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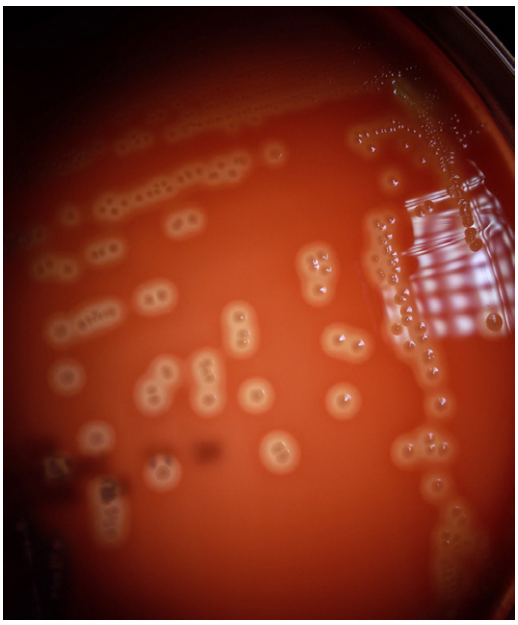

A particular monocyte with cytoplasmic inclusions: what is your diagnosis?

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EDITORIAL

Vitamin B12

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In this issue of the *Netherlands Journal of Medicine*, the authors of the paper 'Association of vitamin B12, methylmalonic acid and functional parameters'[1] recommend increased awareness of the complicated recognition of what they call symptomatic vitamin B12 deficiency. Their study is based on a large NHANES dataset that is a cross-sectional sample of the general US population aged 19 years and older from 2012-2014. The dataset contains much biochemical data, including measurements of serum vitamin B12 and methylmalonic acid levels, in addition to functional parameters of physical and mental state, well-being, and use of medical care, as collected by questionnaires. These functional parameters are the focal point in the current discussion on their eventual relation to vitamin B12 deficiency (vitB12).

The results of this study confirm earlier observations that macrocytic anaemia occurs only in a minority (4.4%) of individuals with subnormal vitB12 levels. The study does not state whether cases with the typical neurological symptoms of ataxia, paraesthesias, and impaired sense of touch had been found. The results confirm the well-known inverse relation between serum concentrations of vitB12 and methylmalonic acid (MMA); MMA is the substrate of the vitB12-dependent enzyme MMCoA-mutase.[2] In addition, the authors show that only 50-60% of the participants with a low serum vitB12 have an increased MMA and that a normal vitB12 does not exclude an increased MMA. Remarkably, the observed mental and physical parameters are not, or only weakly, associated with vitB12, but are significantly associated with MMA. The authors conclude that MMA has proven to be a more reliable predictor of poor functional performance and may assist with diagnosing vitB12 deficiency in uncertain cases where serum vitB12 concentrations are above the lower reference value, thereby assuming that the observed functional performance parameters are related to vitB12 status.

How convincing are these conclusions? In the first place, the list of investigated health and functional parameters includes more aspects than the parameters that are included in the above-mentioned discussion. In fact, they are more closely covered by only the domains 'mental health & depression', 'physical functioning', and 'cognitive functioning'. Even in those three domains, the associations with vitB12 are very weak and are significantly stronger with MMA. The median scores and ranges are however low. These observations, therefore, only weakly support the concept that these three domains indeed belong to the spectrum of symptoms of vitB12 deficiency and should be diagnosed and treated as such. What might then be an explanation for the relation to MMA?

There is no doubt that vitB12 deficiency results in congestion of MMA if MMA is not converted to succinate, and it has been shown that this leads to increased MMA concentrations in the blood. But analysing the absolute numbers rather than the percentages of increased MMA concentrations in the vitB12 categories low, intermediate, and high, one can calculate that, in addition to the 89 cases in the low vitB12 range, another 647 cases have increased MMA in the intermediate (372) and high (275) vitB12 ranges. This is approximately four times the total number of cases in the low range. The reasons for this apparent imbalance between alleged cause (vitB12 deficiency) and consequence (MMA rise) are still unknown.

One possible explanation is that serum vitB12 is not always representative for intracellular vitB12 and that MMA more reliably reflects this intracellular vitB12 status. Either the vitamin deficiency is developing but not yet in a stage of depletion at tissue level (in the category low vitB12), or the tissues are depleted but unusual amounts of vitB12 are bound to other vitB12-binding proteins, such as haptocorrin, that cannot reach the peripheral tissues. This has been reported in typical forms of cancer and chronic inflammation.[3,4] It is however, unlikely that this occurs as frequently as seen in this cross-sectional sample. The variability of serum MMA appears to be influenced by serum vitB12 in fewer than 17% of healthy volunteers,[5] but MMA concentration increases strongly with decreasing glomerular filtration rate (GFR).[6] The percentage of participants in the study with a normal vitB12 and an increased MMA is reduced from 14% to 5% when participants with an estimated GFR < 60 ml/min are

excluded. Of course, in addition to MMA, impaired renal function effects many other plasma components such as homocysteine, which is increased in vitB12-deficiency as well. Therefore, the more relevant question is whether disturbed mental or physical functioning is more closely associated with renal function than with MMA, but unfortunately the data in this paper was not analysed in a manner that can answer this question.

It therefore remains unsure whether there is indeed a functional relation between vitB12 and the functional parameters. An association is not proof of a causal relationship and both vitB12 and MMA can represent other parallel acting parameters. From this perspective, hyperhomocysteinemia has been shown to have an effect on neurotransmitter concentrations in the cerebral spinal fluid and may therefore influence brain functions.[7] Vitamin deficiencies are individually linked to a variety of clinical symptoms but these lists of symptoms overlap considerably. Most B vitamins are active as co-factors in important enzymatic reactions that occur in most tissue cells all over the body. Shortages in vitamins, either single or combinations, may lead to different expression levels in various organs. Nutrient intake might be another modifying factor. Although the body is capable of neutralising large differences in the diet, the differences may have more influence at extreme deviations. Thus, it is conceivable that a methionine-poor diet causes a higher sensitivity to a shortage of vitB12, because of the role of methylcobalamin in the re-methylation of homocysteine to methionine.

Despite the results of the above study, the many outstanding questions prevent complete confidence in the mentioned functional parameters and their relevance to vitB12 status and MMA, and the conclusion that they are an effective diagnostic substitute for vitB12. Further epidemiological studies, with even larger patient numbers and new parameters will not solve the question of whether a specific blood component can bear the role of reliable biomarker as long as it remains uncertain which symptoms are proven to be linked to vitB12 status, and are effectively treated with vitB12 suppletion. The only way to identify this is by taking the initiative to conduct a blinded intervention study with properly defined patients and controls and by applying standard treatment modalities that have established their effectiveness in proven vitB12-deficiency conditions such as pernicious anaemia. Strict standardisation of the scoring of investigated clinical symptoms before, during, and after the intervention period is an essential condition.

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ORIGINAL ARTICLE

Septic patients with cancer: Do prehospital antibiotics improve survival?

A sub-analysis of the PHANTASi trial

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ABSTRACT

Background: Sepsis in patients with cancer is increasingly common and associated with high mortality. To date, no studies have examined the effectiveness of prehospital antibiotics in septic patients with cancer. This study aimed to compare survival of septic patients with cancer to those without and to evaluate the effect of prehospital antibiotics in septic patients with cancer.

Methods: We conducted a post-hoc sub-analysis of the PHANTASi (PreHospital ANTibioitcs Against Sepsis) trial database: a randomised controlled trial which enrolled patients with suspected sepsis who were transported to the emergency department by ambulance. Patients in the intervention group were administered prehospital intravenous antibiotics while those in the control group received usual care. We compared patients who had cancer to those who did not. Primary outcome was 28-day mortality; among the secondary outcomes, we included in-hospital mortality and 90-day mortality.

Results: 357(13.4%) of the 2658 included patients had cancer in the past five years, of which, 209 (58.5%) were included in the intervention and 148 (41.5%) usual care groups; 28-day mortality was significantly higher in patients who were diagnosed with cancer in the past five years than those without cancer in the past five years: 15.2% vs. 7.1%, respectively ($p < 0.001$).

Prehospital antibiotics in the group of patients with cancer in the last five years yielded no significant effect on survival. There were however, significantly fewer 30-day readmissions ($p = 0.031$) in the intervention group of cancer patients (12.2% vs 5.7%).

Conclusion: Prehospital antibiotics did not improve overall survival. However, there was a significant reduction in 30-day readmissions.

KEYWORDS

Cancer, mortality, prehospital antibiotics, readmission, sepsis

INTRODUCTION

Sepsis is a syndrome which often leads to high morbidity and mortality.[1-3] Although absolute mortality has decreased in recent years, incidence is still rising. Several factors are associated with increased mortality in septic patients, including age, gender, presence of organ dysfunction, and active cancer.

Retrospective studies in the last decade have found that early treatment with antibiotics is associated with better outcomes in sepsis patients,[4,5] although a recent prospective study found no benefit of prehospital antibiotics on overall survival (PHANTASi). The Surviving Sepsis Campaign currently recommends antibiotic treatment within one hour after arrival at the hospital.[6] However, whether early antibiotics administration lead to better outcomes in all sepsis patients is a matter of debate.[7,8]

Several studies have investigated the epidemiology of sepsis in patients with cancer.[9-12] The diagnosis of infection in this cohort is difficult as its early signs and symptoms are mimicked by non-infective causes, including the cancer

itself and responses to systemic anti-cancer treatment (SACT).[13,14] Retrospective studies in this sub-group of patients have shown that this population may benefit from early treatment with antibiotics.[15-17] Multiple studies have been conducted in septic cancer patients with neutropenia and shown that delay in administration of the first dose of antibiotics, as well as pneumonia and thrombocytopenia, were risk factors for severe complications.[12]

The aim of this study was to evaluate whether septic patients with cancer have a different survival rate compared to non-cancer patients, who have reached the emergency department (ED) by ambulance. In addition, we investigated the effect of early antibiotics administration in these two sub-groups on patient outcomes.

MATERIALS AND METHODS

Design and setting

A sub-analysis was conducted using the PHANTASi (PreHospital ANTibioitcs Against Sepsis) trial database.[7,18,] In this randomised controlled, open-labelled trial, we investigated whether improved recognition of sepsis and administration of antibiotics in the ambulance led to increased survival when compared to usual care (fluid resuscitation and supplementary oxygen). Patients under usual care received their first dose of antibiotics at the ED. Between June 2014 and June 2016, eligible patients who were transported to one of the 34 participating hospitals in the Netherlands were enrolled.

Sepsis was defined as: a diagnosed or suspected infection, a temperature of > 38°C or < 36°C, and a minimum of one other Severe Inflammatory Response Syndrome (SIRS) criterion (heart rate > 90 beats per minute or a respiratory rate > 20 per minute). Due to the lack of prehospital leucocyte test, this was not used as an inclusion criterion.

