines optimal clinical outcomes.

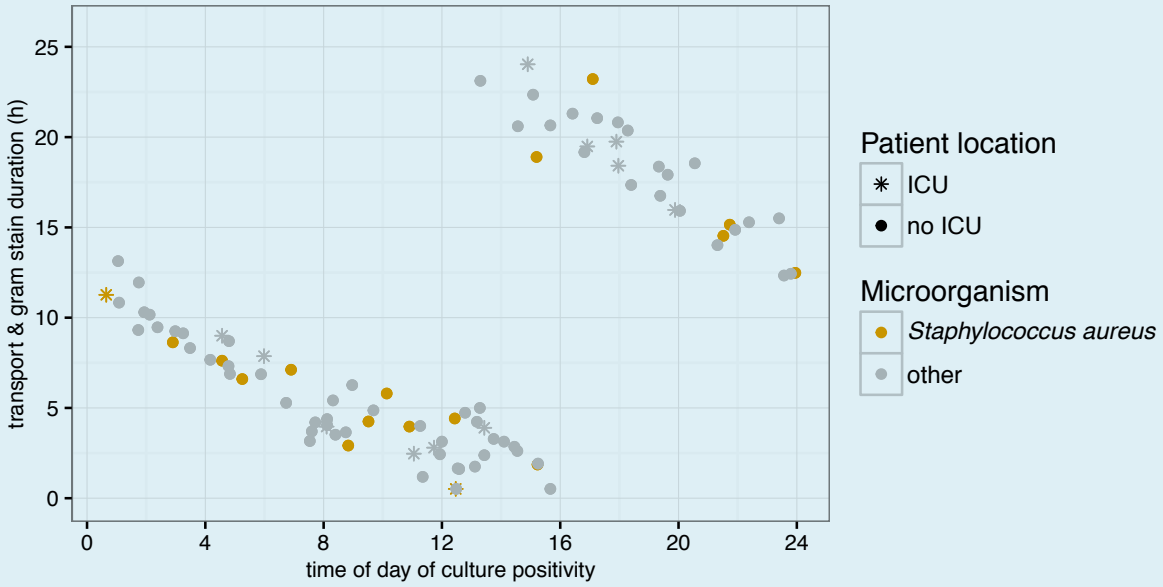
It could be argued that microbiologists might speed up culture processing if they knew that a certain patient was suspected of having a serious infection (e.g. sepsis, endocarditis). To check whether our results also applied to patients with severe infections like those admitted to the ICU or with *Staphylococcus aureus* bloodstream

Figure 1. *Box plots of the duration of each culture-processing step stratified by two office hour variables denoting the moment of culture positivity. Regular office hours were defined as the 08:00 – 17:00 period while optimal office hours were defined as the 02:00 - 14:00 period. Numbers in the boxes represent medians. In each of three culture-processing steps (transport & Gram stain, determination, and cumulative (determination-based), one outlier exceeded the y-axis limit and is thus not shown*



infections, we presented culture processing for these patients separately. We assumed that ICU patient culture transport and processing may have been fast-tracked. Similarly, although clinicians and microbiologists would not yet have known the responsible pathogen for the *Staphylococcus aureus* patients at this stage, we hypothesised that these patients may have presented with more severe or typical symptoms leading to quicker processing as well.[21] However as *figure 2* shows, cultures for these two groups of patients followed the same delay pattern, suggesting that this was not the case.

Figure 2. *Scatterplot of the duration of culture transport & Gram stain by hour of day of incubation completion. Cultures with Staphylococcus aureus and from patients admitted to the ICU are distinctly marked for illustrative purposes. One outlier exceeded the y-axis limit and is thus not shown*



An obvious solution would be to extend transportation and laboratory activity into the evening and night, or to use a transport and stain on-demand solution. We expect that hospitals with similar characteristics to the hospital in our study would process a monthly average of 6.3 positive, new, non-contaminated blood cultures that would complete incubation between 14:00-02:00. Of these cultures, 1.9 (30%) would belong to patients who would thus not receive adequate antimicrobial coverage for the cultured microorganism. Complete culture determination in our study decreased this non-coverage to 9% so the number of patients who would potentially benefit would be 1.3, every month. Assuming that this solution would completely solve the problem of the after-hours delay, it would allow a monthly average of 1.3 patients to receive adequate antimicrobial coverage 13 hours (median value) earlier than in the current situation. In other words, although the delay is substantial, it occurs relatively infrequently and also depends on hospital size and local epidemiology.

Another potential solution can be deduced from our finding that cultures completing incubation as early as 14:00 could be deferred to the next day. This suggests that increased efforts and coordination to ensure that cultures flagged positive between 14:00 and 16:00 are included in the final transport to the laboratory where a Gram stain can be performed before closing time, may prevent unnecessary consequences.

Our results suggest there was no influence of time of day of incubation completion on the speed of culture processing after Gram staining. This is not unexpected because delays in the transport and Gram stain step meant that this step was often completed during office hours, which allowed the subsequent steps to take place during office hours as well. It must be noted that timing of treatment advice from the microbiologist is not the only determinant of the duration of prescription change and drug administration. Other factors may have played a role, such as physician-specific advice adherence rate, or sufficient or insufficient appreciation of the urgency of timely adequate treatment.

Our study has limitations. It contains a relatively small number of cultures, so a comparison of clinical patient outcomes was not feasible. Due to time constraints, we could not collect every culture available in the inclusion period, so selection bias cannot be ruled out. However, the included cultures were from all parts of the calendar year to prevent influence of specific seasons. Moreover, the specific inclusion periods were chosen before data collection took place to prevent outcome bias. The single-centre design and availability of data made it impossible to perform an isolated estimation of the effect of the offsite location of the laboratory. Still, our findings suggest that work completed during or outside office hours results in an unequal standard of care for patients with bacteraemia. This inequality is infrequent, can be substantial, and may be preventable. As outcomes and cost-effectiveness considerations are subject to local circumstances and epidemiology, we advise hospitals

with similar offsite laboratories to investigate the extent of the problem in their centre in order to be able to act accordingly.

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DISCLOSURES

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

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ORIGINAL ARTICLE

Adherence to surveillance guidelines for colorectal adenomatous polyps in the elderly

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ABSTRACT

Objective: Determining adherence to recommended surveillance intervals after polypectomy in elderly patients.
Design: A retrospective cohort study including 531 patients aged above 70 years undergoing polypectomy between 2009-2011 in a large Dutch teaching hospital, identified using the hospital's pathology registry. Outcomes of the index colonoscopy were reviewed. The interval until the next colonoscopy was assessed and compared both to the advised interval according to the Dutch guidelines and the gastroenterologist's recommendation. Reasons for deviating from the guideline were assessed.
Results: The initial recommendation of the gastroenterologist for the surveillance interval was in accordance to the guideline in 59.1% of the patients. In 21.8% the gastroenterologist's advice was not documented. In 15.8% of the patients the gastroenterologist recommended to perform surveillance endoscopy earlier than the guideline, mainly based on polyp characteristics. The gastroenterologist advised endoscopy when the guideline advised no surveillance at all in 1.0%, later than the guideline recommendation in 1.2%, or did not recommend surveillance when the guideline advised to continue in 1.0%. Actual surveillance intervals were in accordance to the guideline in 54.4% and in accordance to the initial advice of the gastroenterologist in 58.4% of the patients.
Conclusion: Only in 41% of patients was the gastroenterologist's recommendation regarding surveillance after polypectomy either absent (21.8%) or not in accordance to the guideline (19.2%).
Future research should focus on developing an evidence-based decision algorithm for elderly patients to support gastroenterologists and patients in the choices regarding cessation of surveillance at a certain level of frailty, comorbidity or remaining life-expectancy.

KEYWORDS

Polypectomy, colonoscopy, elderly, surveillance, adenomas

INTRODUCTION

Colorectal cancer is the third most common type of cancer in both women and men in the Netherlands; every year approximately 4500 patients die from this disease.[1] There is sufficient evidence that a large number of colorectal cancers develop from adenomatous polyps which slowly progress into adenocarcinomas. The incidence of colorectal cancer is therefore reduced by endoscopic polypectomy.[2,3] However, in patients who undergo polypectomy the occurrence of new adenomas is higher than in the general population and the risk of developing colorectal cancer remains higher.[4-7] Therefore effective follow-up regimes for identification and removal of premalignant adenomas in these patients are important. Despite clear guideline recommendations concerning surveillance, multiple studies have shown poor adherence as surveillance recommendations after polypectomy are not followed in around 70% of patients of all ages.[8-10]
Studies that investigated the adherence to surveillance guidelines specifically in elderly patients are scarce. Currently, more than 50% of the patients with colon cancer are over 70 years of age, and due to ageing of the population, the average age at diagnosis of colorectal cancer is rising.[11] Furthermore, the risk of developing new adenomas, and also interval carcinomas, increases with age.[6,12-14] Thus, adequate surveillance seems particularly important in elderly patients.
On the other hand, there are several arguments to limit surveillance amongst the elderly. Firstly, there is considerable evidence that elderly patients are at a higher

risk of developing complications when undergoing a colonoscopy.[15-18] A study by Tran et al. showed that among elderly patients undergoing surveillance colonoscopy, there was a relatively high rate of post-procedure hospitalisation, but also a low incidence of finding colorectal cancer.[19] Second, elderly patients benefit less from preventive diagnostics, as the estimated time of 10 to 20 years in which an adenoma develops into carcinoma may exceed their remaining life expectancy.[16-18,20,21] Risks and benefits should therefore be weighed carefully.

Furthermore, it is important to take cost-effectiveness into account. The Surveillance After Polypectomy study showed that surveillance stops being cost-effective above a certain age, varying from 75 years in low-risk patients to 85 years in high-risk patients.

In this study, we set out to evaluate adherence to surveillance guidelines for elderly patients after polypectomy. The Dutch national guideline that was applicable during the study period recommends cessation of surveillance in patients older than 65 years with only one adenoma in their cumulative history, and in patients over 75 years with only two adenomas.[8,16] In patients with a cumulative number of three or more adenomas, it is recommended to continue surveillance 'as long as the patient's condition and vitality allows it'. In addition to assessing overall guideline adherence, we wanted to examine the criteria and motivations that were used in decision-making regarding continuation or discontinuation of colonoscopic surveillance.

METHODS

Study population and data collection

A retrospective study was performed among patients of 70 years or older who underwent a polypectomy at the Diakonessenhuis, a large teaching hospital in Utrecht, the Netherlands, between 2009 and 2011. Patients were identified using the hospital's pathology registry; all clinically relevant polyps are routinely sent to the pathology laboratory for examination and this registry thus provides a reliable overview of polypectomy patients. Patients were excluded if they had a proven gastrointestinal malignancy, a past history of gastrointestinal malignancies and if the indication for coloscopy was inflammatory bowel disease because there is a different surveillance regime for this category of patients. The medical ethics committee overseeing our hospital provided a written waiver stating that, given the retrospective nature of the study, no formal ethical review was required.

Index colonoscopy

The colonoscopies carried out between 2009 and 2011 in which the first polypectomy was performed – considered the 'index colonoscopy' – were used to select our patients. Using the electronic patient charts, the following data were collected from the time of index endoscopy: age, sex, comorbidity, scored using the Charlson comorbidity index, reason for index colonoscopy, number and localisation of removed polyps, number of pathologically-proven adenomas and hyperplastic polyps, number of proximal adenomas, and presence of adenomas or hyperplastic polyps > 1 cm) (*table 1*).

Table 1. *Baseline characteristics*

Study population (n)	**418**
Sex (male), n (%)	202 (48.3)
Age, mean (SD)	76 (4.5)
70-75 years, n (%)	235 (56.2)
75-80 years, n (%)	113 (27.0)
> 80 years, n (%)	70 (16.7)
Localisation of polyps	
Unknown, n (%)	2 (0.4)
Proximal, n (%)	137 (32.8)
Distal, n (%)	155 (37.1)
Proximal and distal, n (%)	124 (29.7)
Number of polyps, median (range)*	2 (1-12)
Adenomas, median (range)	1 (0-7)
1, n (%)	282 (67.5)
2, n (%)	76 (18.2)
≥ 3, n (%)	28 (6.7)
Hyperplastic polyps, median (range)	0 (0-3)
Size of polyps	
Adenomas > 1 cm, n (%)	105 (25.1)
Hyperplastic polyps > 1 cm, n (%)	4 (1.0)
Charlson index	
0	223 (53.3)
1-2	138 (33.0)
≥ 3	57 (13.6)
Indication colonoscopy†	
Anaemia, n (%)	46 (11.0)
Occult rectal bleeding, n (%)	32 (7.7)
Abdominal pain, n (%)	65 (15.6)
Change in bowel movements, n (%)	178 (42.6)
Rectal bleeding, n (%)	86 (20.6)
Status post-polypectomy, n (%)	111 (26.6)
Family history of CRC, polyps, n (%)	48 (11.5)
Colectomy in medical history because of benign polyp, n (%)	4 (1.0)

CRC = colorectal cancer. *In 4 patients the number of polyps was not documented. †Total percentage > 100%; there was often ≥ 1 indication for colonoscopy.

Initial surveillance recommendations

Based on the number of pathology-proven adenomas at the index colonoscopy, we determined the guideline recommendations regarding surveillance colonoscopy for each patient. The 2008 national guideline concerning surveillance after polypectomy, which was applicable during the study period, recommends a surveillance

colonoscopy after three years for patients with three or more colorectal adenomas at the moment of index colonoscopy and after six years for patients with fewer than three adenomas, provided that the patient is still sufficiently fit. The following classification was made: 1) no indication for follow-up; 2) follow-up colonoscopy after three years provided that the patient is still sufficiently fit; 3) follow-up colonoscopy after six years provided that the patient is still sufficiently fit; 4) rescopy in the short-term because of non-radical resection, a residual polyp or an insufficient cleaned colon at endoscopy; the latter patients were excluded from further analyses.

In addition to determining the guideline recommended surveillance interval, we also extracted the gastroenterologist's recommendations regarding surveillance interval from the patient's chart at the time of index colonoscopy. Of note, the endoscopist performing the index endoscopy and the gastroenterologist making the recommendations regarding surveillance were not necessarily the same person. Recommendations were classified as follows: 1) no recommendation documented; 2) recommendation in accordance with guideline; 3) recommendation earlier than prescribed by guideline; 4) recommendation later than prescribed by guideline; 5) surveillance recommended, despite guideline prescribing no follow-up; 6) no surveillance recommended, despite follow-up prescribed by guideline. Reasons for the gastroenterologist's recommendation were also extracted if recorded.

Actual surveillance colonoscopy

We subsequently investigated when surveillance colonoscopy follow-up took place and whether this was in accordance with the guideline recommendations. Groups were divided into the following: 1) surveillance in accordance with guideline; 2) earlier than guideline; 3) later than guideline; 4) no surveillance, despite recommendation; 5) surveillance, while not recommended. We considered follow-up as being in accordance with the guideline if the actual interval was within six months before or after the guideline recommended interval, or if patients deliberately did not receive a surveillance colonoscopy because of age, comorbidity or vitality. In addition, we investigated if surveillance colonoscopy took place in accordance to the recommendation of the gastroenterologist. The same classification was used as described earlier. Also here, the motivations of the gastroenterologist were investigated. If surveillance had taken place earlier or later or at any moment when guideline and/or gastroenterologist advised cessation of surveillance, this was defined as inadequate surveillance. If surveillance had not taken place, despite the guideline recommendation, this was defined as absent surveillance.

Statistical analysis

Results are presented as descriptive data only. Data analysis was performed using SPSS 23.0.

RESULTS

Patient characteristics

Between 2009 and 2011, a total of 531 patients of 70 years or older underwent a polypectomy with pathology analysis. After exclusion of 113 patients for various reasons (*figure 1*), 418 patients were included. The patient characteristics are listed in *table 1*. The median age was 76 (range 70-90) and 48.3% were male (*table 1*). The Charlson comorbidity index was 0 in 223 patients (53.3%) and ≥ 3 in 138 patients (33.0%). In these 418 patients, all 945 visible polyps were removed during index colonoscopy. Of the polyps examined pathologically, 533 were adenomas and 84 hyperplastic polyps.

Index colonoscopy

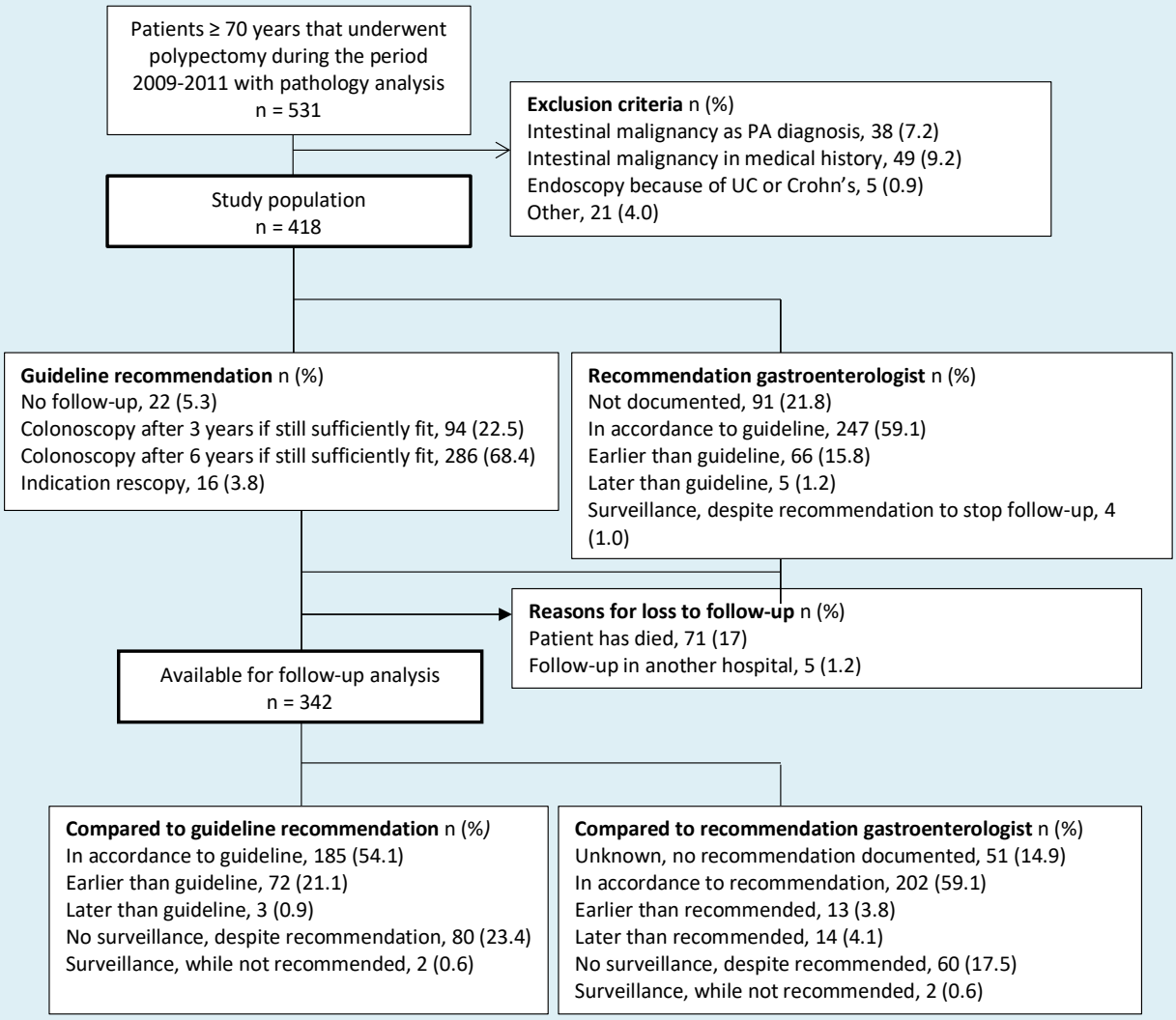
The most common indications for colonoscopy were a change in bowel movements (n = 178, 42.6%), surveillance after prior polypectomy (n = 111, 26.6%) or rectal bleeding (n = 86, 20.6%, *table 1*). The median number of polyps at index colonoscopy was 2 (range 1-12). Twenty-eight patients (6.7%) had three or more pathology-proven adenomas.

Surveillance recommendations according to guideline and gastroenterologist

Based on the guidelines, surveillance was not required in 5.3% (n = 22) of patients; 22.5% (n = 94) should receive surveillance after three years and 68.4% (n = 286) after six years, provided they are still sufficiently fit. In 3.8% (n = 16), there was an indication for scheduling a new endoscopy in the short term (rescopy).

In the patients' charts, no surveillance recommendation was reported for 21.8% of patients (n = 91). The recommendation of the gastroenterologist was in accordance with the guideline in 59.1% of all patients (n = 247). In 15.8% (n = 66) of the patients, the gastroenterologist recommended earlier surveillance than the guideline advised, mainly based on polyp characteristics (n = 28) or because of unknown reasons (n = 35, *table 2a*). Five patients (1.2%) received surveillance endoscopy later than the guideline advised for unknown reasons. For 1.0% of the patients (n = 4), surveillance was recommended despite this not being necessary according to guideline. Conversely, gastroenterologists recommended no surveillance in 1.2% of the patients (n = 5) while the guideline recommended further follow-up. Motivations for these choices varied (*table 2a*).

Figure 1. *Flow chart*



PA = pathological anatomy; UC = ulcerative colitis.

Surveillance colonoscopy

Of the 418 included patients, 71 patients died during follow-up and five patients underwent follow-up in another hospital. Therefore, the actual time of surveillance colonoscopy was evaluated in 342 patients (*figure 1*).

Surveillance was performed in accordance with the guideline in 185 patients (54.1%). Of these 185 patients, there was a subgroup of 107 patients who received a colonoscopy and a subgroup of 78 patients who received no colonoscopy based on age and/or vitality. The median age was 78 years (IQR 75.5-82.3) and 37.0% had a Charlson comorbidity index of 1-2 and 15.4% of ≥ 3. For another 23.4% (n = 80), in which no surveillance colonoscopy was performed in spite of guideline recommendations, the reason for this omission was not reported or unclear to the investigators. For the total population of 342 patients, 21.1% of surveillance colonoscopies (n = 72) were performed earlier and 0.9% (n = 3) later than the guideline recommendation. Finally, two patients (0.6%) underwent a surveillance colonoscopy despite the guideline deeming it unnecessary.

Compared with the initial recommendation of the gastroenterologist, the surveillance colonoscopy was performed on time in 59.1% (n = 202). In 3.8% (n = 13) the colonoscopy was performed earlier than advised by the gastroenterologist because of a new indication for endoscopy, inclusion in the national colon cancer screening program or for unknown reasons (*table 2b*). In 4.1% (n = 14) surveillance endoscopy was performed later than advised. Two patients (0.6%) received surveillance, while initially not recommended based on age and vitality; however, a new indication for endoscopy arose during follow-up. Sixty patients (17.5%) received no surveillance while surveillance was recommended; reasons for this were generally unclear (*table 2b*).

Table 2a. *Motivations for initial advice given by gastroenterologist*

Motivation	Frequency (n, %)
Not documented	91 (21.8)
In accordance with guideline	247 (59.1)
Earlier than guideline Size of polyp(s) Number of polyps PA diagnosis* Control endoscopy after polyp resection (surgery) Difficult view during endoscopy Patient's request ('carcinophobia') Unknown	66 (15.8) 11 (2.6) 11 (2.6) 6 (1.4) 1 (< 1.0) 1 (< 1.0) 1 (< 1.0) 35 (8.4)
Colonoscopy later than guideline recommendation Indication rescopy; but endoscopy took place > 1 year Unknown	5 (1.2) 2 (< 1.0) 3 (< 1.0)
No surveillance, despite recommendation to continue 'No explanation found for symptoms' 'Insufficient relevant abnormal findings' (1 adenoma) Unknown	5 (1.2) 1 (< 1.0) 1 (< 1.0) 3 (< 1.0)
Surveillance, despite recommendation to stop follow-up Request patient Advice given during scopy, pathology report showed later no adenomatous tissue Polyp was seen, but pathology unknown Unknown	4 (1.0) 1 (< 1.0) 1 (< 1.0) 1 (< 1.0) 1 (< 1.0)

* Example given: high-graded dysplasia. PA = pathological anatomy.

Table 2b. *Motivations to deviate from initial advice of gastroenterologist at the moment of surveillance colonoscopy*

Motivation	Frequency (n, %)
Unknown, no recommendation documented	51 (14.9)
In accordance to recommendation	202 (59.1)
Earlier than recommended Participation in CRC screening Other indication for scopy Unknown	13 (3.8) 2 (< 1.0) 6 (1.8) 5 (1.5)
Later than recommended Unknown*	14 (4.1) 14 (4.1)
No surveillance, despite recommended Poor condition of patient Unknown†	60 (17.5) 17 (5.0) 43 (10.0)
Surveillance, while not recommended Earlier advised based on age/vitality to stop surveillance, however continued because of new symptoms at that moment	2 (0.6) 2 (0.6)

CRC = colorectal cancer. *In 9 of the 14 patients surveillance endoscopy was performed in accordance with the guideline. The gastroenterologist initially advised surveillance endoscopy earlier than the guideline, but surveillance was actually performed later, resulting in surveillance on time. †In 11 of the 43 patients a digital invitation for surveillance endoscopy was found in the patient record.

For 51 patients (14.9%), no comparison between actual surveillance and the recommendation of the gastroenterologist could be made because the latter was not documented.

DISCUSSION

In this study, we assessed adherence to post-polypectomy surveillance guidelines in the elderly. The recommendation of the gastroenterologist regarding the surveillance intervals was in accordance with the guideline in 59.1% (n = 247) of all patients. Reasons to deviate from the guideline were amongst others polyp characteristics, but mostly for unknown reasons. The actual time to surveillance colonoscopy was appropriate in only 54.1% of all patients compared with the guideline and in 59.1% compared with the gastroenterologist's recommendation. Overall, inadequate surveillance, i.e. earlier, later or at any time in spite of no follow-up being recommended by guidelines, occurred in 157 patients.