Sepsis severity was categorised into three groups according to the 2001 SSCM/ ESCIM/ ACCP/ ATS/ SIS International Sepsis definitions Conference guidelines:[19] uncomplicated (non-severe) sepsis, severe sepsis, and septic shock.

Data collection methods have been described elsewhere.[7] In short, data were collected by emergency medical services (EMS) personnel and the PHANTASi trial investigators. Variables collected included patient demographics, comorbidities, sepsis severity, mortality, and length of stay, among others. Infection site and microbiological data was also retrieved. The case record form has been published elsewhere.[7]

Methodology

A total of 2,658 patients were included in the PHANTASi trial. In this post-hoc review, we compared patients who had any type of cancer in the past five years to those who were cancer free. Patients who had benign neoplasms in the past five years were categorised into the latter group.

Cancers in our study were categorised into 18 categories: (1) bladder cancer, (2) breast cancer, (3) renal cancer, (4) prostate cancer, (5) leukaemia, (6) colorectal cancer, (7) hepatobiliary cancer, (8) melanoma, (9) lymphoma, (10) upper gastrointestinal cancer, (11) lung cancer, (12) pancreatic cancer, (13) head and neck cancer, including thyroid cancer, (14) myeloma, (15) gynaecological cancer, (16) sarcoma, (17) primary central nervous system (CNS) cancer, and (18) cancer of unknown primary origin.

An overview of cancer types that were included in each category can be found in the appendix (supplementary table 1)*.

Statistical analysis

The primary outcome of this study was 28-day mortality. Secondary outcomes included in-hospital mortality, 90-day mortality, focus of infection, time to antibiotics before arrival at the ED, time to antibiotics after arrival at the ED, 30-day readmission, intensive care unit (ICU) admission, length of hospital stay (LOS), temperature in the ambulance and at the ED, systolic blood pressure in the ambulance and at the ED, thrombocyte count at the ED, positive blood cultures, and ceftriaxone resistance. These clinical parameters were chosen as they have been described in the literature as being associated with mortality in septic patients with and without cancer.[1,12,15,20-28]

Descriptive statistics were used to describe patient characteristics, presented as frequency (proportion), mean ± standard deviation (SD), or as median (interquartile range (IQR)). Comparisons between the groups of patients with and without cancer were performed using the Pearson Chi-square. Confounders were identified and corrected for through logistic regression analyses. Power calculation showed that we were able to detect at least a 3.9% difference in 28-day mortality by using our sample size of 2,658 patients with a power of 80% (two-sided testing). All analyses were performed in IBM SPSS Statistics 22.0 (Chicago, USA), with $p < 0.05$ considered significant.

Ethics

The study protocol of the PHANTASi trial was approved by the medical ethical committee of the Amsterdam University Medical Centre, Location VU University Medical Centre, the coordinating centre, and all ethical committees of each participating hospital. Due to the complexity of the PHANTASi trial, the ethics committees granted approval to obtain deferred consent when necessary. Informed consent before study enrolment or deferred consent was obtained from all patients or their legal representatives or surrogates. All efforts were made by EMS personnel to obtain informed consent before study inclusion.

Table 1. *Type of cancer and mortality*

	n	In-hospital mortality (%)	28-day mortality (%)	90-day mortality (%)
Type of Cancer				
Bladder cancer	34	3 (8.2%)	3 (8.2%)	8 (23.5%)
Breast cancer	22	3 (13.6%)	4 (18.2%)	4 (18.2%)
Renal cancer	5	0 (0%)	0 (0%)	1 (20%)
Prostate cancer	44	5 (11.4%)	7 (15.9%)	8 (18.2%)
Leukaemia	12	2 (16.7%)	5 (41.7%)	5 (41.7%)
Colorectal cancer	43	1 (23.2%)	2 (4.7%)	4 (9.3%)
Hepatobiliary cancer	5	0 (0%)	1 (20%)	2 (40%)
Melanoma	5	0 (0%)	0 (0%)	0 (0%)
Lymphoma	18	2 (11.1%)	3 (16.7%)	4 (22.2%)
Upper gastrointestinal cancer	14	3 (21.4%)	5 (35.7%)	8 (57.1%)
Lung cancer	48	3 (6.3%)	9 (18.8%)	14 (29.1%)
Pancreatic cancer	10	1 (10%)	1 (10%)	2 (20%)
Head and neck cancer (including thyroid)	7	1 (14.2%)	1 (14.2%)	1 (14.2%)
Myeloma	10	2 (20%)	3 (30%)	3 (30%)
Gynaecological cancer	5	0 (0%)	0 (0%)	1 (20%)
Sarcoma	0	0 (0%)	0 (0%)	0 (0%)
Primary CNS cancer	0	0 (0%)	0 (0%)	0 (0%)
Cancer of unknown primary origin	75	7 (9.3%)	10 (13.3%)	17 (22.7%)

CNS = central nervous system; n = number of patients

Table 2. *Sepsis severity and mortality all patients*

	Cancer	No cancer	p-value
In-hospital mortality, n (%)*			
Sepsis	2 (1.5)	7 (0.8)	0.911
Severe sepsis	22 (11.1)	96 (7.3)	0.266
Septic shock	7 (38.9)	21 (25.9)	0.524
Other diagnosis	2 (25.0)	2 (4.8)	0.144
28-day mortality, n (%)*			
Sepsis	7 (5.3)	13 (1.5)	0.278
Severe sepsis	36 (18.2)	125 (9.5)	0.125
Septic shock	9 (50.0)	19 (23.5)	0.349
Other diagnosis	2 (28.6)	5 (12.8)	0.824
90-day mortality, n (%)*			
Sepsis	18 (13.5)	30 (3.5)	0.047
Severe sepsis	51 (25.8)	173 (13.2)	0.044
Septic shock	9 (50.0)	25 (30.9)	0.941
Other diagnosis	4 (50.0)	6 (14.0)	0.303

*Percentage mortality per sepsis category
n = number of patients

RESULTS

Among all included subjects in PHANTASi trial, 357 (13.4%) were diagnosed with cancer within five years prior to their inclusion in the study, while the remaining 2,301 (86.6%) patients did not. The most common types of cancer were colorectal, prostate, and lung cancer. Table 1 provides an overview of the mortality rates per cancer type. In the group of patients with cancer, 148 patients (41.5%) were in the usual care group and 209 patients (58.5%) were in the intervention group with a mean age of 74.8 ± 9.4 years and 74.7 ± 9.7 years, respectively. See table 2 and 3 for more details on sepsis severity and mortality.

Mortality

Difference between patients with and without cancer in the total population

Overall, in patients with and without cancer there was a significantly higher age-adjusted in-hospital mortality (9.2% vs. 5.5%, respectively; p = 0.008), 28-day mortality (15.2% vs. 7.1%, respectively; p < 0.001) and 90-day mortality (23.0% vs. 10.2%, respectively; p < 0.001) (table 4).

Usual care group

Among patients in the usual care group, the age-adjusted in-hospital mortality between patients with and without cancer did not differ significantly (6.8% vs. 5.8%, respectively; p = 0.715). However, those with cancer had a significantly higher age-adjusted 28-day mortality (15.5% vs. 7.5%, respectively; p = 0.002) and 90-day mortality (26.4% vs. 10.1%, respectively; p < 0.001) than those without cancer (table 4).

Intervention group

Among subjects in the intervention group, patients with cancer had a significantly higher age-adjusted mortality than patients without cancer in all outcomes: in-hospital mortality (11.0% vs 5.2%, respectively; p = 0.002), 28-mortality (14.9% vs 6.8%, respectively; p < 0.001) and 90-day mortality (20.6% vs 10.3%, respectively; p < 0.001) (table 4).

Patients with cancer < 5 years

In the group of patients with cancer, there were no significant differences between the usual care compared to the intervention group for age-adjusted in-hospital (6.8% vs 11.0%, respectively; p = 0.173), 28-day (15.5% vs 14.9%, respectively; p = 0.203) and 90-day (26.4% vs 20.6%, respectively; p = 0.870) mortality (table 5). See supplementary figures 1-3 and supplementary tables 21-24 for more details on mortality.

Table 3. *Sepsis severity and mortality in patients with cancer < 5 years*

	Intervention	Usual care	p-value
In-hospital mortality, n (%)*			
Sepsis	2 (2.6)	0	0.336
Severe sepsis	14 (11.8)	8 (10.1)	0.284
Septic shock	5 (50)	2 (25.0)	0.911
Other diagnosis	2 (50)	0	0.336
28-day mortality, n (%)*			
Sepsis	5 (6.6)	2 (3.5)	0.421
Severe sepsis	19 (16.0)	17 (21.5)	0.331
Septic shock	6 (60.0)	3 (37.5)	0.538
Other diagnosis	1 (33.3)	1 (25.0)	0.829
90-day mortality, n (%)*			
Sepsis	9 (11.8)	9 (15.8)	0.815
Severe sepsis	27 (22.7)	24 (30.4)	0.907
Septic shock	6 (60.0)	3 (37.5)	0.365
Other diagnosis	1 (25.0)	3 (75.0)	0.260

*Percentage mortality per sepsis category
n = number of patients

Table 4. *Mortality between patients with and without cancer < 5 years*

	Cancer < 5 years (%)	No cancer (%)	p-value	p-value, age-adjusted
All patients				
In-hospital mortality	33 (9.2)	126 (5.5)	0.005	0.008
28-day mortality	54 (15.2)	162 (7.1)	< 0.001	< 0.001
90-day mortality	82 (23.0)	234 (10.2)	< 0.001	< 0.001
Intervention group				
In-hospital mortality	23 (11.0)	69 (5.2)	0.001	0.002
28-day mortality	31 (14.9)	89 (6.8)	< 0.001	< 0.001
90-day mortality	43 (20.6)	135 (10.3)	< 0.001	< 0.001
Usual care group				
In-hospital mortality	10 (6.8)	57 (5.8)	0.643	0.715
28-day mortality	23 (15.5)	73 (7.5)	0.001	0.002
90-day mortality	39 (26.4)	99 (10.1)	< 0.001	< 0.001

Table 5. *Mortality patients with cancer < 5 years*

	Intervention (%)	Usual care (%)	p-value	p-value, age-adjusted
In-hospital mortality	23 (11.0)	10 (6.8)	0.172	0.173
28-day mortality	31 (14.9)	23 (15.5)	0.869	0.870
90-day mortality	43 (20.6)	39 (26.4)	0.201	0.203

Secondary outcomes

Difference between patients with and without cancer

No significant difference was observed for LOS between subjects with and without cancer (8.1 vs. 8.0 days, p = 0.867). An overview of vital parameters and laboratory findings of both groups can be found in the supplementary tables 2 and supplementary tables 11-21.

Usual care group

In the usual care group, no significant difference was observed between patients with and without cancer in terms of the frequency of ICU admissions (p = 0.589) and 30-day readmissions (p = 0.076). The average LOS in patients with vs. without cancer were 9 days (IQR 3.25-9.0) and 7.8 days (IQR 3.0-9.0), respectively (p = 0.922).

Intervention group

In the intervention group, there was no significant difference in frequency of ICU admissions or 30-day readmissions between patients with and without cancer (p = 0.155 vs. p = 0.290, respectively). Mean LOS in patients with vs. without cancer were 8.3 days (IQR 3.25-9.0) and 8.2 days (IQR 3.0-9.0), respectively (p = 0.898).

Patients with cancer < 5 years: usual care group vs. intervention group

Patients with cancer had a median time to antibiotics of 26 minutes (IQR 19-34) prior to arrival at the ED in the intervention group; in the usual care group, patients had a median time to antibiotics of 76 minutes (IQR 36-134.5) after arriving at the ED, leading to a mean time advantage of 102 minutes for the intervention group.

In patients with cancer, there was no significant difference in ICU admissions (p = 0.947) between the intervention group and the usual care group. The average LOS in the intervention group and usual care group was 8.3 ± 8.1 days and 7.9 ± 10.7 days, respectively (p = 0.714). However, 30-day readmission was significantly lower in the intervention group (p < 0.031).

Chemotherapy one month prior to ED was not significantly correlated with increased in-hospital, 28-day, or 90-day

mortality (supplementary tables 3 and 4). Information on micriobiological data can be found in supplementary tables 5-10.

DISCUSSION

To the best of our knowledge, this is the first study to compare the effect of prehospital antibiotics in septic patients with and without cancer. In the complete patient population included in the PHANTASi trial, when comparing patients with and without cancer, we found a significantly higher in-hospital (9.2% vs. 5.5%, respectively), 28-day (15.2% vs. 7.1%, respectively) and 90-day (23.0% vs. 10.2%, respectively) mortality rates. Despite receiving their first dose of intravenous antibiotics 102 minutes earlier, septic patients with cancer in the intervention group did not have significantly different mortality rates to those receiving usual care. However, septic patients with cancer in the intervention group did have a significantly lower readmission rate.

Previous studies compared clinical outcomes of patients with and without sepsis. However, these studies only included non-ED patients or patients without prehospital intravenous antibiotics.[20] Nurse-led protocols for sepsis and cancer patients have been shown to be an effective, safe, and sustainable method for early antibiotic administration but have not demonstrated a significant decrease in mortality.[29] Similarly, in the PHANTASi trial, EMS personnel were trained in the recognition of sepsis, which lead to an improvement in the recognition of sepsis as well as time to antibiotics.

Following SACT, cancer patients may produce vasoactive pro-inflammatory cytokines such as interleukin (IL)-2, IL-6, and tumor necrosis factor. This, together with the ability of the malignancy itself to mimic an infective driver of SIRS, can give a false impression that a cancer patient has sepsis.[30] This alternative pathway is responsible for a proportion of sepsis presentations in patients with cancer and may partially explain the ineffective prehospital antibiotics on clinical outcomes in these patients.

Our study has a number of strengths. First, this study had a large sample size of septic patients of whom 357 had cancer. Previous studies in similar populations had significantly smaller sample sizes.[20,31] Second, this is the first study to compare the effect of prehospital intravenous antibiotics by EMS personnel in septic patients with and without cancer. Other studies have investigated either septic patients[1-3,32] or cancer patients with febrile neutropenia.[11,12] Third, a retrospective chart analysis was conducted on PHANTASi trial data by two acute physicians and an infectious diseases specialist, which allowed us to efficiently include patients with sepsis. In addition, in the PHANTASi trial, sepsis severity was classified by reviewing available information such as admission letters, vital parameters, lab results, and discharge papers available in electronic patient charts.[7] These thorough reviews ensured high-quality samples and data. Fourth, this study investigated several factors associated with mortality in septic patients with and without cancer, described in literature, including time to antibiotics, systolic blood pressure, temperature, thrombocyte count, haemoglobin levels, C-reactive protein levels, leucocyte count, neutrophil count, gram-negative and gram-positive bacteraemia, type of cancer, and chemotherapy prior to ED admission.[1,12,15,20-28]

Septic patients have an increased risk of readmission and unplanned admissions after discharge. This risk is higher in septic patients with cancer.[23] Interestingly, we found a lower 30-day readmission in cancer patients who received prehospital antibiotics compared to those who did not. This same trend was found in the PHANTASi trial.[7] A possible explanation has been suggested for this effect that early antibiotics administration prevents the development of organ failure during the initial hospital admission which, in turn prevents readmission.[33]

Despite the smaller sample size of studies mentioned and minor differences in categorising cancer types, the main groups of cancer are similar in our study compared to others.[15,20]

Despite these strengths, our study also has a few limitations. First, our study was a sub-analysis of the PHANTASi trial, which inevitably leads to limitations. Although we did not apply stratified randomisation on the basis of cancer, we had a relatively large sample size compared to other studies on septic patients with cancer. Second, we had relatively limited amount of data on the cause of death as we did not have long-term follow-up. We could only retrieve data on in-hospital mortality and documented mortality by family members. Third, we included all patients with cancer in the past five years rather than only those with a currently an active form of malignancy due to absence of data. Despite these limitations, we did find a significantly higher mortality rate in septic patients with cancer (five years prior to the ED admission) compared to those who are cancer free.

Early antibiotic administration remains a paradigm of care for cancer patients presenting with sepsis and many studies have demonstrated the challenges of achieving this through routine care. This study shows that paramedic administration of intravenous antibiotics is a safe and effective strategy for decreasing the time to intravenous antibiotics in septic patients. However, in this unselected group of cancer patients with sepsis, this did not improve mortality and further studies are required to determine markers of high-risk patients who will benefit from this intervention.

CONCLUSION

Septic patients with cancer had higher in-hospital, 28-day, and 90-day mortalities compared to those without. Prehospital antibiotics did not lead to a decrease in mortality in patients with cancer, but did reduce 30-day readmission rate. Future studies should focus on optimisation of the treatment of septic patients with active malignancy.

* For supplementary tables and figures, see njmonline.nl

DISCLOSURES

All authors declare no conflict of interest.

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ORIGINAL ARTICLE

Association of vitamin B12, methylmalonic acid, and functional parameters

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ABSTRACT

Introduction: Diagnosis of vitamin B12 deficiency is difficult, as there is no conclusive single test for this disorder. We evaluated the association of serum B12 and methylmalonic acid (MMA) with haematologic parameters and physical and cognitive functioning in an effort to use such clinical parameters to improve the interpretation of serum values.

Methods: We used data of participants > 19 years of age from NHANES 2011-2012 and 2013-2014, a cross-sectional survey in the United States. Functional status was assessed with questionnaires on current health condition, disability, hospital utilisation, cognitive functioning, mental health and depression, and physical functioning. Muscle strength assessed with a handgrip dynamometer was used as a performance parameter. Results were evaluated both for the entire population and participants of Western European descent. Because renal function influences MMA concentrations and is a proxy for both frailty and comorbidity, all results were additionally stratified for individuals with normal vs impaired renal function (eGFR < 60 ml/min).

Results: In total, data of 9645 participants (mean age 49 (SD 17) years, 49.3% males) were included. Out of all participants with serum B12 < 140, 140-300, and 301-1000 pmol/l, 56.2%, 13.5%, and 4.1%, respectively had elevated MMA. MMA concentrations were more strongly associated with poor functional status and physical performance than serum B12. We identified a significant and independent association of MMA concentrations, as well as haemoglobin and co-morbidity with muscle strength.

Conclusions/interpretations: A large proportion of individuals with a decreased serum B12 concentration still has normal MMA concentrations. Elevated MMA concentrations were more strongly associated with poor functional performance than serum B12.

KEYWORDS

Epidemiology, methylmalonic acid, muscle strength, NHANES, vitamin B12

INTRODUCTION

The recognition of symptomatic cobalamin (vitamin B12) deficiency poses several challenges. First, the spectrum of complaints may be diverse, and symptoms such as paraesthesia in the hands and feet, muscle cramps, dizziness, cognitive disturbances, ataxia, fatigue, and depression may vary considerably between patients.[1-6] Second, the prevalence of anaemia in vitamin B12 deficiency is lower than anticipated[3,7], and neurological signs often occur in the absence of anaemia.[8] Third, serum B12 as a diagnostic test for tissue B12 deficiency may fail, as many people with such symptoms may have serum B12 concentrations above the lower population reference limit, which may cause individuals with relevant deficiency to be missed.[9,10] In some people, this may be caused by the recent use of oral supplementation with multivitamins, high-dose oral vitamin B12 preparations or even B12-fortified energy drinks.[11,12] Fourth, there is a poor correlation between serum B12 concentrations and complaints related to deficiency,[3,5,13] and some people with serum B12 concentrations below the lower reference limit may not have symptoms or may have normal active B12 concentrations.[14] Better information on the association between serum B12 concentrations and clinical symptoms is therefore warranted.

Yet, in daily practice, many general practitioners consider serum B12 concentrations within the reference interval for the population (i.e., 140-700 pmol/l) as proof of sufficiency, and possible complaints in this situation are determined to not be related to deficiency. Several authors have shown that many people with vitamin B12 deficiency

would be overlooked by incorrectly using only total serum B12 concentrations as status marker.[9,15,16] They therefore advocate measurement of one or more biomarkers, including methylmalonic acid (MMA), homocysteine (HCys), and/or holotranscobalamin in people with serum B12 concentrations in the grey zone of 140 to 300 pmol/l, in order to establish a possible diagnosis of deficiency.[9,17-19] Vitamin B12 is a pivotal cofactor in various enzymatic systems, and its deficiency will influence enzymes such as methylmalonyl-CoA mutase (MCM) and methionine synthase. As a consequence, vitamin B12 deficiency may result in high concentrations of MMA and HCys.[20] The sensitivity and specificity of elevated MMA concentrations as an indicator of symptomatic B12 deficiency are unknown. Earlier studies have even suggested that MMA concentrations are a poor predictor of symptom score or neurological complaints.[21] In addition, MMA concentrations may be elevated in people with severely impaired renal function.[22,23] Chronic kidney disease and impaired renal function are associated with more comorbidity and a higher risk of frailty, and there is evidence that chronic kidney disease is linked with impairments of physical and cognitive function and quality of life.[24,25] Similarly, elevated HCys concentrations may suggest symptomatic B12 deficiency, but HCys is also elevated in cases of folate deficiency or impaired renal function. Thus, although elevated MMA and HCys concentrations may be indicative of vitamin B12 deficiency, normal concentrations of these biomarkers do not rule out deficiency[26] or a favourable response to cobalamin therapy.[9] There are few large-scale studies that have reported on the association between serum B12, MMA, and functional status in the general population. A study in people > 70 years in Sweden showed that half of them had abnormal MMA or homocysteine concentrations, suggesting a latent or overt tissue deficiency of cobalamin or folate.[11] The 2001-2002 and 2003-2004 National Health and Nutrition Examination Survey (NHANES), a long-term epidemiologic survey in the USA,[27] showed that there is a large intermediate group of people whose B12 status is difficult to interpret.[20] In NHANES participants > 60 years, vitamin B12 deficiency was associated with an almost 10-fold increased risk in peripheral neuropathy and a 20-fold increased risk of total disability.[3] NHANES-based population reference values for MMA showed an age-related increase, due to both a gradual decline in kidney function with ageing, as well as vitamin B12 status.[28]

We combined data from two consecutive NHANES surveys of 2011-2012 and 2013-2014 in order to evaluate the association between serum B12 and MMA with haematological parameters and physical and cognitive functioning parameters. Furthermore, we also aimed to study the potential role of variation in serum B12 and MMA concentrations due to age and renal function on these associations.