In an earlier, large retrospective study in non-elderly patients (mean age 59 years) in the Netherlands, guideline adherence was even lower than in our study, with only 25% of patients receiving surveillance at the appropriate interval.[10] The study, published in 2015, compared the guidelines before and after 2002. Before 2002, the guideline recommended strict surveillance with a two to three year surveillance interval for patients with one adenoma and a one year surveillance interval for patients with more than one adenoma. After 2002, the same intervals were recommended as in our study. The researchers found that before 2002, surveillance was mainly too late or absent (57% of cases), while after 2002 patients received surveillance too early in 48% of cases.

In our study, there were legitimate reasons to deviate from the guideline (*table 2a*) for some patients. However, often the reasons for recommending earlier surveillance were factors such as size, number or pathology results of polyps. In these cases, the gastroenterologist might have been unnecessarily defensive, placing elderly patients at risk for complications without clinical benefit and also creating unnecessary healthcare costs.[15-19] On the other hand, the new national guideline which was implemented

in 2013 has incorporated several independent risk factors for developing colorectal cancer into its surveillance recommendation algorithm; these include the patient's age, sex and more polyp characteristics, such as location, size and villous or serrated histology classification.[24] It is possible that gastroenterologists were already aware of these risk factors, and that this subsequently influenced their choice of surveillance interval.

While timely surveillance is important, we believe that timely cessation of follow-up is just as relevant for elderly patients. In our population, reasons for not performing surveillance colonoscopy were not always well documented. For the majority, no outpatient visit to the hospital was documented at the time the decision to withhold colonoscopy was made; this suggests that this decision was likely to be based on age or earlier documented comorbidity but not necessarily on the patient's actual health status at the time surveillance colonoscopy was due.

There is general consensus that basing treatment decisions on age alone is insufficient.[22] Ageing is a highly individual process, resulting in increasing heterogeneity with increasing age; this heterogeneity extends to remaining life expectancy as well as a patient's ability to tolerate diagnostic procedures and treatment. Thus, vitality or frailty of a patient is much more relevant to treatment decisions than age itself. Our study shows that documentation of this vitality or frailty was very limited in the period of investigation. There are no validated checklists or tools for gastro-enterologists to measure frailty and vitality as part of the decision-making regarding surveillance colonoscopy. Interestingly, while the new guideline of 2013 addresses additional factors, such as specific polyp characteristics, to guide the timing of surveillance, very little guidance is provided regarding the decision to end follow-up. It is still left to the gastroenterologist's own subjective judgement. Clearer clinical criteria or a specific tool to define frailty and comorbidity could improve the quality of decision-making. We believe this should be developed, validated and incorporated in future guidelines, so that the decision-making process regarding ending surveillance in elderly patients will become more objective and transparent. In this context, collaboration between gastroenterologists, specialised in intestinal polyps and their treatment, and geriatricians, specialised in assessing ageing and frailty, might be valuable. This has become particularly pertinent with the introduction of the national colon cancer population screening program in the year 2013. This has increased the total number of colonoscopies performed and makes correct and efficient surveillance intervals even more important considering possible unnecessary complications of too frequent surveillance colonoscopies.

To our knowledge, this is the first study specifically addressing surveillance after polypectomy in patients aged 70 years and over. However, this study has some limitations. Inherent to the retrospective design, we could only use the data that were available. In the patient records, data regarding comorbidity, frailty status and the specific reasons for deviating from the guideline were not always documented. In addition, due to insufficient information about previous adenomas and pathology records, we were unable to apply the part of the guideline that justifies stopping surveillance based on cumulative one or two adenomas in patients over 65 and 75 years, respectively. However, as surveillance was not performed when the guideline advised to continue in only 1.0% of patients, this limitation is unlikely to have significantly affected our findings.

CONCLUSION

Only in 41% of patients was the gastroenterologist's recommendation regarding surveillance after polypectomy either absent (21.8%) or not in accordance to the guideline (19.2%). The imminent ageing of Western societies and developments such as the national cancer screening program mean that improving guidance on surveillance decisions will become increasingly important. Future research should focus on developing an evidence-based decision algorithm for elderly patients to support gastroenterologists and patients in the choices regarding cessation of surveillance at a certain level of frailty, comorbidity or remaining life-expectancy.

DISCLOSURES

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ORIGINAL ARTICLE

Variable workup calls for guideline development for type 2A hereditary haemochromatosis

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ABSTRACT

Background: Type 2A hereditary haemochromatosis (type 2A HH) is a rare iron-loading disorder caused by mutations in the *HFE2* gene, which encodes the HJV protein. We present characteristics, treatment and follow-up of subjects diagnosed with type 2A HH in the Netherlands to increase awareness of the disease and its treatment, and to define knowledge gaps.

Methods: We collected clinical, biochemical and genetic data from seven patients (two female; five probands) from six families genetically diagnosed with type 2A HH at the Expertise Center for Iron Disorders, Radboud University Medical Centre between 2006 and 2016.

Results: The five probands presented with heterogeneous complaints between the ages of 19 and 39. One of two patients with delayed clinical diagnosis developed hypogonadism and *Y. enterocolitica* sepsis. Diagnostic workup and follow-up varied. When assessed, elevated transferrin saturation (79-98%), ferritin (1400-6200 µg/l) and severely elevated liver iron levels were found, and in all subjects, phlebotomies were initiated. One subject was switched to erythrocytapheresis. Target ferritin levels varied. Despite long-term iron depletion, two subjects developed clinical complications. Sanger sequencing revealed two pathogenic *HFE2* variants (homozygous or compound heterozygous) for the five families of Dutch descent and one new pathogenic variant in the family of non-Dutch descent.

Conclusion: Three genetic variants caused type 2A HH in six families. Clinical diagnosis was delayed in two subjects. We observed variance in presentation, workup, follow-up and treatment. We found new complications in long-term iron-depleted patients. We recommend research and guidelines for optimal workup, follow-up and treatment of type 2A HH.

KEYWORDS

Diagnosis, follow-up, iron, juvenile haemochromatosis, workup

INTRODUCTION

Hereditary haemochromatosis (HH) is a genetically heterogeneous disorder that results in body iron accumulation. The most common form is attributed to homozygosity for the p.Cys282Tyr (c.845G > A) variant of the haemochromatosis (*HFE*) gene on chromosome 6p21.[1] Most male patients with *HFE*-associated hereditary

haemochromatosis (*HFE*-HH; also called type 1 HH) do not present until middle age, and women not until after menopause; the disorder is mainly restricted to patients of Northern European descent.[2]

Other HH subtypes are rare and less restricted to certain populations.[3-9] Juvenile forms of HH are caused by variants in genes encoding for haemojuvelin (*HFE2* or *HJV,* located on chromosome 1q21, type 2A HH) and hepcidin (*HAMP,* located on chromosome 19q13, type 2B HH). These forms of HH are generally characterised by their early onset and a particularly severe phenotype, with patients typically presenting before the age of 30 with severe systemic iron overload, heart failure and endocrine disorders.[10,11] Several studies have reported that of these two forms of juvenile haemochromatosis (JH), type 2A HH is by far the most common, comprising 90% of the approximately 110 reported patients diagnosed with JH.[1,5,6,10-14] The phenotype of JH was first described in 1979 in a young woman with heart failure, insulin-dependent diabetes, amenorrhoea and hepatomegaly.[14] In this report, 52 previously unreported cases were reviewed. In the following decades, the disease was further studied and outlined.[15-17] In 2001, the JH phenotype was first linked to the *HFE2* gene on chromosome 1 in a family of Greek descent.[13,18]

The haemojuvelin protein (HJV) is involved in the BMP receptor complex, which senses body iron stores and circulating iron, and subsequently stimulates hepcidin expression via the SMAD pathway.[19] Pathogenic variants of HJV thus cause an inappropriately low level of hepcidin relative to body iron levels.[20,21] This, in turn, leads to inadequate or ineffective hepcidin-mediated down-regulation of ferroportin on the basolateral membrane of enterocytes and the membrane of macrophages, and subsequent dietary increased iron absorption, relatively low iron content in the reticulo-endothelial system and high parenchymal and circulating iron levels. Therefore, pathogenic variants of *HFE2* lead to an HH phenotype (type 2A HH), and present for example with normal haemoglobin (Hb) levels, elevated ferritin levels and transferrin saturation (TSAT), and iron overload in the parenchymal tissues, such as the liver and exocrine pancreas.[13]

In order to define knowledge gaps and to assess the need for improvement of awareness and clinical management of type 2A HH in the Netherlands, we retrospectively collected genotype, biochemical and clinical presentation, diagnostic workup and treatment strategy in two women and five men (seven patients total; five probands and two siblings) from six families who were genetically and clinically diagnosed with type 2A HH in the Netherlands.

MATERIALS AND METHODS

Patients

We retrospectively collected and reviewed data on clinical presentation, biochemical tests and DNA analysis of seven patients who were genetically diagnosed with type 2A HH at the Radboud University Medical Centre between 2006 and 2016. This includes one patient who has been described previously,[22,23] for whom we now provide follow-up data.

Laboratory methods

Liver iron content (LIC) was estimated by liver biopsy or magnetic resonance imaging (MRI) with a T1-weighted sequence. Free testosterone levels were calculated as described by Ross et al.[24] Genotyping was performed by DNA sequence analysis of the full coding part of the genes and intron-exon boundaries, using Sanger sequencing. The pathogenicity of identified variants was assessed by association of the variant with the phenotype within a family, *in silico* tools and review of the literature. The *in silico* analysis was performed with Alamut Visual (Interactive Biosoftware), which comprises several predictive programmes to assess the consequence of missense and splice site mutations.[25]

CASE REPORTS

Patient 1

Patient 1 is a 62-year-old male of Dutch descent. At age 16, family screening was performed after his sister was diagnosed with HH, which revealed increased serum iron levels and heavy (grade IV) iron accumulation in hepatocytes.[22] At that time, he complained of lower back pain and had freckled skin. His glucose tolerance test was normal. His TSAT was elevated (95%) and a Desferal test revealed a urine iron removal of 159 µmol iron/24 h (reference range (ref) < 35 µmol iron/24 h). Workup consisted of a liver biopsy, where LIC was found to be 324 µmol per gram dry weight (= 18 mg/g; ref < 36 µmol/g), in the presence of an intact liver architecture *(table 1)*.[22] Weekly phlebotomies were administered upon diagnosis of HH.

During maintenance therapy, from ages 45 to 56, his ferritin values varied between 12 and 120 µg/l, and his TSAT between 13.4% and 87.0%. Alanine-aminotransferase (ALAT) was within the normal range.

At age 50, the patient was found to be homozygous for the *HFE2* variant c.494T > A (p.Leu165*) *(table 2)*.[23] Despite a healthy lifestyle, he was diagnosed at age 52 with diabetes and hypertension and since then, has been taking metformin *(table 1)*. At the age of 56, the patient developed a cerebrovascular accident, for which anticoagulant treatment was initiated. Further examination

Table 1. *Patient characteristics*

				At presentation										During maintenance therapy									
Pt	Sex	*HFE2* genotype		Age (years)	Ft (µg/l)	TSAT (%)	Workup								Age	Ft (µg/l)	TSAT (%)	Follow-up						
		cDNA	Protein				LIC	Fi	Hf	G	T	D	O	Other symptoms				LIC	Fi	Hf	G	T	D	O	Other symptoms
1	M	c.494T>A c.494T>A	p.Leu165* p.Leu165*	16	95	na	+	-	na	na	na	-	na	Lower back pain, freckled skin	61	20	47	na	na	+	na	na	+	na	Stroke (PFO), Hypertension
2.1[P]	M	c.494T>A c.959G>T	p.Leu165* p.Gly320Val	24	4000	90	+	-	-	+	-	-	+	Arthralgia, sepsis, fatigue	27	67	20	na	na	na	-	-	-	+	Arthralgia
2.2	M	c.494T>A c.959G>T	p.Leu165* p.Gly320Val	20	1428	98	+	-	+	-	-	-	+	Arthralgia, stroke (PFO)	23	80	82	na	na	na	-	na	-	+	None
3[P]	F	c.494T>A c.959G>T	p.Leu165* p.Gly320Val	29	2854	91	na	-	na	-	-	-	+	Abdominal complaints, rectal bleeding, fatigue	31	189	85	na	na	na	-	-	-	+	Arthralgia, infection
4[P]	F	c.959G>T c.959G>T	p.Gly320Val p.Gly320Val	39	6200	98	+	+	-	na	na	na	na	Rheumatoid arthritis, fatigue	44	25	85	na	na	na	na	-	-	na	Arthralgia
5[P]	M	c.959G>T c.959G>T	p.Gly320Val p.Gly320Val	31	2680	na	+	+	na	na	-	-	na	None	60	140	na	na	na	-	-	-	na	na	Arthralgia
6[P]	M	c.739T>A c.739T>A	p.Phe247Ile p.Phe247Ile	31	2711	79	+	+	na	-	+	+	na	Arthralgia	34	35	20	na	na	na	na	+	+	na	Arthralgia

Pt = patient; Ft = ferritin level; TSAT = transferrin saturation; LIC = liver iron content; Fi = hepatic fibrosis; Hf = heart function; G = hypogonadism; T = hypothyroidism; D = diabetes; O = osteoporosis; M = male; na = not available; + = tested and not within reference values; - = tested and within reference values; PFO = patent foramen ovale; [P] = proband; F = female. Patient 2.1 and 2.2 are brothers

Table 2. *Results of in silico analysis for pathogenicity of observed HFE2 variants*

HFE2 variant		*In silico* analysis and frequency					
cDNA	Protein	ExAC	ESP	PolyPhen-2[1]	Align GVGD[2]	SIFT[3]	Reported in literature
c.739T>A	p.Phe247Ile	np	np	1.0: probably damaging	Class C15: intermediate	0: Deleterious	No
c.494T>A	p.Leu165*	np	np	Not applicable	Not applicable	Not applicable	Yes, pathogenic[23]
c.959G>T	p.Gly320Val	np	np	1.0: probably damaging	Class C0: neutral	0: Deleterious	Yes, pathogenic[63]

np = not present.
[1]**Polyphen** predicts impact of the amino substitution on the form and structure of the human protein. Scores of 0.0-0.15 are considered benign, 0.15-0.85 are considered possibly damaging, 0.85-1.0 are more confidently predicted as damaging.[64]
[2]**Align GVGD** scores missense mutations on a 7-scale scoring system. C0 is considered neutral, C15 and C25 are considered intermediate, C35 and above are considered likely deleterious.[65]
[3]**SIFT** algorithm combines sequence homology and physical properties of amino acid substitutions to analyse whether or not amino acid substitutions are tolerated with regards to predicted effect on protein structure. SIFT scores range from 0 to 1. A variant predicted as damaging is scored < 0.05; variants predicted to be tolerated are scored > 0.5.[66]

revealed a patent foramen ovale (PFO) and 50-90% stenosis of the carotid arteries. It is unclear whether his type 2A HH contributed to the development of his diabetes and cardiovascular symptoms. Currently, he still undergoes maintenance phlebotomy therapy 3-4 times per year.

Patient 2.1

This patient is a 28-year-old male of Dutch descent. He presented at age 21 with ejaculatory failure and arthralgia of knees, elbows and hands. At the age of 23, he developed *Yersinia enterocolitica* sepsis and was subsequently hospitalised in the intensive care unit (ICU). During a check-up visit six months later, his ferritin level was severely elevated (4000 µg/l). The patient was clinically diagnosed with HH and started weekly phlebotomy treatments. Full evaluation showed a ferritin level of 2259 µg/l, with increased TSAT (99%) and immeasurably low testosterone level (< 0.1 nmol/l; ref 10.5 - 37 nmol/l), low luteinising hormone (LH) and follicle-stimulating hormone (FSH) levels (both < 1 U/l; ref in men 1.5-11 U/l and 1.4-8.5 U/l, respectively), indicating secondary hypogonadism, for which the patient started treatment with testosterone gel. The thyroid axis was unaffected. Shortly after, the patient developed an intercurrent *E. coli* sepsis as well. Genetic testing at this time showed compound heterozygosity for the *HFE2* variants c.494T > A (p.Leu165*) and c.959G > T (p.Gly320Val) *(table 2)*.

Workup in the two years after diagnosis consisted of several tests. Bone densitometry revealed osteopenia of the lumbar spine and femur collum. ALAT was found to be increased (90 U/l). An MRI scan of the liver and heart showed an LIC of > 350 µmol/g dry weight and cardiac iron content within reference values. Stroke and end-diastolic volume of the heart also remained within reference values. Testosterone was still low (1.6 nmol/l) with FSH and LH below the detection limit (< 1 U/l). The patient eventually switched from testosterone gel to subcutaneously applied choriongonadotrophin, which raised his testosterone level to 33.0 nmol/l and resolved his sexual problems.

As weekly phlebotomies did not appropriately decrease his ferritin level (3600 µg/l after six months of treatment), the patient switched to erythrocytapheresis (once per two weeks) at age 24, which lowered his ferritin value to 67 µg/l over the course of two years. Thereafter, maintenance therapy consisted of four phlebotomies per year.

After iron depletion, arthralgia decreased but did not completely disappear. Bone densitometry revealed bone density of the lumbar spine to be significantly improved, but no significant change was seen for the femur collum. Blood free thyroxine (FT4) levels remained within reference values *(table 1)*. His latest ferritin level, at age 28, is 69 µg/l with an elevated TSAT of 85%.

Patient 2.2

This patient is 24 years old and the brother of Patient 2.1. He first presented with a cerebellar stroke at the age of 17. Diagnostic workup for this event resulted in identification of a PFO.

Upon clinical diagnosis of his brother, family screening at age 20 revealed an increased ferritin value of 1188 µg/l with an elevated TSAT of 90%. Furthermore, the patient reported mild arthralgia of the ankle, but no gonadal problems. His ALAT was normal. The patient was clinically diagnosed with HH and weekly phlebotomies were started. Initially, ferritin inexplicably rose to 2007 µg/l after one month, but after two-and-a-half months and eight phlebotomies, his ferritin level decreased to 1428 µg/l with a TSAT of 99% and kept decreasing during the following months.

Diagnostic workup took place during the two years post clinical diagnosis. Genetic screening confirmed compound heterozygosity for the *HFE2* mutations c.494T > A (p.Leu165*) and c.959G > T (p.Gly320Val) *(table 2)*. Bone densitometry revealed osteopenia of the lumbar spine. MRI of the liver revealed increased LIC of 310 (± 50) µmol/g dry weight, whereas iron levels in his heart were within reference values. Heart function was assessed as normal by ultrasound, and hormone analysis showed normal thyroid function. Four months into his phlebotomy treatment, at a ferritin level of 1125 µg/l, testosterone was low (3.1 nmol/l); FSH and LH were not measured at this point in time. Several months later, the testosterone (10.6 nmol/l) and free testosterone (120 pmol/l; ref 120-750 pmol/l)[26] were just within reference range. FSH and LH were normal as well. No other abnormalities were reported *(table 1)*. One year after the first testosterone measurement, testosterone levels had increased to 20.5 nmol/l (free testosterone 266 pmol/l) with a ferritin value of 147 µg/l. At 24 years of age, TSAT was 82% and depletion of iron stores was achieved with a ferritin level of 80 µg/l *(table 1)*. Thereafter, maintenance phlebotomy therapy consisting of six phlebotomies per year was started. Upon iron depletion, bone density of the lumbar spine, but not that of the femur collum, was found to be significantly improved, and the patient did not report arthralgia.

Patient 3

This patient is a 32-year-old female proband of Dutch descent. She had had complaints of severe fatigue and several infections since childhood, but despite extensive hospital examination, the cause was not identified until age 29. At this age, she presented with abdominal complaints and rectal bleeding. Evaluation revealed an elevated TSAT (91%) and a severely increased ferritin value (2854 µg/l). Although the common *HFE* variants (C282Y and H63D) were not identified, the patient was diagnosed with HH and weekly phlebotomies were planned. Almost a year after

starting treatment, having received only 13 phlebotomies due to lack of compliance, her ferritin level was 1994 µg/l, and she presented with arthralgia in her wrists and fingers. Genetic testing revealed compound heterozygosity for c.494T > A (p.Leu165*) and c.959G > T (p.Gly320Val) in the *HFE2* gene *(table 2)*.

During the two years after diagnosis (age 29-31 years), workup consisted of i) bone densitometry revealing osteoporosis of the lumbar spine and femur collum; ii) computed tomography scan of the hand revealing diffuse osteopenic aspect; iii) analysis of TSH, which was found to be within the reference range; and iv) evaluation of the gonadal axis, which showed no abnormalities *(table 1)*. ALAT was normal and ultrasound of the abdomen showed no indication for the presence of cirrhosis or hepatocellular carcinoma. Further examinations to assess liver iron and imaging studies of the heart were not performed.

At age 30, at a ferritin level of 838 µg/l, the patient switched to biweekly phlebotomies, since she found the weekly phlebotomies too taxing. At this age, a Pseudomonas infection of the peripherally inserted central catheter (PICC) was found. At age 31, the TSH was still within reference range and her ferritin levels reached 189 µg/l *(table 1)*. Currently, the patient is doing well, with enough energy to work full time.

Patient 4

This patient is a 44-year-old female proband of Dutch descent. At age 39, she presented with seronegative rheumatoid arthritis of the metatarsal-phalangeal and proximal interphalangeal joints and reported fatigue. Laboratory testing showed a severely increased ferritin level of 6200 µg/l with elevated TSAT (98%) and ALAT (150 U/l) levels. Weekly phlebotomies were started upon clinical diagnosis of HH, after which arthralgia persisted. In the workup of the following months, no bone densitometry was performed. Echography and subsequent MRI of the liver revealed multiple lesions (not metastases) as well as steatosis hepatis, iron accumulation and mild fibrosis, but no hepatomegaly. Using tissue Doppler imaging, which may be used as a screening tool to detect cardiac involvement in patients with HH, no cardiac iron accumulation was found. Liver biopsy revealed severe (grade IV) iron accumulation *(table 1)*. TSH, FT4 and the gonadal axis were not evaluated at this point in time. After eight months of phlebotomy, her liver enzyme levels had normalised.

Three years after presentation, her TSH was high normal (4.2 mU/l), FT4 was normal and diabetes mellitus was absent. Ferritin levels varied between 24 and 64 µg/l with a TSAT around 85%. Four years after clinical diagnosis, genetic testing revealed a homozygous c.959G > T (p.Gly320Val) variant in *HFE2 (table 2)*. Her arthralgia has decreased, but not completely disappeared *(table 1)*.

Patient 5

This patient is a 60-year-old male proband of Dutch descent. He was first noticed in routine medical evaluation at age 31, as his liver enzymes were elevated with a ferritin of 2680 µg/l. Diagnostic workup consisted of liver biopsy showing grade IV iron accumulation with fibrosis. Blood tests revealed normal thyroid status: TSH and total thyroxine were within normal limits *(table 1)*. HH was diagnosed and phlebotomies were started. Evaluation of the gonadal axis and bone density was considered but not deemed necessary.

At age 55, the patient presented with arthrosis in both halluces *(table 1)*. Of note, this had existed for 11 years, during which arthrodesis had been performed. The patient's treatment at the time consisted of phlebotomies once every eight weeks. Ferritin was 140 µg/l. Genetic testing of the *HFE2* gene revealed homozygosity for the pathogenic variant c.959G > T (p.Gly320Val) *(table 2)*. After this diagnosis, an ECG and ultrasound of the heart were performed, and no abnormalities were found. Iron loading of the heart was not tested. Laboratory results did not indicate any abnormalities of liver, gonadal axis or thyroid. His most recent ferritin level, measured at age 60, was 29 µg/l. He still undergoes phlebotomies once every eight weeks and feels well, although he does have painful shoulders and wrists.

Patient 6

This patient is a male proband, 35 years of age and of Turkish origin. At age 31, he presented with arthralgia in his hands, fatigue and weight loss and was diagnosed with diabetes and microcytic anaemia (Hb 7.8 mmol/l, mean corpuscular volume 78 fl). Diagnostic workup of his anaemia showed heterozygous β-thalassaemia (or β-thalassaemia minor), a TSAT of 79% and a ferritin value of 2711 µg/l. Furthermore, MRI of the liver yielded LIC in excess of 350 µmol/g dry weight and a liver biopsy identified severe iron accumulation in the hepatocytes and mild fibrosis. T2* imaging of his heart showed normal cardiac iron content. ALAT was high normal (54 U/l). His testosterone, LH and FSH levels were within reference ranges. The patient also had subclinical hypothyroidism: TSH levels were increased (7.6 mU/l, ref 0.4-4.0 mU/l) and FT4 was normal *(table 1)*. Combined, and since β-thalassaemia minor is not associated with iron overload, these findings led to the clinical diagnosis of HH, and weekly phlebotomies were started.

At age 31, genetic diagnosis showed that the patient was a homozygous carrier of the c.739T > A (p.Phe247Ile) *HFE2* variant. This variant was not previously described in literature, but was predicted as pathogenic by *in silico* analysis (Align GVGD, SIFT, PolyPhen-2) *(table 2)*. His brother and sister were diagnosed with the same HJV genotype and both show a phenotype consistent with type

2A HH. However, as they never presented at our centre, we do not have any clinical data on these two siblings.

Phlebotomies were well tolerated and at age 34, iron depletion was achieved (TSAT 20%; ferritin 35 µg/l) and maintenance therapy with phlebotomies every three months was planned. However, due to lack of compliance, the patient did not receive therapy for one year, during which TSAT and ferritin levels increased to 76% and 135 µg/l, respectively. Currently, his diabetes is well regulated with metformin and his hypothyroidism has remained subclinical with a TSH of 6.1 mU/l and an FT4 of 14 pmol/l (ref 8.0-22.0 pmol/l). His ALAT has normalised, but a mild arthralgia is still present.