MATERIALS AND METHODS

NHANES structure and inclusions

In short, NHANES is a cross-sectional survey in the U.S. that uses a complex, stratified, multistage probability sampling design.[20,27] NHANES has obtained written informed consent from all participants. The survey protocol was approved by the Research Ethics Review Board of the National Center for Health Statistics, Centers for Disease Control and Prevention. Interview questionnaires and examination response rates are publicly available.[29] Participants were first interviewed in their homes, during which demographic information and a variety of health-related information were collected. One to two weeks later, they underwent a standardised physical examination, as well as additional investigations such as exercise testing, 24-hour (h) dietary recall, and a blood draw in a mobile examination centre. Blood samples were taken with the participant fasting. Participants who visited the examination in the morning were requested to fast for nine hours; those visiting in the afternoon or evening were requested to fast for six hours. For this study, we created a dataset of NHANES 2011-2012 and 2013-2014 participants who were older than 19 years and had available serum B12 measurements. The NHANES survey included people from several ethnicities and the sample design for NHANES 2011-14 included an oversample of Asian Americans.

Outcomes

To estimate clinical complaints and functioning, we used data from the following NHANES questionnaires: current health condition, disability, hospital utilisation, medical conditions, cognitive functioning, mental health and depression, and physical functioning (supplementary table 1). Based on these questionnaires, we calculated symptom scores for current health status, mental health and depression, and physical functioning and disability, taking into account the most relevant questions/variables for each entity, as described in supplementary table 1. A higher symptom score conforms to a higher number of symptoms, complaints, and disturbance of functioning. We also evaluated the results of muscle strength which was measured through a grip test using a handgrip dynamometer as a separate parameter of physical functioning, and the results of cognitive functioning tests (the CERAD Word Learning sub-test, the Animal Fluency test, and the Digit Symbol Substitution Test (DSST), for details see supplementary table 1). The latter tests were only performed in participants aged 60 years and older.

Reference values were calculated for participants who could be considered 'healthy', i.e., defined as those participants with serum B12 between 301 and 1000 pmol/l, normal serum MMA, estimated glomerular filtration rate (eGFR) > 60, and no medication use.

Exposures

Haemoglobin and mean corpuscular volume (MCV) measurement were performed with a Beckman Coulter MAXM for 2011-2012 and the Beckman Coulter DxH 800 for 2013-2014 (Beckman Coulter Inc, Brea, CA, USA). No significant trend changes for haemoglobin and MCV were reported from NHANES 2011-2012 to NHANES 2013-2014. Serum B12 concentrations were measured with electrochemiluminescence immunoassay on a Modular Analytics E170® system (Roche Diagnostics, Indianapolis, IN). Serum MMA concentrations were analysed by LC-MS/MS as dibutylester after extraction from serum with tert-butylmethylether and derivatisation with butanol.[30] Serum creatinine was measured with the Jaffe rate method (kinetic alkaline picrate) on a Beckman Synchron DxC800 modular chemistry analyser. All information regarding these methods are publicly available on the NHANES website.[20,31]

On the basis of serum B12 concentrations, three groups were constructed: 1) probable vitamin B12 deficiency, defined as a serum B12 concentration < 140 pmol/l; 2) 'possible deficiency', serum B12 concentrations between 140 and 300 pmol/l; 3) normal concentrations, serum B12 > 300 pmol/l. Serum B12 concentrations greater than 1000 pmol/l were considered suggestive for supplementation with (parenteral) B12-containing preparations, and these participants were not included in the calculations. Serum MMA concentration ≥ 300 nmol/l was considered elevated. Anaemia was defined according to the World Health Organization criteria: haemoglobin concentration in men < 8.0 mmol/l and in women < 7.5 mmol/l, with MCV > 100 fl used as a definition for increased MCV.

Other variables

Medication use was scored in NHANES by the unique generic drug code from the Multum Lexicon Drug Database. The number of different medications reported by a participant was considered as a proxy for comorbidity.[32] Current smoking was defined as a positive answer to question SMQ690A/691A: Have you used tobacco/nicotine during the last 5 days? Renal function was calculated as eGFR with the formula developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).[33] Impaired renal function influences MMA concentrations, and thereby the prognostic influence of elevated MMA concentrations may be different in people with impaired renal function. Also, impaired renal function itself is associated with more comorbidity and a higher risk of frailty.[24,25]

Statistics

Functional outcomes were assessed in relation to serum B12 and MMA concentrations. We calculated all variables for the entire population of participants > 19 years of age, and separately for people from Western European descent (described in NHANES as Non-Hispanic Whites, supplementary table 2) to evaluate for generalisability to Dutch individuals. Means were compared between groups with analysis of variance. When variables were not normally distributed, medians were compared with the nonparametric Mann-Whitney U or Kruskal Wallis test. Chi-square test was used to analyse categorical variables.

Univariable and multivariable linear regression analyses were performed to examine the association between relevant factors like serum B12 and MMA concentrations (both log transformed) and muscle strength. As age, sex, haemoglobin, serum creatinine (both log transformed), current smoking, and comorbidity are strong determinants of physical functioning and have been included in the multivariable model as co-factors.

NHANES has created specific sampling weights to account for its complex survey design (including oversampling), survey non-response, and post-stratification.[34] The incorporation of sampling weights into estimated regression coefficients helps protect against the potential existence of missing regressors. In addition, the linearisation variance estimator is suggested to be robust against the likelihood of correlated errors and the possibility of heteroscedasticity.[35,36] Although we did not intend to extrapolate our findings to the U.S. civilian non-institutionalised census population, we calculated our multivariable regression models with application of these weights. A p-value < 0.01 was used as a cut-off for statistical significance. Analyses were conducted using IBM SPSS Statistics (Version 24, IBM, Armonk, NY, USA) and Stata Statistical Software (version 16.0; Stata Corp).

RESULTS

NHANES 2011-2014 included 19931 participants. A total of 10286 participants were excluded because of age ≤ 18 years, unavailability of serum B12 measurements, serum B12 > 1000 pmol/l, pregnancy or breastfeeding (supplementary figure 1). The final study population included 9645 participants with a mean age of 49 (standard deviation (SD) 17) years; 50.1% were males. Mean haemoglobin was 8.7 (SD 1.0) mmol/l (in men: 9.2 ± 0.8 mmol/l, in women 8.2 ± 0.8 mmol/l), eGFR 96 (SD 24) ml/min, and median serum vitamin B12 concentrations and MMA concentrations were 377 pmol/l (IQR 280-509) and 140 nmol/l (IQR 108-189), respectively. In total, 159 (1.6%) participants had serum B12 concentrations < 140 pmol/l, while 2760 (28.6%) had concentrations between 140 and 300 pmol/l.

Table 1. *Association between serum B12 levels, methylmalonic acid, haematologic variables, and renal function*

	B12 < 140 pmol/l	B12 140-300 pmol/l	B12 301-1000 pmol/l	p-value
All	**n = 159**	**n = 2760**	**n = 6726**	
Males (%)	45	52	49	0.030
Age (years)	56 ± 17	49 ± 17	49 ± 18	< 0.001
MMA (nmol/l)	366 (69-5540)	161 (27-3020)	131 (37-1730)	< 0.001
MMA ≥ 300 (%)	56.2	13.5	4.1	< 0.001
Haemoglobin (mmol/l)	8.6 ± 0.9	8.8 ± 1.0	8.7 ± 1.0	0.038
Anaemia (%)	13.8	10.4	10.2	0.336
MCV (fl)	90 ± 7	89 ± 6	89 ± 6	0.176
MCV > 100 (%)	4.4	2.6	2.1	0.025
eGFR (ml/min)	87 ± 25	94 ± 23	94 ± 24	0.001
eGFR < 60 (%)	13.2	7.9	8.8	0.047
Western Europeans	**n = 65**	**n = 1249**	**n = 2635**	
Males (%)	47	49	51	0.390
Age (years)	58 ± 18	50 ± 18	51 ± 19	0.002
MMA (nmol/l)	309 (77-1890)	177 (59-1470)	151 (43-1240)	< 0.001
MMA ≥ 300 (%)	52.3	16.8	5.7	< 0.001
Haemoglobin (mmol/l)	8.8 ± 0.8	8.9 ± 0.9	8.9 ± 0.9	0.283
Anaemia (%)	7.6	6.2	5.9	0.805
MCV (fl)	91 ± 5	91 ± 5	91 ± 5	0.420
MCV > 100 (%)	4.5	2.5	3.0	0.472
eGFR (ml/min)	82 ± 23	89 ± 22	88 ± 23	0.032
eGFR < 60 (%)	12.1	11.4	12.3	0.689

Data are given as mean ± SD, median (range), or percentage.
eGFR = estimate glomerular filtration rate; MMA = methylmalonic acid; MCV = mean corpuscular volume; SD = standard deviation

Figure 1. *Relationship between levels of serum B12 and methylmalonic acid (MMA) in the entire population. The lower smoothed line shows the regression curve for participants with eGFR > 60 ml/min, the upper smoothed line is the regression curve for eGFR < 60 ml/min.*

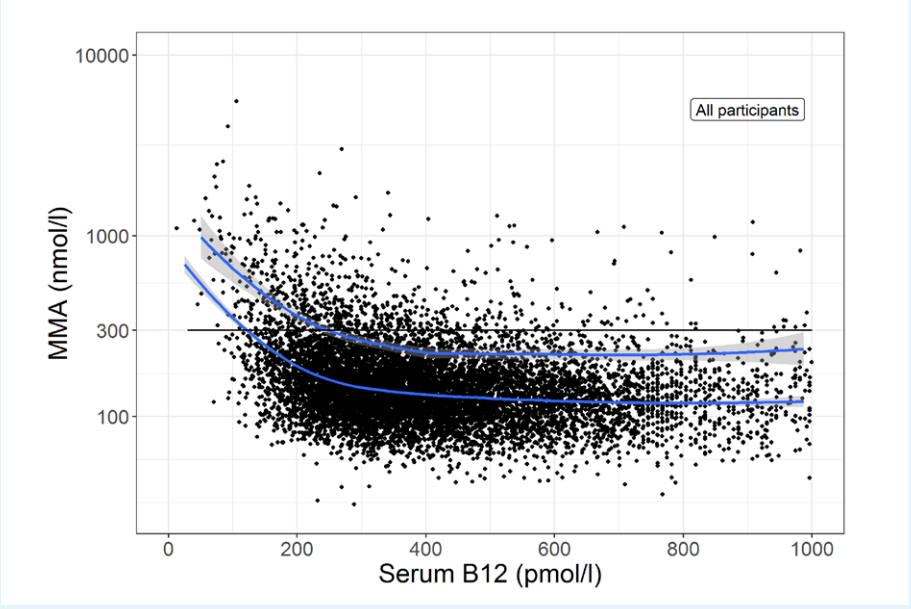

Table 2. *Association between methylmalonic acid, serum B12 levels, haematologic variables, and renal function*

	MMA < 300 nmol/l	MMA ≥ 300 nmol/l	p-value
All	**n = 8898**	**n = 742**	
Males (%)	50	51	0.581
Age (years)	48 ± 17	61 ± 16	< 0.001
Serum B12 (pmol/l)	385 (71-999)	260 (13-992)	< 0.001
Serum B12 < 140 (%)	0.8	12.1	< 0.001
Haemoglobin (mmol/l)	8.8 ± 0.9	8.5 ± 1.1	< 0.001
Anaemia (%)	9.7	18.1	< 0.001
MCV (fl)	89 ± 6	90 ± 6	0.008
MCV > 100 (%)	2.2	2.3	0.484
eGFR (ml/min)	96 ± 22	72 ± 29	< 0.001
eGFR < 60 (%)	6.6	32.5	< 0.001
Western Europeans	**n = 3553**	**n = 396**	
Males (%)	51	48	0.327
Age (years)	50 ± 18	64 ± 17	< 0.001
Serum B12 (pmol/l)	370 (87-999)	265 (70-992)	< 0.001
Serum B12 < 140 (%)	0.9	8.6	< 0.001
Haemoglobin (mmol/l)	9.0 ± 0.9	8.6 ± 1.0	< 0.001
Anaemia (%)	5.1	14.6	< 0.001
MCV (fl)	91 ± 5	91 ± 5	0.077
MCV > 100 (%)	2.9	2.5	0.410
eGFR (ml/min)	90 ± 21	70 ± 26	< 0.001
eGFR < 60 (%)	9.4	35.6	< 0.001

Data are given as mean ± SD, median (range), or percentage.
eGFR = estimate glomerular filtration rate; MMA = methylmalonic acid; MCV = mean corpuscular volume; SD = standard deviation

Of those participants with serum B12 < 140 pmol/l, 56.2% had elevated MMA concentrations, while only 4.4% had elevated MCV (table 1). In those with serum B12 < 100 pmol/l, only 32 of 42 (76.2%) had elevated MMA concentrations. In participants with serum B12 in the grey zone between 140 and 300 pmol/l, 13.5% had elevated MMA, while this was 4.1% in subjects considered to have normal serum B12 (301-1000 pmol/l). Similar results were obtained when only participants of Western European descent were studied. In these, only half (52.3%) had elevated MMA when serum B12 was < 140 pmol/l. Elevated MMA concentrations ≥ 300 nmol/l (table 2) were associated with significantly lower serum B12 and haemoglobin concentrations, and lower eGFR, but not with MCV or percentage of participants with elevated MCV.

There was a curvilinear association of serum B12 with MMA by which lower serum B12 was associated with higher MMA (figure 1). Elevated concentrations of MMA can be found across the entire spectrum of serum B12 concentrations (figure 1). Participants with an eGFR < 60 ml/min had a higher MMA for a given serum B12 concentration. In participants with serum B12 < 140 pmol/l, we identified that 43.8% had a normal MMA concentration (table 1). In participants with serum B12 in the grey zone and elevated MMA concentrations, 25.4% had an eGFR ≥ 60 ml/min, whereas in those with serum B12 between 301 and 1000 pmol/l, 46.8% of those with elevated MMA had an eGFR < 60 ml/min (supplementary table 3). Impaired renal function was found in 5.2-7.2% participants with normal serum MMA (supplementary table 3).

Table 3. *Health and functional parameters according to serum B12 levels*

	B12 < 140 pmol/l	B12 140-300 pmol/l	B12 301-1000 pmol/l	p-value
All	**n = 159**	**n = 2760**	**n = 6726**	
Current health status	186 (157-229)	180 (143-220)	180 (143-220)	0.052
Mental health & depression	14 (0-43)	17 (0-43)	17 (0-50)	0.115
Physical functioning	0 (0-40)	0 (0-20)	0 (0-18)	0.036
Any disability (%)[a]	28.4	20.3	20.5	0.108
Muscle strength (kg)	64 ± 19	72 ± 22	72 ± 23	0.001
Western Europeans	**n = 65**	**n = 1249**	**n = 2635**	
Current health status	200 (169-241)	180 (143-220)	180 (143-220)	0.037
Mental health & depression	14 (0-52)	17 (0-50)	17 (0-50)	0.889
Physical functioning	0 (0-40)	0 (0-26)	0 (0-30)	0.202
Any disability (%)[b]	23.5	22.9	23.4	0.955
Muscle strength (kg)	66 ± 20	71 ± 22	72 ± 23	0.192

Data are given as mean ± SD, median (IQR), or percentage.
Current health status, score 0-400, reference values 157 (129-180); mental health & depression, score 0-250, reference values 20 (0-40); physical functioning, score 0-190, reference values 0 (0-0);
for all three variables, higher score means worse performance.
Any disability: reference value 4%.
Muscle strength: the sum of the largest reading from each hand, reference values 78 ± 23 kg.
[a] only available in 116, 1863, and 4453 participants in the three different groups (B12 < 140 pmol/l, 140-300 pmol/l, and 301-1000 pmol/l, respectively)
[b] only available in 51, 892, and 1873 participants in the three different groups (B12 < 140 pmol/l, 140-300 pmol/l, and 301-1000 pmol/l, respectively)
n = number of patients

Table 4. *Health and functional parameters according to serum MMA levels and renal function*

	MMA < 300 nmol/l	MMA ≥ 300 nmol/l	p-value
All	**n = 8898**	**n = 742**	
Current health status	180 (143-220)	200 (160-240)	< 0.001
Mental health & depression	17 (0-50)	17 (0-57)	0.001
Physical functioning	0 (0-10)	12 (0-62)	< 0.001
Any disability (%)[a]	19.1	35.6	< 0.001
Muscle strength (kg)	72 ± 22	62 ± 21	< 0.001
Western Europeans with eGFR > 60 ml/min	**n = 3218**	**n = 255**	
Current health status	180 (143-220)	186 (157-240)	< 0.001
Mental health & depression	17 (0-50)	17 (0-50)	0.041
Physical functioning	0 (0-18)	9 (0-50)	< 0.001
Any disability (%)[b]	19.4	32.0	< 0.001
Muscle strength (kg)	74 ± 23	66 ± 21	< 0.001
Western-Europeans with eGFR < 60 ml/min	**n = 334**	**n = 141**	
Current health status	200 (169-240)	220 (183-260)	< 0.001
Mental health & depression	17 (0-52)	29 (0-62)	0.114
Physical functioning	27 (0-73)	50 (18-89)	< 0.001
Any disability (%)[c]	33.6	46.0	0.009
Muscle strength (kg)	59 ± 19	51 ± 19	0.001

Data are given as mean ± SD, median (IQR), or percentage.
For explanation: see legends for table 3.
[a] only available in 5831 and 596 participants of the two groups (< 300 vs ≥ 300 nmol/l MMA, respectively)
[b] only available in 2158 and 200 participants of the two groups (< 300 vs ≥ 300 nmol/l MMA, respectively)
[c] only available in 318 and 137 participants of the two groups (< 300 vs ≥ 300 nmol/l MMA, respectively)
eGFR = estimated glomerular filtration rate; IQR = interquartile range; kg = kilogram; MMA = methylmalonic acid
n = number of patients

Table 5. *Cognitive functioning in participants aged 60 years and older*

All participants	B12 < 140 pmol/l n = 69	B12 140-300 pmol/l n = 777	B12 301-1000 pmol/l n = 1896	p-value
CERAD total score	17.6 ± 5.2	18.6 ± 4.7	18.7 ± 4.9	0.248
CERAD score recall	5.3 ± 2.7	5.8 ± 2.3	5.8 ± 2.4	0.215
Animal Fluency Test	14.6 ± 5.2	16.7 ± 5.6	16.4 ± 5.6	0.009
Digit Symbol Substitution Test	40.2 ± 18.0	44.8 ± 17.1	46.4 ± 17.5	0.004

Reference values are:
CERAD total score 19.5 ± 4.6;
CERAD score recall 6.2 ± 2.3;
Animal fluency test 17.5 ± 5.8;
Digit symbol substitution test 50.0 ± 18.0

CERAD = Consortium to Establish a Registry for Alzheimer's Disease; MMA = methylmalonic acid
n = number of patients

Participants with serum B12 < 140 pmol/l reported significantly worse physical functioning and had lower muscle strength compared to those with serum B12 levels between 140-300 pmol/l and > 300 pmol/l (table 3). Both differences were lost when only people of Western European descent were evaluated. In the total population and in Western Europeans, we observed a significantly lower score in all functional outcomes, including muscle strength, for those with MMA concentrations > 300 nmol/l in comparison with people with normal MMA concentrations (table 4). In order to correct for impaired renal function, we re-calculated the composite outcomes and muscle strength for participants with an eGFR > 60 ml/min. Again, we observed lower performance with MMA ≥ 300 nmol/l, but the overall scores were slightly better for participants with an eGFR > 60 compared to those with an eGFR < 60 ml/min.