DISCUSSION

In our sample consisting of seven patients from six families diagnosed with type 2A HH in the Netherlands, we observed: i) a highly pleiotropic presentation; ii) a significant delay to the clinical diagnosis in two patients; iii) only two variants that are responsible for the six biallelically-affected patients from five families of Dutch origin; iv) a practice variation in the diagnostic workup, follow-up and applied treatment strategy; and v) novel complications that may be attributed to type 2A HH occurring with ageing despite the long-term iron depletion therapy.

Probands (three men and two women) first presented in adulthood, but their ages varied. Female probands presented at a later age (range 31-39) than male probands (range 19-31). Our female patients had not been pregnant prior to presentation, but blood loss (and thus loss of iron) due to menstruation may explain the later onset. Findings of a JH case series and a study comparing JH with type 1 and 3 HH are in agreement with our findings.[10,11] The prevalence of presenting symptoms of our seven patients compared to three previously reported case series of JH patients (N, respectively 13 (> 8 years old, defined as < 30 years old at presentation), 26 (adults, defined as presentation < 30 years old), 37 (adults, linked to chromosome 1q)) can be summarised as follows: arthralgia 57% in our patients vs. 27-30% in previous reports; hypogonadotropic hypogonadism 14% vs. 77-96%; diabetes/reduced glucose tolerance 29% vs. 31-58%; and cardiac problems 29% vs. 35-54%.[10-12] In addition, we report one patient out of seven with subclinical hypothyroidism, in comparison to one out of 13 that had hypothyroidism in one of the aforementioned case series.[12] The others do not report hypothyroidism, which corresponds to the notion that the development of hypothyroidism in juvenile haemochromatosis is rare.[12,28,29] Hashimoto thyroiditis characterised by thyromegaly, hypothyroidism and elevated serum concentrations of anti-thyroid peroxidase (anti-TPO) antibodies has been documented as a cause of thyroid dysfunction in two patients suspected for JH.[30] To the best of our knowledge, it is not fully known whether hypothyroidism in JH is coincidental, a primary thyroid dysfunction or secondary anterior pituitary failure.[29,30]

Three of our patients were diagnosed with osteopenia or osteoporosis, one of whom also had hypogonadism. Osteopenia and osteoporosis are reported as common complications in type 2A HH.[31] Proposed underlying mechanisms include hypogonadism, liver failure and iron overload,[32] of which liver failure is unlikely since liver fibrosis was absent in our patients diagnosed with osteoporosis. Furthermore, one patient developed sepsis from *Yersinia enterocolitica* and *E. coli* in his early 20s. From the literature, these infections have been described in states of iron overload and related high iron availability, and are the result of hepcidin deficiency. Among the causal micro-organisms are – apart from *Yersinia enterocolitica* and *E. coli* – also *Vibrio vulnificus* and *Listeria monocytogenes*.[33-38] Another patient (patient 3) presented with a central-line infection caused by Pseudomonas, which has also been associated with iron overload.[39]

Overall, the differences in prevalence of most of the presenting symptoms in our series compared to the literature might be characterised as chance findings due to our small sample size. The lower prevalence of most symptoms may be attributed to rising timely diagnosis and treatment of patients in the last decade, as recently reviewed.[6]

Two out of our five probands (patients 2.1 and 3) had a significant delay to clinical diagnosis. One of these probands, patient 2.1, presented with multiple concomitant symptoms: arthralgia, hypogonadism and sepsis. Delay to clinical diagnosis has been reported by others in patients with type 2A HH,[40] who developed severe cardiac complications. Since severe complications also occurred in one proband (patient 2.1) with delay to clinical diagnosis, we urge physicians to be mindful of possible iron overload if systemic symptoms in young patients go unexplained for several months.

In this respect, for patients without *HFE* C282Y homozygosity or C282Y/H63D compound heterozygosity and with hyperferritinaemia and TSAT > 45%, existing guidelines[41] recommend direct assessment of liver iron by MRI or liver biopsy. If iron excess has been proven (i.e. > 3-6 times the upper limit of normal)[42,43] and other (hepatic or haematological) diseases have been ruled out, genetic testing for rare defects in *HFE* and other non-*HFE* haemochromatosis genes should be performed.

We found the previously unreported c.739T > A (p.Phe247Ile) variant in our non-Dutch patient. Moreover, we report that the common c. 959C > G (p.Gly320Val) variant and the c.494T > A (p.Leu165*) variant to be the causative mutations for all of our patients of Dutch descent.

To date, the latter variant has only been described in Dutch patients,[23] suggesting a founder effect. Since numbers are low, we were unable to assess if there was a difference in iron accumulation between patients with the different genotypes. To the best of our knowledge, a genotype-phenotype relationship between *HFE2* mutations and iron accumulation has not yet been described in the literature. Alternatively, other genes or factors aside from menstrual blood loss may play a modifying role in rate of body iron accumulation.

We found that after the diagnosis of type 2A HH was made, workup, follow-up and treatment strategies differed between subjects. Currently, no guideline or evidence exists for the optimal workup, follow-up and treatment of type 2A HH. Nevertheless, workup for organ damage by iron accumulation is generally performed by physicians in JH patients, prompted either by genetic diagnosis or abnormally elevated ferritin levels. In the current case series, this workup was variable: heart function and the gonadal axis were only studied in some of the patients, and evaluation of liver iron content by MRI (n = 2), liver biopsy (n = 2) or both (n = 2) was performed in all subjects except one. For the latter patient, ultrasound of the liver was performed, but this technique is not suitable for the detection of iron overload. Of the four patients who presented elevated liver enzymes at diagnosis, a liver biopsy was performed in three of them. Follow-up also differed between patients, with symptoms of hypogonadism and glucose intolerance-initiated workup in two patients. However, in the absence of complaints, the patients were not always tested for endocrine abnormalities and possible associated osteoporosis.

In the absence of guidelines for treatment of type 2A HH, treatment strategies for the patients described here were similar to that of HFE-HH,[6] as laid out in the European Association for the Study of the Liver (EASL) Guidelines for HFE haemochromatosis.[41] In accordance with the EASL Guidelines, six out of seven patients were phlebotomised until iron depletion was achieved; of these, five underwent weekly phlebotomies to obtain full depletion. One patient however, switched to biweekly phlebotomies and another switched from phlebotomy to erythrocytapheresis. In all patients, this depletion therapy took at least 24 months. Notably, we observed variation between patients in target ferritin levels for both the depletion and maintenance phase. Erythrocytapheresis was successfully used for iron depletion in one of the patients. A recent observational study showed beneficial effects of this therapy.[44] However, a Cochrane review concluded that there is currently insufficient evidence to determine whether erythrocytapheresis is beneficial or harmful compared to phlebotomy therapy.[45]

While iron-depleted, two of our patients developed new (co)morbidities with ageing. These comprised stroke, diabetes and articular problems. Although the prevalence of these morbidities increases with ageing in the general population,[46] it is also conceivable that they can be attributed to HJV function in cells other than hepatocytes, since *HFE2* is also expressed in myocardial cells,[13,47] and β and acinar cells of the pancreas.[48] Another possibility is that long-term exposure to non-transferrin bound iron (NTBI) contributes to the development of these morbidities. Indeed, even after iron depletion, in the maintenance phase, TSAT in most patients remains elevated (above 70%), producing NTBI in the circulation.[49] NTBI has been documented as toxic for parenchymal cells,[47,50-54] including pancreas β cells and cardiomyocytes, where it has been reported to be taken up in an unregulated manner by ZIP14 or L-type Ca^{2+} channels.[55,56] Interestingly, increased circulating iron has been associated with increased atherosclerosis in a mouse model of hereditary haemochromatosis[57,58] and with coronary artery disease in patients with stable angina pectoris.[59] Long-term exposure to high TSAT-induced NTBI levels may also predispose patients to arthralgia. Indeed, articular manifestation is a common occurrence in type 2A HH,[10-12,31] but how exactly iron overload damages joints in HH – in general and in JH in particular – is not yet clear.[60,61] Therefore, we speculate that the chronic presence of NTBI underlies the development of complications in type 2A HH over time, despite the depletion of patient iron stores. If this is indeed the case, adequate treatment remains a challenge: phlebotomy therapy to lower TSAT in these patients would require extensive venesection, which could lead to anaemia. It is however, conceivable that novel hepcidin agonists, which are currently under development as novel therapies for diseases that are characterised by low hepcidin levels relative to body iron stores,[62] may provide a solution in the future.

In conclusion, even though we note that awareness of type 2A HH is increasing among physicians, much remains unclear for the optimal management of this disease regarding workup and follow-up, and for the significance of chronically elevated TSAT for the development of long-term complications in patients with type 2A HH. Therefore, we advocate research on the natural course of the disease and the cost-effectiveness of the various workups, follow-ups and diagnostic strategies. Timely referral, a global registry and management of these patients within networks of expertise centres, such as the recently initiated European Reference Networks, will help achieve these goals. Until this has been accomplished, we recommend to adhere to existing clinical guidelines for HFE-HH: to screen for the *HFE2* genotype and iron overload phenotype of all first-degree relatives, and to assess diagnosed patients for complications including liver fibrosis and cirrhosis, diabetes mellitus, joint disease, endocrine deficiency (hypothyroidism and hypogonadism),

cardiac disease and osteoporosis, preferably before the start of intensive iron depletion therapy.

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CASE REPORT

Case series on acute HCV in HIV-negative men in regular clinical practice: a call for action

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ABSTRACT

Background: The evidence that HIV treatment as prevention (TasP) and HIV pre-exposure prophylaxis (PrEP) reduces the risk of HIV transmission is overwhelming. But as PrEP and TasP can lead to increased sexual mixing between HIV positive and negative men who have sex with men (MSM), sexually transmitted infections such as acute hepatitis C (HCV), which were thought to be limited to HIV-infected MSM, could become more frequent in HIV uninfected MSM as well. The objective of this study was to describe a series of cases of sexually transmitted HCV infections in HIV-uninfected MSM in the Netherlands and Belgium.

Methods: Through the Dutch Acute HCV in HIV Study (a Dutch-Belgian prospective multicentre study on the treatment of acute HCV infection, NCT02600325) and the Be-PrEP-ared study (a PrEP project in Antwerp, EudraCT2015-000054-37) several acute HCV infections were detected in HIV-negative men.

Results: A newly acquired HCV infection was diagnosed in ten HIV-negative MSM. HCV was diagnosed at a sexually transmitted infection (STI) clinic (n = 2), by their general practitioner (n = 2), by their HIV physician (n = 1) or at a PrEP clinic (n = 5). Ten patients reported unprotected anal intercourse and four had a concomitant STI at the time of HCV diagnosis. Six patients reported using drugs during sex.

Conclusions: Our observation calls for a larger nationwide epidemiological study on the prevalence, incidence and risk factors of HCV infection in HIV-uninfected MSM. In the changing landscape of TasP and PrEP, reliable and up-to-date epidemiological data on HCV among HIV-uninfected MSM are needed and will help in developing evidence-based testing policies.

What was known on this topic?

Sexually acquired acute hepatitis C infection used to be regarded as limited to HIV-positive men who have sex with men (MSM). Several large cohort studies showed a very low prevalence in HIV-negative MSM, comparable to the HCV prevalence in the general population.

What does this add?

With this case series we want to raise awareness among a broad range of Dutch clinicians. Sexually transmitted hepatitis C infection seems to be no longer limited to HIV-positive MSM, a subgroup of HIV-negative MSM are probably at increased risk. With the advent of HIV pre-exposure prophylaxis (PrEP), clinicians should be aware of the possibility of acute HCV infections in HIV-negative MSM and at least surveillance should be in place to gain inside the prevalence and incidence among this risk group.

KEYWORDS

Acute hepatitis C, men who have sex with men (MSM), sexually transmitted infections

INTRODUCTION

The World Health Organisation recently released targets for hepatitis B and hepatitis C (HCV) elimination by 2030. They included a 90% reduction in new infections and a 65% reduction in hepatitis-related mortality by 2030.[1]

One of the key populations at risk for HCV infection is HIV-infected men who have sex with men (MSM). Among HIV-infected individuals worldwide, it has been estimated that 2.4% are co-infected with HCV, yet this rises to 6.4% in HIV-infected MSM.[2] Because HIV-infected MSM in Western Europe are receiving care for their HIV, treating all HIV-infected patients with an HCV co-infection for their HCV should be straightforward and HCV elimination in this specific subgroup might be possible.[3,4] Previous epidemiological data suggested that transmission of HCV among MSM was largely limited to HIV-infected MSM.[5-7] The evidence that HIV treatment as prevention (TasP) and HIV pre-exposure prophylaxis (PrEP) reduces the risk of HIV transmission is overwhelming.[8-11] Although currently only four European countries have made PrEP available free of charge, generic tenofovir-disoproxil fumarate and emtricitabine in a single combination tablet will soon become available in certain European countries.[12] In Germany as well as the Netherlands negotiations have resulted in a substantial price reduction and will make PrEP affordable for many MSM. Without any doubt, PrEP and TasP will prevent many new HIV infections in MSM. However, it can be expected that PrEP and TasP will also lead to increased sexual mixing between HIV positive and negative MSM. As such, sexually transmitted infections (STIs) such as HCV that were thought to be limited to HIV-infected MSM are likely to become more frequent in HIV-uninfected MSM as well. A recent modelling study seems to confirm this and showed that sexual behaviour patterns are likely to drive the HCV infection pattern among HIV-positive MSM. If changes in these patterns occur, they could lead to HCV dissemination amongst HIV-negative MSM and may decrease the impact of unrestricted HCV treatment for HIV-infected MSM on the HCV epidemic in MSM in general.[13] Very recently, Hoornenborg et al. showed that at the start of the Amsterdam PrEP study, the prevalence of HCV infection was 4% as 15 of the 375 MSM were chronically infected with HCV.[14] This illustrates that in a subgroup of HIV-uninfected MSM, the prevalence of HCV infection may be very substantial and this contrasts with what has been reported earlier about the HCV prevalence and incidence in HIV-negative MSM.[6,15]

The objective of this study was to describe a series of cases and therefore create increased awareness about newly acquired HCV infections in HIV-uninfected MSM in the Netherlands and Belgium. All had tested negative for HCV in the recent past.