Table 5 shows the results of the cognitive function tests for all participants aged 60 years and older. The participants with serum B12 < 140 pmol/l had a lower score on the Animal Fluency Test and the Digit Symbol Substitution Test, compared to participants in the other groups, whereas the scores on the CERAD Word Learning and Recall test were similar. Participants with MMA ≥ 300 nmol/l had a lower score on all domains. Scores for the Intrusion Word Count were not different between the groups; 20.7% of participants with normal MMA compared with 19.5% with elevated MMA produced one or more intrusion words. Similar results were obtained for participants of Western European descent. Participants with normal renal function had significantly better score ($p < 0.001$ for all three domains) compared to those with impaired renal function. Comparison of participants with serum B12 < 140 pmol/l with normal or with elevated serum MMA concentrations showed that those with MMA ≥ 300 nmol/l were older, had slightly lower median serum B12 concentrations and more frequently impaired renal function; their haemoglobin, MCV concentrations and functional status were not different. Although the cognitive function tests showed lower scores for those with elevated MMA, these differences were not statistically significant due to low numbers (supplementary table 4).

Serum B12 concentration (log transformed) was not significantly associated with muscle strength (coefficient -0.279, SE 0.82, $p = 0.737$), while serum MMA (log transformed) was significantly associated with muscle strength (coefficient -6.90, SE 0.79, $p < 0.001$). The results of the multivariable regression analyses for muscle strength are depicted in table 6. For the entire population, we observed a significant and independent contribution for gender, age, and MMA concentrations, as well as haemoglobin concentrations and co-morbidity. Serum B12 was not independently associated with muscle strength. Similarly, age, serum B12, MMA concentrations, and co-morbidity were independently associated with the physical functioning symptom score, whereas only age and co-morbidity were significantly associated with current health status and mental health.

DISCUSSION

In this study, we have shown that a large proportion of individuals with a decreased serum B12 concentration still has normal MMA concentrations. In addition, in people with serum B12 concentrations in the grey zone between 140 and 300 pmol/l, 13.5% had elevated MMA concentrations. Only a very small proportion of participants with low serum B12 and elevated MMA had anaemia, with or without elevated MCV. Participants with serum B12 concentrations < 140 pmol/l had lower physical functioning and muscle strength in the entire

Table 6. *Multivariable regression analysis for muscle strength/combined grip test, corrected for NHANES survey weights*

A. Entire population (n = 8134)				
Variable	Coefficient	Linearized SE	t	p-value
Male	-28.700	0.644	-44.56	< 0.001
Age (years)	-0.324	0.021	-15.77	< 0.001
Log serum B12 (pmol/l)	-1.275	0.615	-2.07	0.046
Log MMA (nmol/l)	-4.414	0.607	-7.27	< 0.001
Log haemoglobin (mmol/l)	17.276	2.567	6.73	< 0.001
Log creatinine (mcmol/l)	8.989	1.191	7.55	< 0.001
Log N prescription meds	-1.746	0.278	-6.27	< 0.001
Current smoking	-0.353	0.459	-0.77	0.447
R-squared 0.648				
B. Western Europeans (n = 3482)				
Variable	Coefficient	Linearised SE	t	p-value
Male	-29.929	0.834	-35.89	< 0.001
Age (years)	-0.341	0.027	-12.73	< 0.001
Log serum B12 (pmol/l)	-1.463	0.825	-1.77	0.086
Log MMA (nmol/l)	-4.335	0.791	-5.48	< 0.001
Log haemoglobin (mmol/l)	21.012	3.724	5.64	< 0.001
Log Creatinine (mcmol/l)	6.035	1.486	4.06	< 0.001
Log N prescription meds	-2.321	0.372	-6.23	< 0.001
Current smoking	-0.762	0.610	-1.25	0.220
R-squared 0.678				

MMA = methylmalonic acid; N = number; SE = standard error of the mean; t = result of t-statistic test

population, but not in the subgroup of participants of Western European descent. Serum MMA concentrations were strongly associated with all clinical outcomes and with muscle strength in both the total population as well as participants of Western European descent.

When evaluating the functional status or performance of the participants, serum MMA concentrations proved to be a better indicator of poor functional status than serum B12 concentrations. In all domains, participants with elevated MMA ≥ 300 nmol/l had worse scores compared to those with MMA < 300 nmol/l. This is confirmed in our evaluation of muscle strength as a functional marker. Regression analysis showed that MMA, but not serum B12, was a significant and independent predictor of muscle strength in all subpopulations evaluated; i.e., the entire population, participants of Western European descent, and those with normal renal function. In an earlier study in NHANES participants > 60 years, it was demonstrated that vitamin B12 deficiency was associated with an almost 10-fold increased risk in peripheral neuropathy for participants with serum B12 < 200 pmol/l and homocysteine > 20 µmol/l, but only a 1.4 fold increase for participants with B12 (< 258 pmol/l) or MMA (> 210 nmol/l).[3]

We aimed to investigate cognitive domains in relation to serum B12 and MMA concentrations. As shown in tables 3, 4, and 6, elevated MMA concentrations were more strongly associated with poor functional performance than serum B12. There is limited literature on the relationship between serum B12, its biomarkers, and cognitive performance. Hooshmand et al. reported that higher serum homocysteine concentrations were associated with poorer performance in global cognition, memory, executive functions, and verbal expression, while higher baseline holotranscobalamin (holoTC) was significantly associated with better performance in global cognition,

executive functioning, and psychomotor speed.[37] In the Maine-Syracuse study, serum B12 concentrations and total homocysteine concentrations were positively and negatively associated, respectively, with cognitive performance.[38] Lewis et al. reported that elevated MMA concentrations appeared to be more reflective of cognitive impairment than serum B12, even when corrected for serum creatinine concentreations,[39] and similarly, high plasma homocysteine and serum MMA concentrations correlated inversely with movement and cognitive performance.[40] Taken together, these studies support our observation that biomarkers show a stronger association with functional outcome than serum B12 measurements. A study in people aged 75 and above showed that serum folate concentration was a more important determinant of cognitive performance than serum B12.[41]

The results of this observational study are of clinical importance for our approach towards patients with presumed or possible vitamin B12 deficiency. Some clinicians and clinical chemists consider elevated MMA as the single proof of existing B12 deficiency, and they base their diagnostic algorithms on this. However, almost 25% of people with serum B12 concentrations < 100 pmol/l had normal MMA concentrations. This has been previously observed: approximately 63% of people with low holo-transcobalamin (holoTc) levels < 20 pmol/l, indicative of true deficiency, had normal serum MMA concentrations.[26] This supports observations that serum MMA is not a very sensitive indicator of tissue B12 deficiency. Indeed, Schrempf W. et al. reported that both serum B12 and holoTC levels were weak predictors of abnormal MMA levels.[15] Other studies have confirmed that normal levels of MMA may be measured even in situations of very low B12 levels.[42] In addition, there are isolated reports showing that serum B12, homocysteine, and MMA levels are unreliable predictors of B12-responsive neurological disorders.[43]

The curvilinear association between serum B12 and MMA has been shown by Bailey et al. [20] in an earlier subset of NHANES participants, combining all available data from three consecutive NHANES screenings (1999-2000, 2001-2002, 2003-2004). These authors also showed that for each level of serum B12, MMA concentrations were higher in groups of participants with higher age.[20] Possibly, reductions in eGFR may mediate some of these differences, and they also raise the question whether age-specific reference values for MMA should be used.[23] This is supported by the observation that eGFR is independently associated with MMA in multivariable analysis. In the current paper, we confirmed that a serum B12 within the normal reference range, i.e., > 140 pmol/l, does not definitively reflect normal tissue B12 activity as estimated by serum MMA concentrations. This is also supported by our regression analysis, as depicted in figure 1, where the curvilinear course has an inflection point between 300 and 400 pmol/l of vitamin B12. This clearly supports the assumption that the area of vitamin B12 insufficiency extends above the lower reference value of serum vitamin B12.

Clinicians must consider that an impairment in renal function may increase serum MMA concentrations. As shown, NHANES participants with impaired renal function (eGFR < 60 ml/min) had poorer functional outcomes compared to those with an eGFR above 60 ml/min. A recent publication has provided information on how to adjust serum MMA concentrations for a reduction in eGFR in people with serum B12 levels between 90 and 300 pmol/l, and these calculations were intended to reduce the number of patients classified as vitamin B12 deficient.[23] However, based on the current data (table 1 and supplementary table 4), as well as earlier observations, serum MMA below 300 nmol/l does not exclude vitamin B12 deficiency.[15,26]

Classically, vitamin B12 deficiency has been associated with macrocytic anaemia. However, neurological signs of vitamin B12 deficiency are often present in the absence of anaemia.[8] The prevalence of anaemia in vitamin B12 deficiency appears to be lower than anticipated.[7] In NHANES participants, fewer than 10% of people considered to be vitamin B12 deficient had macrocytosis.[3] In the current study, the number of people with serum B12 < 140 pmol/l and anaemia and/or elevated MCV is small: anaemia was observed in 13.8% of these participants and elevated MCV in 4.4%. Prevalence of anaemia in those with MMA ≥ 300 nmol/l was 18.1%. Causes of anaemia may be complex and concomitant iron deficiency may mask macrocytosis.[44] It should be noted however, that we currently report data from an epidemiological survey and not data regarding patients referred for suspected vitamin B12 deficiency. In the latter group, prevalence of anaemia may be higher. Nevertheless, as anaemia is only seen in a minority of patients with vitamin B12 deficiency, its absence should not be considered as proof that vitamin B12 status is normal.

The results of the current study may help clinicians to identify pitfalls in diagnosing vitamin B12 deficiency. First, since 13.5% of these people with serum B12 concentrations > 140 and < 300 pmol/l have elevated MMA provides evidence that such serum B12 concentrations should not always be interpreted as normal, which is in accordance with several earlier reports.[6,9,15,19] Second, serum MMA is not a sensitive marker; the high percentage of people with low serum B12 but normal MMA suggests that the prevalence of tissue B12 deficiency may even be higher than can be estimated based on abnormal serum MMA concentrations. As the natural course of vitamin B12 deficiency is not well-known, it cannot be excluded that participants with low serum B12 but normal MMA may be in the early, still asymptomatic phase of their

deficiency.[45] Earlier studies have shown the importance of treatment response. In one of his papers, Solomon concluded that if cobalamin therapy had been restricted to symptomatic patients with both low or intermediate serum B12 concentrations and increased MMA or homocysteine concentrations, 63% of responders would not have been treated.[9] Functional vitamin B12 deficiency can be present in patients with apparently normal serum B12 concentrations, either related to defects in intracellular transport of B12,[46] due to interference of serum B12 assays by intrinsic factor antibodies,[47-51] or by masking due to the use of oral vitamin B12-containing supplements.[11,12] The high prevalence of elevated MMA in people with serum B12 > 140 pmol/l refutes the proposed algorithm for cost minimisation which was reported in this journal in 2013.[52] Applying the proposed algorithm will therefore, leave several people undiagnosed, who do have a high probability of vitamin B12 deficiency. Taken together, there is a great need of a generally accepted definition of vitamin B12 deficiency, which takes into account complaints, baseline biochemical results, and response to treatment.[9] Most studies have until now focus on normalisation of serum B12 or MMA, and do not specifically address the clinical syndrome, complaints, or quality of life.[6,53]

Strengths and limitations

This study was adequately powered to study the associations of interest, because we used a large NHANES dataset that reflects the general U.S. population. In addition, we considered potential effects of renal function and age, and have been able to provide sub-analyses in participants of Western European descent to evaluate for generalisability to Dutch individuals. Earlier studies have suggested that ethnicity may influence this association.[20] Because of its cross-sectional nature, we cannot be sure that the same results will apply to patients who are evaluated because of specific (neurological) complaints and are found to have low serum B12 concentrations. In NHANES 2011-2012 and 2013-2014, no direct assessment of peripheral neuropathy was available. The observational nature of this study does not allow for conclusions regarding causality. Also, the use of the NHANES dataset could limit the generalisability to the Dutch population.

CONCLUSIONS

MMA concentrations are elevated in only 56% of people with serum B12 concentrations < 140 pmol/l, and in 13.5% of people with serum B12 in the grey zone of 140-300 pmol/l. MMA concentrations proved to be a more reliable predictor of complaints, functional status, and physical performance than serum B12. Measuring serum MMA may also assist with diagnosing tissue B12 deficiency in cases of doubt when serum B12 concentrations are higher than 140 pmol/l, but this biomarker may also be elevated in people with (severely) impaired renal function.

DISCLOSURES

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Availability of data and material

We used publicly available and de-identified NHANES data collected by the National Center for Health Statistics, Centers for Disease Control and Prevention for the present study. https://www.cdc.gov/nchs/nhanes/index.htm

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Conflicts of interest

All authors declare no conflicts of interest.

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APPENDIX

Supplementary table 1. ***Questionnaire data*** *For each item, the total sum of score was calculated and divided by the number of answers given, to correct for missing answers/data. For calculation purposes, the resulting sum was multiplied by 100. If needed, scores were recoded, with the highest score indicating a worse performance or a higher level of complaints or dysfunctioning.*

Domain 1. Current health status **HSQ = current health status; maximum score 400**	
2011-2012 Questions	**Scoring**
HSD010 - General health condition	1 = Excellent; 2 = Very Good; 3 = Good; 4 = Fair; 5 = Poor
HSQ493 - Pain makes it hard for usual activities (number of days)	Scores recoded: 0-5 days = 1; 6-12 days = 2; > 12 days = 3
HSQ496 - How many days feel anxious (number of days)	Scores recoded: 0-5 days = 1; 6-12 days = 2; > 12 days = 3
2013-2014* Question	**Scoring**
HSD010 - General health condition	1 = Excellent; 2 = Very good; 3 = Good; 4 = Fair; 5 = Poor
* In the years 2013-2014, only question HSD010 was available, no t the other questions HSQ493 and HSQ496, In 2014, these questions were dropped from the survey.	
Domain 1. Current health status **HUQ = hospital utilisation and access to care**	
2011-2012 Questions	**Scoring**
HUQ010 - General health condition	1 = Excellent; 2 = Very Good; 3 = Good; 4 = Fair; 5 = Poor
HUQ020 - Health now compared with 1 year ago	1 = better; 2 = worse; 3 = about the same Scores recoded: 1 = 1; 2 = 4; 3 = 2
HUQ050 - # times receive healthcare over past year	0 = None; 1 = 1; 2 = 2 to 3; 3 = 4 to 9; 4 = 10 to 12; 5 = 13 or more
HUQ090 - Seen mental health professional/past year	Recoded: 0 = no; 1 = yes
2013-2014* Questions	**Scoring**
Same as 2011-2012, except HUQ050 and HUQ051	
HUQ051 - # times receive healthcare over past year	Scores recoded to match the scores of question HUQ050 which was used in 2011-2012.
Domain 2. Mental health & depression **DPQ = mental health and depression**	
Questions	**Scoring**
DPQ010 - Have little interest in doing things	Scores recoded: 0 = 0; 1 = 1; 2 = 2; 3 = 3; 7, 9 = missing
DPQ020 - Feeling down, depressed, or hopeless	Scores recoded: 0 = 0; 1 = 1; 2 = 2; 3 = 3; 7, 9 = missing
DPQ040 - Feeling tired or having little energy	Scores recoded: 0 = 0; 1 = 1; 2 = 2; 3 = 3; 7, 9 = missing
DPQ070 - Trouble concentrating on things	Scores recoded: 0 = 0; 1 = 1; 2 = 2; 3 = 3; 7, 9 = missing
DPQ100 - Difficulty these problems have caused	Scores recoded: 0 = 0; 1 = 1; 2 = 2; 3 = 3; 7, 9 = missing
HUQ090 - Seen mental health professional/past year	0 = no; 1 = yes
MCQ084 - Difficulties in thinking or remembering	0 = no; 1 = yes

Domain 3. Physical functioning **PFQ = physical functioning**	
Questions	**Scoring**
PFQ049 - Limitations keeping you from working	Scores recoded: 1 = 1; 2 = 0; 7, 9 = missing
PFQ051 - Limited in amount of work you can do	Scores recoded: 1 = 1; 2 = 0; 7, 9 = missing
PFQ057 - Experience confusion/memory problems	Scores recoded: 1 = 1; 2 = 0; 7, 9 = missing
PFQ059 - Physical, mental, emotional limitations	Scores recoded: 1 = 1; 2 = 0; 7, 9 = missing
PFQ061B - Walking for a quarter of a mile difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
PFQ061C - Walking up ten steps difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
PFQ061D - Stooping, crouching, kneeling difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
PFQ061I - Standing up from armless chair difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
PFQ061M - Standing for long periods difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
PFQ061P - Grasp/holding small objects difficulty	Scores recoded: 1 = 0; 2 = 1; 3 = 2; 4 = 3; 5, 7, 9 = missing
Original scores for PFQ061B through P 1 = no difficulty; 2 = some difficulty; 3 = much difficulty; 4 = unable to do	
Domain 4. Any disability **DLQ = disability (only for 2013-2014 survey). Maximum score 100**	
Questions	**Scoring**
MCQ084 - Difficulties in thinking or remembering	0 = no; 1 = yes.
DLQ040 - Have serious difficulty concentrating?	0 = no; 1 = yes.
DLQ050 - Have serious difficulty walking?	0 = no; 1 = yes.
DLQ060 - Have difficulty dressing or bathing?	0 = no; 1 = yes.
DLQ080 - Have difficulty doing errands alone?	0 = no; 1 = yes.
Domain 5. **MGX = muscle strength**	
The muscle strength/grip test component measured the isometric grip strength using a handgrip dynamometer. We used the combined grip strength (kg), i.e., the sum of the largest reading from each hand.	
Domain 6.	
CFQ = cognitive functioning, only available in participants aged 60 years and older **CFDCSR - CERAD: Score delayed recall, maximum score 10.** **CFDCIR - CERAD: Intrusion word count recall, maximum score 8.**	
The word learning and recall modules from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) assesses immediate and delayed learning ability for new verbal information (memory sub-domain). The test consists of three consecutive learning trials and a delayed recall. Participants are instructed to read aloud 10 unrelated words, one at a time, and immediately following the presentation of the words, they recall as many words as possible. In each of the three learning trials, the order of the 10 words is changed. The delayed word recall occurred after the other two cognitive exercises (Animal Fluency and DSST) were completed (approximately 8-10 minutes from the start of the word learning trials). In addition to scores for each word learning trial and the delayed word recall, a score for the number of intrusions (incorrect words that were not on the list) is included in the data file.	
CFDAST - Animal Fluency Test, maximum score 39	
The Animal Fluency test examines categorical verbal fluency, a component of executive function. Participants are asked to name as many animals as possible in one minute. A point is given for each named animal.	
CFDDS - Digit Symbol Substitution Test, maximum score 105	
Digit Symbol Substitution test (DSST), a performance module from the Wechsler Adult Intelligence Scale, relies on processing speed, sustained attention, and working memory. The exercise is conducted using a paper form that has a key at the top containing nine numbers paired with symbols. Participants have two minutes to copy the corresponding symbols in the 133 boxes that adjoin the numbers.	

Supplementary table 2. *Distribution of ethnicity in the current dataset of 9645 NHANES 2011-2014 participants*

		Number	Percent
1	Mexican American	1140	11.8
2	Other Hispanic	918	9.5
3	Non-Hispanic White	3951	41.0
4	Non-Hispanic Black	2141	22.2
6	Non-Hispanic Asian	1213	12.6
7	Other Race, Including Multi-Racial	282	2.9

Supplementary table 3. *Distribution of renal function (eGFR) according to groups of serum B12 and MMA concentrations*

		MMA < 300 nmol/l		MMA ≥ 300 nmol/l	
Serum B12 (pmol/l)	n	eGFR > 60 ml/min	eGFR ≤ 60 ml/min	eGFR > 60 ml/min	eGFR ≤ 60 ml/min
< 140	157	63 (94.0%)	4 (6.0%)	74 (8.2%)	16 (17.8%)
140-300	2757	2259 (94.8%)	124 (5.2%)	279 (74.6%)	95 (25.4%)
301-1000	6722	5983 (92.8%)	461 (7.2%)	148 (53.2%)	130 (46.8%)

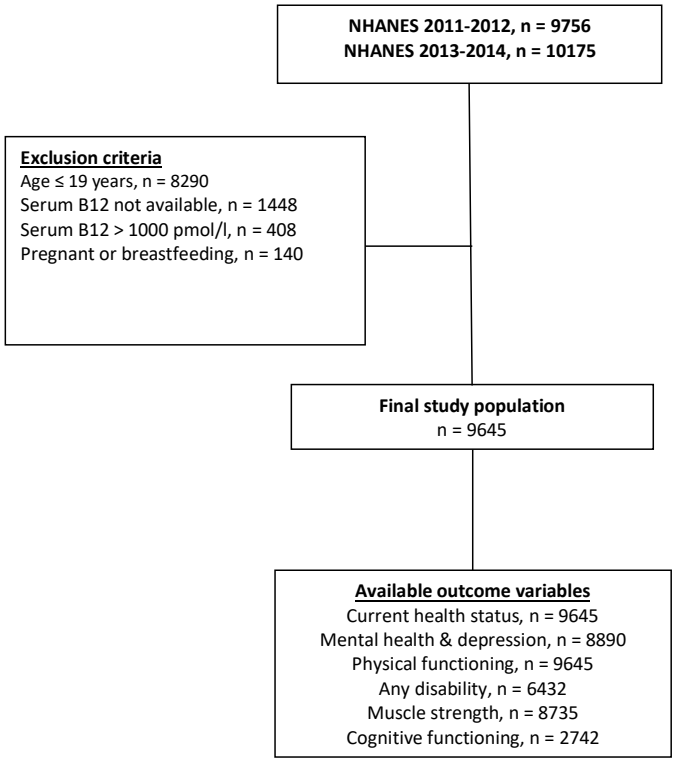
eGFR = estimate glomerular filtration rate; MMA = methylmalonic acid