METHODS

Cases of HIV-negative MSM with a newly acquired HCV infection were collected in the context of an acute HCV treatment study (the Dutch-Belgian prospective multicentre study on the treatment of acute HCV infection (DAHHS-2, NCT02600325) or within an PrEP-project in Antwerp (Be-PrEP-ared; EudraCT2015-000054-37). Patients were initially diagnosed by their GP, their STI clinic, their HIV specialist or the PrEP project before they were referred to the DAHHS-2 study team.

All reported patients had tested negative for HCV in the recent past. Patients were screened for HCV for different reasons in different settings as stated above and in *table 1*. HCV testing was done according to the local standard of care, which in all cases consisted of screening for HCV with HCV antibodies. Acute HCV was defined as a positive anti-HCV immunoglobulin G and a documented negative anti-HCV IgG in the previous 12 months.[1] Patient characteristics and risk factors were retrieved from the patient files by the treating physician and transferred to the study coordinators after anonymisation.

Both studies were approved by the institutional medical ethics committees. Enrolment in these studies was voluntary and written informed consent was obtained in which the patients described in this report agreed that data and blood samples could be used for research purposes.

RESULTS

From 1 January 2016 to July 2017 a total of ten HIV-negative MSM with a recently acquired HCV infection were reported *(table 1)*. HCV infection was diagnosed at a sexually transmitted infection (STI) clinic (n = 2), by the general practitioner (n = 2), by their infectiologist (n = 1) or at their PrEP clinic (n = 5). All patients had a documented negative HCV test within the year preceding the HCV diagnosis. Of the patients diagnosed at the PrEP clinic, one was diagnosed before the start of PrEP and four after the start of PrEP. Median age was 39.5 years (range 25-59). HCV genotype 1 was found in four patients, genotype 4 in two patients and the genotype was unknown in four patients. All patients reported unprotected anal intercourse, four had a concomitant STI at the time of HCV diagnosis and six reported drugs use during sex (chemsex). One patient reported intravenous drug use during sex (slamming). Clinical symptoms were non-specific or absent. Two patients were diagnosed after they had been informed of a HCV diagnosis in a partner.

DISCUSSION

Our case series shows that, even without an active screening policy, HCV infections are diagnosed in HIV-negative MSM as we were able to describe 10 cases of newly acquired HCV infections in Dutch and Belgian

Table 1. *Overview of the baseline characteristics, HCV diagnosis and treatment outcome of the patients described in this case series*

Risk factors	Genotype	Symptomatic HCV infection?	Comorbidities at time of acute HCV infection	Earlier HCV infections?	Year of acute HCV infection	Prior negative HCV test	HCV test indication	Tested by	Treatment given	SVR12
UAI CS SS	4	No	Chlamydia	No	2016	2014	PN	STI clinic Breda	Treated after HCV infection became chronic with sofosbuvir ledipasvir 8 weeks	Yes
UAI	1a	No	Non	No	2016	2016	During PrEP study	PrEP clinic Amsterdam	Grazoprevir elbasvir 8 weeks[$]	Yes
UAI CS	Undetectable	Fatigue	LGV, syphilis, gonorrhoea, suspicion of AIN	No	2017	2016	LGV	STI clinic Rotterdam	No, spontaneous clearance	N/A
UAI CS	4	Erythema multiforme	Gonorrhoea pharynx, anal chlamydia	No	2016	2015	Routine testing	GP from Leuven area	Grazoprevir elbasvir 8 weeks[$]	Yes
UAI CS	1a	No	Non	No	2016	2016	Routine testing	GP from West-Flanders area	No, spontaneous clearance	N/A
UAI	1a	No	Chlamydia, mycoplasma genitalium	No	2016	2016	During PrEP study	PrEP clinic Antwerp (patient from Brussel area)	Ongoing chronic infection	N/A
UAI	Undetectable	Fatigue	Non	No	2016	2016	During PrEP study	PrEP clinic Antwerp, (patient from Antwerp area)	No, spontaneous clearance	N/A
UAI CS	Undetectable	No	Depression, post-traumatic stress syndrome	No	2016	2015	During PrEP study	PrEP clinic Antwerp, (patient from Antwerp area)	No, spontaneous clearance	N/A
UAI	Unknown	Proteinuria	Non	Unknown	2016	2016	Before start PrEP	PrEP clinic Antwerp, patient from East-Flanders)	Unknown	N/A
UAI CS	1a	Fatigue	Non	No	2017	2017	PEP use	Hospital, Eindhoven	Grazoprevir elbasvir 8 weeks[$]	N/A

SS = Slamsex, i.e. use of intravenous drugs during sex; UAI = unprotected anal intercourse; PN = partner notification (acute HCV is a reportable disease which means that the public health service contacts all traceable recent sex partners and offers them HCV testing); CS = Chemsex, i.e. use of oral drugs during sex; PrEP = pre-exposure prophylaxis for HIV; PEP = postexposure prophylaxis for HIV; STD = sexually transmitted disease; LGV = Lymphogranuloma venereum; SVR12 = sustained virological response 12 weeks after treatment; GP = general practitioner; AIN = anal intraepithelial neoplasia; N/A = Not applicable; [$] = $ DAHHS-2 study; NCT02600325.

HIV-uninfected MSM. Other recent publications on HCV infections in HIV-negative MSM were the result of an active screening policy as part of an observational study or a PrEP program.[14,16] Furthermore, very few studies on the epidemiology of HCV in HIV-uninfected MSM are available and none were collected in a way that incidence rates of acute HCV infection in HIV-negative MSM can be calculated to properly address the problem.[6,7,14,16-18] By design, case series cannot help to reliably estimate the size of the problem. We therefore call for a nationwide epidemiological study to get a reliable estimate of the prevalence and incidence of and insight into risk factors for HCV infection in HIV-uninfected MSM.

Not surprisingly, all reported unprotected anal intercourse and most had other concomitant STI diagnoses and six used non-injection drugs during sex. These are known risk factors for sexual HCV transmission in HIV-positive MSM.[19] In the UK, the British Association of Sexual Health and HIV (BASHH) recommends to at least consider testing MSM for HCV if they are considered at high risk for HCV infection (independent of HIV status).[20] In the Netherlands guidelines for the STI clinics advise testing HIV-positive MSM and MSM notified for HCV, MSM diagnosed with a lymphogranuloma venereum infection and MSM refusing an HIV test.[21] In Belgium, there is no national guideline for HCV testing in HIV-negative MSM. Our case series, together with the high prevalence of chronic HCV infection in the AmPrEP project,[14] call for HCV testing of MSM at Dutch and Belgian STI clinics in order to get reliable data of the HCV prevalence and incidence in 2018. PrEP programs should include regular HCV testing and MSM who start using PrEP outside the context of an official PrEP program should be tested at STI clinics.[22] In a recently published systematic review, daily oral PrEP use was associated with a significant increase in rectal chlamydia and increase in any STI diagnosis,[23] which emphasises the need for STI as well as HCV prevention strategies for PrEP users and their partners.

Second, as multiple parties are involved in the care of MSM (HIV centres, STI clinics, general practitioners) collaboration is needed if HCV elimination is to be pursued. Last but not least, the development and validation of an HCV risk score for HIV-negative MSM, as has been done before for HIV-infected MSM, could facilitate targeted HCV testing in the future.[24]

According to the definition for acute HCV infection by the European AIDS Treatment Network consensus panel,[1] all our patients fulfilled the criteria for acute HCV, except for one patient in whom an earlier negative test was missing. And although he was 'directly' diagnosed after a recent partner notification, we cannot say for sure that he had an acute infection. Perhaps this patient is better defined as a recent infection according to definitions of Hajarizadeh et al.[25]

Our case series has several limitations. Because our cases were not identified during a prospective surveillance study, risk factors for HCV infection cannot be identified as we cannot compare our cases with HCV-negative controls. Furthermore, we have no denominator and therefore no estimate of the prevalence and incidence can be given. Also, we report on Dutch and Belgium cases. This is due to the fact that the acute HCV treatment study (DAHHS) is recruiting patients in the two neighbouring countries. Although the healthcare systems in these two countries are very alike, two important differences should be mentioned. In Belgium HCV therapy for patients with F0 to F2 fibrosis is currently restricted to patients that are HIV/HCV-coinfected, while in the Netherlands restrictions are no longer in place. In contrast, at the time of writing this manuscript, PrEP was available free of charge in Belgium but not in the Netherlands, except for a small group of MSM participating in the AmPrEP program.

In conclusion, in the changing landscape of TasP and PrEP, close monitoring of HCV infection among HIV-uninfected MSM is needed to improve case finding of HCV infection and decide upon the best testing policies of HCV infection in HIV-negative MSM.

DISCLOSURES

AB: no conflicts. KW: no conflicts (the PrEP in the Belgian Be-Prepared project is donated by Gilead). HA: no conflicts. HG: no conflicts. ML: no conflicts. BR has received a research grant from Merck Sharp & Dohme (ongoing, 2014–17) within the context of this article. Outside the context of this article he has received research grants from Gilead Sciences (ongoing, 2013–17), has been an investigator of trials sponsored by Merck Sharp & Dohme, Gilead Sciences and Janssen-Cilag, has been an invited speaker for Gilead Sciences, Merck Sharp & Dohme, Pfizer and Janssen-Cilag, has participated on advisory boards and has received conference invitations for Bristol-Myers Squibb, AbbVie, Merck Sharp & Dohme, Gilead Sciences and Janssen-Cilag and has been a consultant to GL pharmaceuticals.

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CASE REPORT

Urine changing from clear to milky-white

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ABSTRACT

After sedation with propofol a young man developed milky-white urine. Urinalysis showed a high concentration of uric acid crystals as being responsible. This phenomenon appears to be dose-dependent and is explained in this report. Since it is harmless and self-limiting no extensive analysis is needed when observed.

KEYWORDS

Propofol, uric acid crystals, urine discoloration

What was known on this topic?
A variety of causes of discoloration of urine are known, such as different types of diseases and some medication. Administration of propofol may result in the production of green urine, and rarely in milky-white urine, which is caused by the production of uric acid crystals in the urine. Since propofol is quite often used in daily practice, the chance of observing this phenomenon is substantial. The phenomenon is self-limiting and does not harm kidney function.

What does this add?
Discoloration of urine to milky-white during infusion of propofol is described. The phenomenon appears to occur in a dose-dependent fashion. In our patient induction of sedation with a high dose of propofol induced the production of milky-white urine, followed by normalisation of the urine during maintenance of low-dose infusion of propofol. During surgery the dose was increased causing recurrence of the milky-white urine. We assume that a higher dose of propofol results in higher levels of uric acid crystals in the urine, after a 'threshold' has been passed.

CASE REPORT

A 22-year-old man presented to the emergency department. He was arrested because of irrational, aggressive behaviour after jumping out of the window of his house. Because of severe agitation an attempt to sedate him with 15 mg midazolam in the ambulance was unsuccessful. His medical history revealed only substance abuse, and no other medication was used.

After admission to the ICU where sedation with propofol was initiated, no abnormalities were found on physical and radiological examination apart from a soft tissue wound with tendon injury to his right wrist. Because substance abuse was suspected, toxicity screening was performed after insertion of a urinary catheter, revealing the presence of amphetamines, benzodiazepines and cannabinoids. Ethanol could not be detected.

Initially, clear yellow urine was drained; however, within 30 minutes after initiation of propofol infusion (160 mg bolus dose and 240 mg/h) the aspect of the urine changed into cloudy, milky-white, yellowish urine (*figure 1*). The urine was analysed microscopically, showing the presence of vast amounts of uric acid crystals. Serum uric acid concentration was elevated (1.14, normal 0.20-0.42 mmol/l) as was the creatinine kinase concentration (2100 mmol/l). Kidney and liver function was normal.

Within three hours the discoloration disappeared and the urine returned to normal, despite continuation of a low-dose propofol infusion. Ten hours later the patient was transferred to the operating room for surgical repair of his injured tendons. After an extra dose of 160 mg of propofol, and infusion of 400 mg propofol/h, the urine turned milky-white again. After surgery the propofol infusion was stopped and the urine again normalised. The following day the serum uric acid levels returned to normal and no uric acid crystals were detected on urinalysis.

DISCUSSION

The patient developed milky-white urine after anaesthesia with propofol, which is a rare phenomenon. Other types of discoloration of urine due to propofol anaesthesia have

Figure 1. *Milky-white yellow urine after administration of propofol*

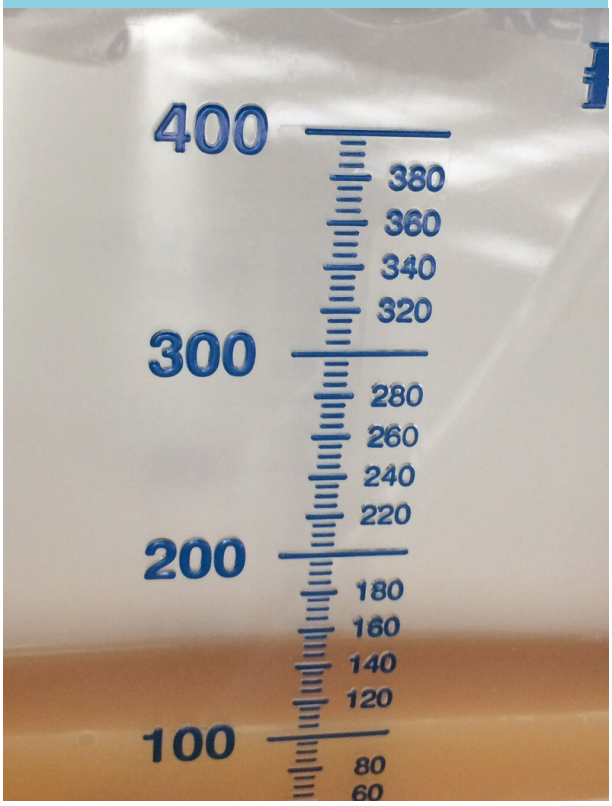
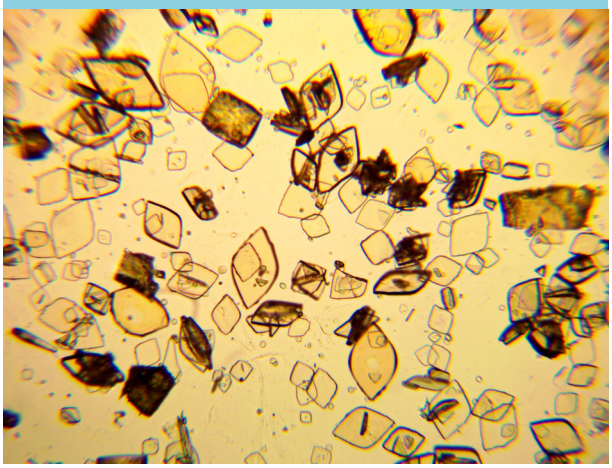


Figure 2. *Uric acid crystals in milky-white cloudy urine*



been described such as green, due to its phenol group, or pink.[1]

The differential diagnosis of discoloration of urine includes a urinary tract infection due to pyuria, increased excretion of phosphate crystals, as occurs in hyperparathyroidism, and the use of drugs (e.g. rifampicin and methylene blue).[2] Discoloration due to pyuria was very unlikely, since there were no signs of infection. Moreover, urinalysis did not show any leukocytes and nitrite was negative. Hyperparathyroidism was not present nor were phosphate crystals seen during microscopic analysis.

We assume that the milky-white, cloudy appearance was due to uric acid crystallisation which was confirmed by urinalysis (*figure 2*).

Propofol is known to be uricosuric.[3] It has a structural similarity to probenecid, which competes with uric acid at the anion transport exchanger at the renal tubules, hence inhibiting reabsorption of uricacid.[3] Propofol, or any of its metabolites, probably competes at the same anion transporter, inducing excretion of uric acid.[3] A number of risk factors are described for uric acid precipitation with propofol,[4] such as hyperuricaemia, low urine pH, obesity and low urine volume. Uric acid can precipitate in the presence of an acidic urine pH, which was present in our case with a pH of 5.0. Increasing the urine pH by adding bicarbonate could theoretically cause the uric acid crystals to dissolve, leading to normalisation of the urine.

In addition, our patient had hyperuricaemia, which was probably related to mild rhabdomyolysis (creatinine kinase was initially 2100 mmol/l), as a result of his aggressive behaviour prior to admission. He had a normal urine volume and no obesity.

Remarkably a high dose of propofol twice resulted in milky-white urine, whereas a lower dose did not, thus suggesting that the formation of uric acid crystals is a dose-dependent phenomenon. To our knowledge, this observation has not been described before.

Since propofol is frequently used, the described phenomenon might be observed more often in the future. It is important to realise that this transient phenomenon appears not to be harmful and has no negative consequences for kidney function. Therefore, unnecessary investigations should be avoided.

DISCLOSURES

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

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PHOTO QUIZ

Jaundice and fever in a patient with psoriasis

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CASE REPORT

A 39-year-old male was admitted with joint pain, skin plaques, and fever. He was diagnosed with psoriasis 20 years ago and was treated with local steroids. On this admission, he had fever (39 °C, tympanic), jaundice, pain in the right upper abdominal region, pain at the axial and peripheral joints, disseminated psoriatic rashes, a sausage-shaped digit (left hand, third finger), pustules on psoriatic rashes on both ankles, and pitting of all fingernails.

Laboratory investigations included a white cell count of 18.2 x 10^9/l, alanine aminotransferase (ALAT) level 310 IU/l, aspartate aminotransferase (ASAT) level 220 IU/l, gamma glutamyl transpeptidase 256 IU/l, alkaline phosphatase 600 IU/l, and total bilirubin 87.2 µmol/l (n = 0-17). He had right-sided sacroiliitis on MRI. Blood cultures were obtained and he was given antibiotics (piperacillin/tazobactam). Blood cultures remained sterile. Hepatobiliary ultrasound and magnetic resonance cholangiography remained negative. Fever, rash, and joint pain persisted. A liver biopsy was obtained (*figures 1 and 2*).

WHAT IS YOUR DIAGNOSIS?

See page 382 for the answer to this photo quiz.

Figure 1. *Liver biopsy revealing neutrophilic infiltration (arrows) of the portal tract (haematoxylin and eosin's staining, x1000)*

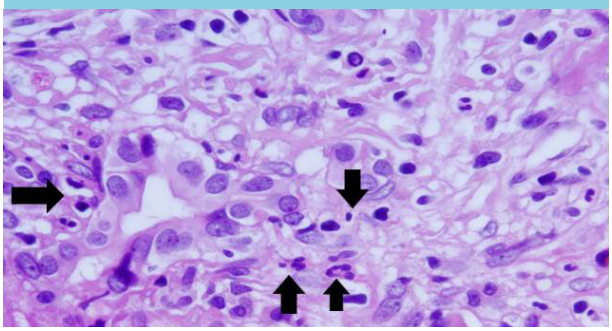
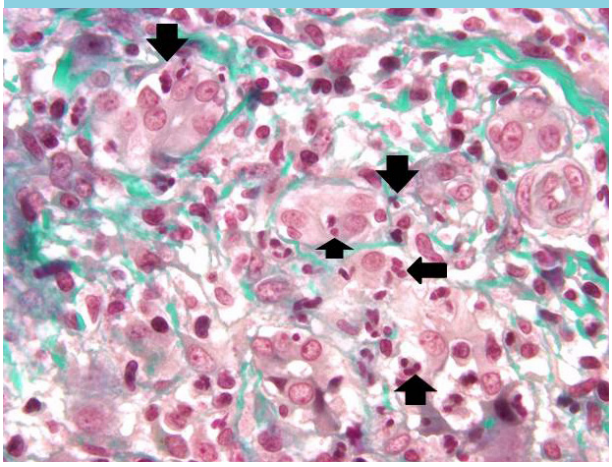


Figure 2. *Liver biopsy revealing neutrophilic infiltration (arrows) of the portal tract (Masson's trichrome staining, x1000)*



ANSWER TO PHOTO QUIZ (PAGE 381)

JAUNDICE AND FEVER IN A PATIENT WITH PSORIASIS

DIAGNOSIS

The liver biopsy revealed neutrophilic infiltration of the portal tract and of the epithelium of the bile ducts (*figures 1 and 2*). The findings on liver biopsy were compatible with neutrophilic cholangitis. Antibiotics were discontinued. He was given pulsed steroids (1 g/day intravenous methylprednisolone for three days), then oral methylprednisolone 20 mg/day. His body temperature returned to normal after the first dose of steroids and the rash evanesced. The pain in his right upper abdomen improved. Methotrexate was added to the therapy and the steroid was tapered. ALAT, ASAT, bilirubin, and alkaline phosphatase returned to normal.

Psoriasis is a common chronic inflammatory disease, affecting approximately 2% of the world's population.[1] Although it primarily involves the skin, systemic involvement including the liver is common. Cholangitis in a psoriatic patient is a diagnostic challenge since these patients are frequently given immunosuppressive or immunomodulatory drugs. Neutrophilic cholangitis is a recently described entity characterised by neutrophilic infiltration of the biliary tree causing cholestasis.[2] It has been described in patients with neutrophilic dermatological infiltrations such as Sweet syndrome, generalised pustular psoriasis and/or psoriatic arthritis.[3] Increasing evidence shows that neutrophilic cholangitis is an extracutaneous manifestation of psoriasis and the frequency of liver involvement in psoriasis is high.[3]

Neutrophilic infiltration, in addition to involvement of the portal tract, is a predominant feature of cutaneous and extracutaneous lesions of pustular psoriasis, especially when the mucous membranes [4] and synovial membranes in patients with polyarthritis are involved.[5]

Recent data suggest that neutrophilic cholangitis has been underestimated and physicians should be aware of this involvement in psoriasis patients.[3] Psoriatic skin lesions and neutrophilic leucocytosis supports a specific involvement of the biliary tract. Neutrophilic cholangitis should be considered in the differential diagnosis in a psoriatic patient with cholangitis.

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PHOTO QUIZ

Back and joint pain according to good old Virchow

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CASE REPORT

A 58-year-old Iraqi male presented to our internal medicine outpatient clinic with long-term complaints of pain in the lower back, accompanied by stiffness and arthralgia. In an attempt to alleviate his symptoms, he underwent a neurosurgical intervention some years earlier, in which a spinal schwannoma was removed. However, this intervention did not relieve his symptoms. Family history unveiled that two of his eight siblings cope with similar issues. The patient had a history of diabetes mellitus, obesity and bladder stones. Physical examination revealed a wheelchair bound man, with pigmentation abnormalities of facial skin, auricles, sclerae and hands (*figure 1*). He had arthralgia of all of his large and small joints, as well as lumbago upon movement, but no signs of acute inflammation or radicular pain. Screening blood tests were normal, with regular levels of vitamin D, calcium, phosphate, normal renal function and no indications of autoimmune antibodies or inflammatory response. X-ray imaging of the lumbar spine, prior to neurosurgical intervention, showed multilevel narrowing of the disc spaces, disc calcifications and anterior osteophytes (*figure 2*).

Figure 1. *Photo of the right hand as a demonstration of bluish-black skin pigmentations*

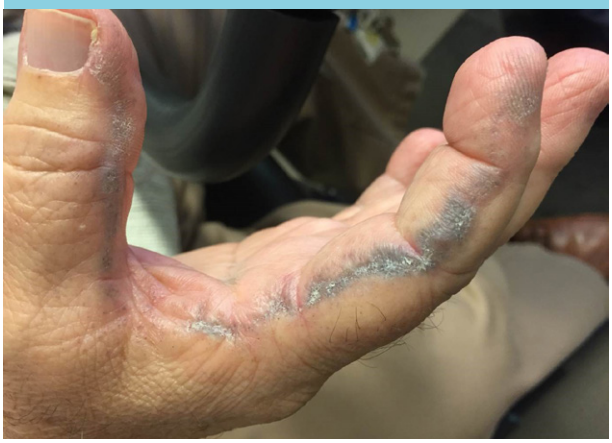
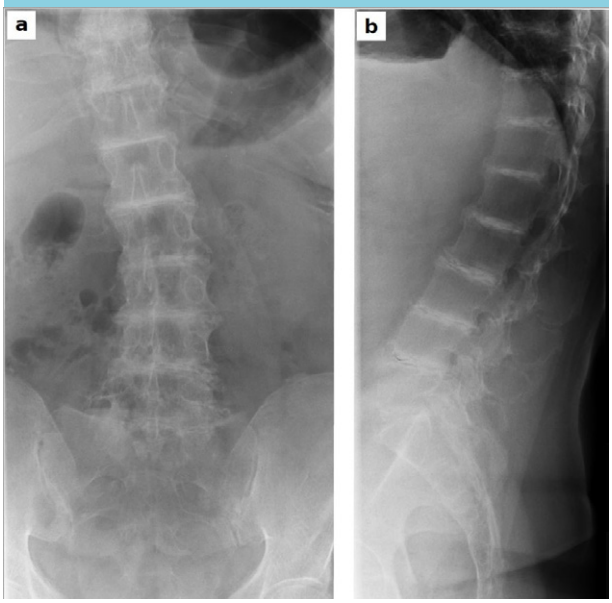


Figure 2. *X-ray image of the lumbar spine, posterior-anterior (A) and lateral (B) view, showing multilevel narrowing of the disc spaces, disc calcifications and anterior osteophytes*



WHAT IS YOUR DIAGNOSIS?

See page 384 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 383)

BACK AND JOINT PAIN ACCORDING TO GOOD OLD VIRCHOW

DIAGNOSIS

The typical skin discoloration and spinal changes, combined with a positive family history, fit with ochronosis caused by alkaptonuria, an inborn error of metabolism. Ochronosis was first described and named by Virchow in 1866.[1]

Alkaptonuria is a rare autosomal recessive disorder with a mutation in the homogentisate 1,2-dioxygenase (HGO) gene. Absence of the HGO enzyme results in accumulation of homogentisic acid (HGA), an intermediate in the tyrosine degradation pathway. Excretion of high HGA levels by the kidneys leads to darkened urine, a characteristic symptom of alkaptonuria that is usually present from birth.[2] This patient did indeed have increased HGA levels, confirming alkaptonuria.

Accumulation of HGA and its metabolites in connective tissues causes pigmentation and eventually deterioration of large joints (ochronosis), followed by involvement of the cardiovascular system, kidneys, skin and glands. Ochronotic changes usually develop at a relatively young age (around the third decade of life), leading to damage to joints. Other complications include aortic valve stenosis, ligament ruptures and urolithiasis.[3] Worldwide, the disease has a prevalence of one in 250,000-1,000,000 births, with more frequent reports in genetically isolated populations.[2] Although alkaptonuria does not reduce life expectancy, it considerably diminishes quality of life. A proposed treatment to slow disease progression is administration of nitisinone, a drug proven effective in reducing the level of HGA.[3] Unfortunately, it causes many side effects, such as corneal irritation, thrombocytopenia, leukopenia and porphyria. Consequently, neither a cure nor an effective therapy is available yet. The treatment of our patient focuses on symptom control; reducing joint complaints with physiotherapy, replacement surgery and painkillers.[2,3] Currently, he is under evaluation for aortic stenosis.

CONCLUSION

Alkaptonuria is a rare metabolic disease, characterised by HGA deposition in connective tissues and cardiac valves. It is important to diagnose this disease early to prevent serious complications and to avoid unnecessary therapeutic interventions.

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PHOTO QUIZ

A granulomatous inflammation in a renal transplant biopsy

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CASE REPORT

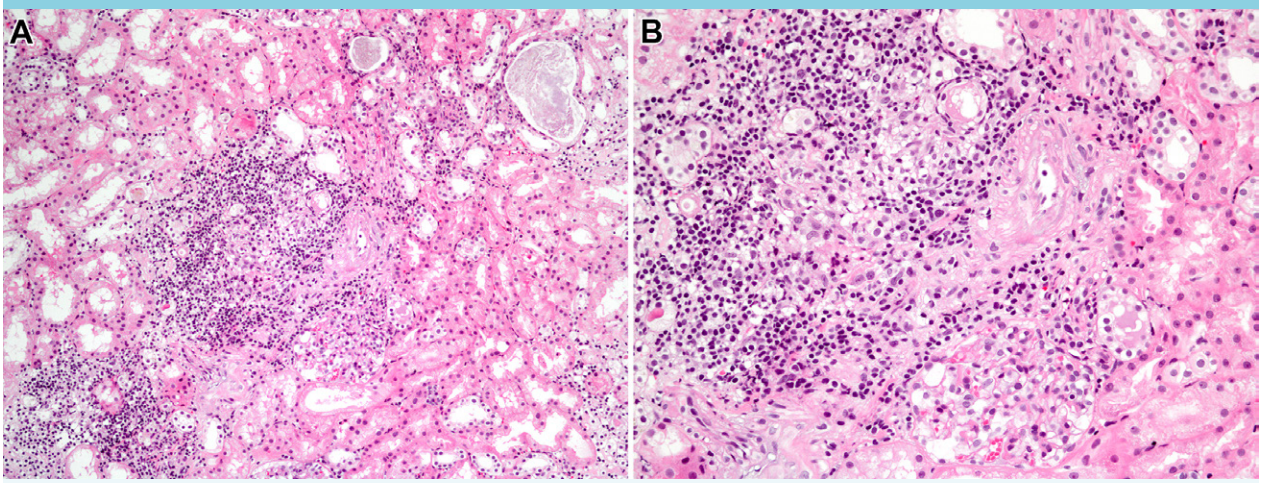
A 79-year-old female of North African descent with end-stage renal disease caused by focal segmental glomerulosclerosis secondary to hypertension started intermittent haemodialysis in 2013. In 2014, she developed erythema nodosum, possibly due to latent tuberculosis infection (she tested positive for Mantoux and Quantiferon). Tuberculostatic treatment (rifampicin/isoniazid) was started but then discontinued because of side effects (increasing malaise and gastrointestinal complaints). As latent tuberculosis infection is a relative contraindication for renal transplantation and the erythema nodosum relapsed, another attempt was made to treat her in 2016. She was successfully treated with isoniazid monotherapy for a duration of nine months.

In April 2017 she received a first, deceased-after-circulatory death donor kidney transplant from a 66-year-old male. The transplantation was complicated by delayed graft function. On postoperative day 6, a kidney transplant biopsy was performed. Light microscopy demonstrated tubulitis, possible arteritis, as well as acute tubular necrosis and mild mesangiolysis. Of note, in the tubulo-interstitial compartment, a patchy infiltrate consisting of lymphocytes and histiocytes was seen, with non-caseating granuloma formation (*figure 1*). Immunohistochemical staining for C4d was negative. The delayed graft function was attributed to the combination of acute tubular necrosis and tacrolimus nephrotoxicity. The tacrolimus dose was reduced.

WHAT IS YOUR DIAGNOSIS?

See page 386 for the answer to this photo quiz.

Figure 1. *Tubulo-interstitial histiocytic infiltrate in a transplant renal biopsy*



Haematoxylin & eosin staining; 10x (left) and 20x (right) magnification.

ANSWER TO PHOTO QUIZ (PAGE 385)

A GRANULOMATOUS INFLAMMATION IN A RENAL TRANSPLANT BIOPSY

DIAGNOSIS

First, the possibility of renal tuberculosis was considered. However, the patient had been treated adequately before transplantation and both the Ziehl-Neelsen and auramine staining for acid-fast bacteria were negative. Other causes of granulomatous tubulo-interstitial nephritis (GTIN) that were considered included acute T-cell mediated rejection, drug-related toxicity, infections, sarcoidosis, tubulointerstitial nephritis and uveitis syndrome, paraproteinaemia and Wegener's granulomatosis. Finally, GTIN can be idiopathic.[1]

To find out if the histiocytic infiltrate in the biopsy was patient- or donor-derived, we performed fluorescent *in situ* hybridisation with probes directed to centromeres of the X- and Y-chromosome (the transplantation was from a male donor to a female recipient). This experiment showed that in the inflammatory infiltrate, both XX and XY cells were present. A double staining with CD68 indicated that the histiocytic cells were indeed donor-derived (*figure 2*). The diagnosis of donor-derived renal sarcoidosis was made.

The contralateral kidney of this same donor was also transplanted in another centre. In the pre-implantation biopsy a patchy infiltrate in the interstitium with giant cells and formation of granulomas without necrosis was present too.

Sarcoidosis is a chronic, idiopathic multisystem, inflammatory disease characterised by the presence of non-caseating granulomas in one or more organs, most often involving the lungs and hilar lymph nodes.[1,2] Renal manifestations of sarcoidosis most commonly include hypercalciuria, nephrocalcinosis and nephrolithiasis as a result of a derangement in calcium homeostasis.[1-3] Sarcoidosis may also directly involve the kidney in the form of GTIN. GTIN is unusual with a reported incidence ranging between 0.7 and 30%.[3]

Only ten cases of recurrent renal sarcoidosis in the form of GTIN in a renal allograft have been described previously.[3] To the best of our knowledge, donor-derived, sarcoid granulomas in a renal allograft have never been described before.

Following the biopsy, her renal function recovered without additional treatment and she could be discharged from hospital in a good clinical condition two weeks after transplantation.

In conclusion, if GTIN is present in a recently transplanted kidney, clinicians should consider the possibility of donor-derived sarcoid granulomas and should not immediately attribute this finding to an acute T-cell mediated rejection.

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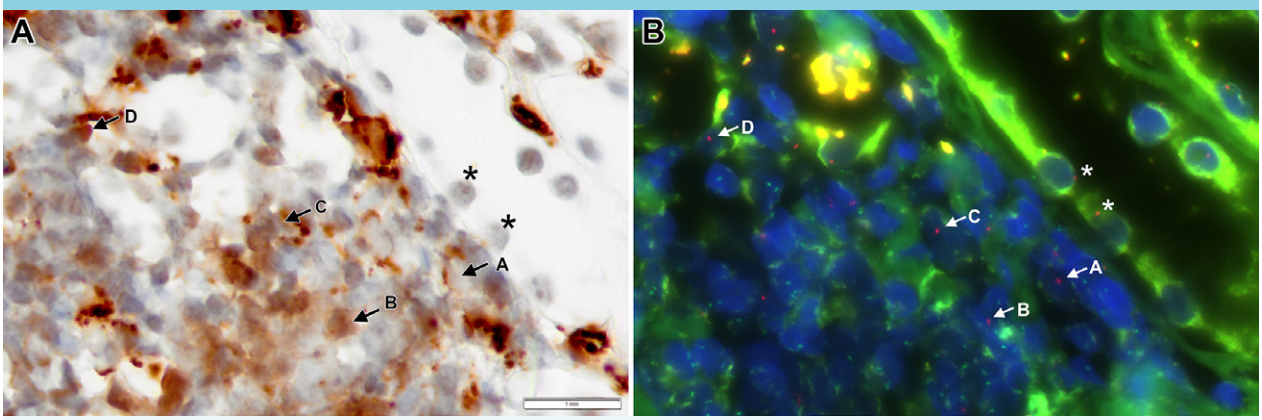
Figure 2. *Donor-derived histiocytes in the tubulointerstitial inflammatory infiltrate*



A (left): CD68 immunostaining. B (right): X- and Y-chromatin fluorescent in situ hybridisation (FISH): X = green signal and Y = red signal. Arrows A-D point to histiocytes with cytoplasmic staining for CD68 (left) and with presence of a Y-chromosome (red signal) in the corresponding cells (right), confirming that they are donor-derived. ** = donor-derived tubular epithelial cells, also containing a Y-chromosome.

LETTER

Hyponatraemia related to hypopituitarism

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I read with interest the two cases of hyponatraemia secondary to hypopituitarism by Van Tienhoven et al.[1] The authors remind us that an endocrine cause of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) must always be excluded. Glucocorticoid deficiency in hypopituitarism leads to inappropriately elevated antidiuretic hormone levels and mimics SIADH. The authors did not mention the bicarbonate level of their two patients, the addition of which would have been relevant because these concentrations can help to identify the true cause of hyponatraemia. We have shown that a low bicarbonate level is frequently seen in hyponatraemia related to adrenocorticotropin deficiency (TCO_2 20.5 ± 3 mmol/l and HCO_3- 20 ± 2 mmol/l)[2] while bicarbonate is normal in non-endocrine SIADH (TCO_2 25.5 ± 2.4 mmol/l and HCO_3- 25 ± 1.7 mmol/l). In subjects with a non-endocrine cause of acute hyponatraemia, a normal bicarbonate and blood acid-base equilibrium is observed, whereas during chronic hyponatraemia (> 24 h) bicarbonate is still normal but the blood acid-base equilibrium shows a mixed respiratory and metabolic alkalosis.[2,3] In hyponatraemia related to SIADH mean aldosterone levels are usually normal despite mild volume expansion. This relative hyperaldosteronism has been well documented in animals[4] and humans.[5] However, the relative hyperaldosteronism which is typically seen in SIADH and causes the aforementioned metabolic alkalosis is only present when there is adequate availability of corticosteroids.[6] In adrenocorticotropin deficiency with hyponatraemia, the relative hypoaldosteronism explains why a metabolic alkalosis does not develop and only respiratory alkalosis is observed, which explains their lower serum bicarbonate levels. Similarly, it has been shown that plasma renin activity and aldosterone are normal in nonhyponatraemic hypopituitarism patients (reflecting euvolaemia) but that cortisol plays a permissive role in the glomerulosa response to a potassium load. Under potassium chloride stimulus the aldosterone response in hypopituitarism patients was only observed when cortisol was given.[7]

This observation (a low TCO_2 level < 22 mmol/l) could be helpful as a diagnostic tool for patients with adrenocorticotropin deficiency presenting with hyponatraemia.[2]

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