Supplementary table 4. *Comparison of participants with serum B12 < 140 pmol/l with normal vs. elevated serum MMA concentrations*

	B12 < 140 and MMA < 300 nmol/l	B12 < 140 and MMA ≥ 300 nmol/l	p-value
All	**n = 67**	**n = 90**	
% males	43.3	46.7	0.746
Age (years)	49 ± 17	61 ± 16	< 0.001
Serum B12 (pmol/l)	121 (71-140)	113 (13-140)	0.005
Serum MMA (nmol/l)	200 (77-298)	698 (301-5540)	N.A.
Haemoglobin (mmol/l)	8.7 ± 1.0	8.6 ± 0.9	0.553
Anaemia (%)	14.9	12.2	0.396
MCV (fl)	90 ± 6	89 ± 7	0.343
MCV > 100 (%)	6.0	3.3	0.340
eGFR (ml/min)	98 ± 21	79 ± 25	< 0.001
eGFR < 60 (%)	6.0	17.8	0.023
Current health status	186 (157-220)	186 (143-241)	0.589
Mental health & depression	20 (0-60)	17 (9-50)	0.526
Physical functioning	0 (0-30)	0 (0-52)	0.127
Grip strength	69 ± 18	62 ± 20	0.033
CERAD total score *	18.9 ± 4.9	17.2 ± 5.3	0.213
CERAD score recall *	6.0 ± 2.4	5.0 ± 2.7	0.154
Animal Fluency Test *	16.1 ± 4.9	14.0 ± 5.3	0.136
Digit Symbol Substitution Test *	45.7 ± 12.7	37.8 ± 19.5	0.109

Data are given as mean ± SD, median (range), or percentage.
CERAD = Consortium to Establish a Registry for Alzheimer's disease; eGFR = estimate glomerular filtration rate; MMA = methylmalonic acid; MCV = mean corpuscular volume.
* only available in 20 and 49 participants aged 60 years and older, for groups MMA < 300 nmol/l and MMA ≥ 300 nmol/l, respectively

Supplementary figure 1.



ORIGINAL ARTICLE

The APOP screener and clinical outcomes in older hospitalised internal medicine patients

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ABSTRACT

Background: Acutely hospitalised older patients with indications related to internal medicine have high risks of adverse outcomes. We investigated whether risk stratification using the Acutely Presenting Older Patient (APOP) screening tool associates with clinical outcomes in this patient group.

Methods: Patients aged ≥ 70 years who visited the Emergency Department (ED) and were acutely hospitalised for internal medicine were followed prospectively. The APOP screener assesses demographics, physical and cognitive function at ED presentation, and predicts 3-month mortality and functional decline in the older ED population. Patients with a predicted risk ≥ 45% were considered 'high risk'. Clinical outcome was hospital length of stay (LOS), and adverse outcomes were mortality and functional decline, 3 and 12 months after hospitalisation.

Results: We included 319 patients, with a median age of 80 (IQR 74-85) years, of whom 94 (29.5%) were categorised as 'high risk' by the APOP screener. These patients had a longer hospital LOS compared to 'low risk' patients (5 (IQR 3-10) vs. 3 (IQR 1-7) days, respectively; p = 0.006). At 3 months, adverse outcomes were more frequent in 'high risk' patients compared to 'low risk' patients (59.6% vs. 34.7%, respectively; p < 0.001). At 12 months, adverse outcomes (67.0% vs. 46.2%, respectively; p = 0.001) and mortality (48.9% vs. 28.0%, respectively; p < 0.001) were greater in 'high risk' compared to 'low risk' patients.

Conclusion: The APOP screener identifies acutely hospitalised internal medicine patients at high risk for poor short and long-term outcomes. Early risk stratification at admission could aid in individualised treatment decisions to optimise outcomes for older patients.

KEYWORDS

Frailty, geriatric emergency medicine, internal medicine, older people, risk stratification

INTRODUCTION

Older patients acutely hospitalised for complaints within the remit of internal medicine are at high risk of adverse health outcomes, with 25-35% showing functional decline during hospitalisation,[1,2] which rises to 23-43% at three months, together with 10-20% mortality rates three months after acute admission.[3-5] Patients with high risks of adverse outcomes require adaptations of care and extra attention to prevent further decline.[6] Risk stratification during the initial stages of an acute care episode is therefore an important first step in targeting interventions and improving outcomes for individual older patients.[7-9] However, the identification of patients at highest risk is challenging and therefore rarely used in practice.

The Acutely Presenting Older Patient (APOP) screener is a validated instrument to predict risk for functional decline and mortality within three months for the total population of older patients presenting to the Emergency Department (ED).[10,11] After arrival in the ED, patients can be screened for their individual risk of adverse outcomes in less than two minutes using the APOP screener, and APOP screening has already been implemented in routine ED

care in several Dutch hospitals. However, how predicted risk for adverse outcomes based on APOP screening relates to various clinical outcomes in older patients who are acutely hospitalised for internal medicine needs to be further defined. For example, if the APOP screener can predict a long hospital length of stay (LOS) and 12-month adverse outcomes in this patient group, it could also be used to guide treatment decisions and care planning from a very early stage onwards during hospital admission.
Therefore, the aim of the present study was to investigate the association between predicted risk of adverse outcomes, as assessed by the APOP screener, and clinical outcomes during hospitalisation and at 3 and 12-month follow-ups in acutely hospitalised older internal medicine patients. This information could be a first step in exploring whether routine APOP-based risk stratification can predict individual prognoses useful in tailoring clinical approaches in this vulnerable patient group.

MATERIALS AND METHODS

Study design and setting

This paper describes a secondary analysis of the Acutely Presenting Older Patient (APOP) study, a prospective multicentre study which was performed in four Dutch hospitals. A detailed description has been published elsewhere.[10] Briefly, consecutive older patients visiting the ED of the participating hospitals were included from September to November 2014 at Leiden University Medical Centre (LUMC); from March to June 2015 at Alrijne hospital; from May to July 2016 at Haaglanden Medical Centre (HMC, location Bronovo); and from July 2016 to January 2017 at Erasmus University Medical Center (Erasmus MC). Patients were included 24 hours a day at the LUMC; seven days a week (from 10 a.m.-10 p.m.) at Alrijne; six days a week (from 10 a.m.-10 p.m.) at HMC Bronovo; and four days a week (from 10 a.m.-10 p.m.) at Erasmus MC.

Study participants

In the APOP study, all consecutive patients aged 70 years or older visiting the ED were included. Patients who were triaged 'red' according to the Manchester Triage System (MTS),[12] patients with an unstable medical condition, patients with an impaired mental status without a proxy to provide informed consent, patients with a language barrier and patients who refused to participate were excluded. For the purposes of the present study, we included all acutely hospitalised patients allocated to the specialism internal medicine, and with an APOP screening result at baseline. The participating hospitals had no separate geriatric departments. We excluded patients who were transferred from the ED for hospitalisation elsewhere. The Medical Ethics Committees of the four hospitals approved the study and written informed consent was obtained from all patients.

Outcomes

For the present study, we defined the following outcomes at hospitalisation: hospital LOS in days, in-hospital mortality, and discharge destination. Adverse outcomes assessed were functional decline and mortality, 3 months and 12 months after acute hospitalisation. The 3-month adverse outcome was met if a patient had died or showed functional decline at the 3-month follow-up compared to baseline functioning. The 12-month adverse outcome was met if a patient had died or showed functional decline at the 12-month follow-up compared to baseline functioning. Functional decline was defined as at least one-point increase in the Katz Index of Activities of Daily Living (ADL) score or new institutionalisation (higher level of assisted living).[13] Patients with a maximum Katz ADL score at baseline, institutionalisation at baseline, or patients who were lost to follow-up were considered as having no functional decline.

Data collection

Patient characteristics

Three domains were assessed at baseline in the ED: demographics, disease severity, and geriatric measurements. Demographics consisted of age, sex, living arrangements, and level of education. Disease severity consisted of characteristics related to the ED visit, including arrival by ambulance, triage urgency according to MTS, chief complaint, and a fall-related ED visit. Geriatric measurements consisted of the number of different medications as stated by the patient (≥ 5 medications meaning polypharmacy), use of a walking device, Katz ADL questionnaire (functional status two weeks before the ED visit),[13] the Six-item Cognitive Impairment Test (6-CIT),[14] and a history of diagnosed dementia reported by the patient or a proxy.

The APOP screening result

The APOP screening instrument was developed and validated to identify older patients at risk for the composite outcome of mortality and/or functional decline within three months.[11] The screener comprises seven predictors which are collected at baseline in the ED: age, sex, arrival by ambulance, need of regular help, need for help with bathing and showering, hospitalisation in the past six months and impaired cognition (defined as having dementia or an incorrect answer on at least one out of two 6-CIT questions ['what year is it now?' and/or 'say the months in reverse order'] or no data on cognition). For the purposes of the present study, we retrospectively calculated

the APOP screening results for all acutely hospitalised patients allocated to internal medicine, meaning that the medical staff, at the time, were unaware of the screening results during admission. Validation and threshold testing of APOP screening has been described previously.[11] The threshold for a 'high risk' APOP screening result is a predicted risk ≥ 45% on the composite outcome of mortality and/or functional decline within three months. The final APOP screening model is calibrated to identify the approximately 20% of patients with a predicted risk ≥ 45%. Previously, we compared the APOP screener with the Identification of Seniors At Risk - Hospitalised Patients (ISAR-HP), another frequently used screening tool in the Netherlands, and found that the APOP screener demonstrated better predicting performance for this composite outcome.[15]

Follow-up data

The outcomes at hospitalisation including hospital LOS, in-hospital mortality, and discharge destination were collected from the electronic health records of the participating hospitals. Hospital LOS was measured by subtracting the date of admission to the hospital ward after the ED visit from the hospital discharge date. The discharge destination was compared with the patient's former place of residence before hospital admission. We divided discharge destination into two groups: discharge to the former place of residence (either living at home or in a nursing home) or new institutionalisation at discharge. To obtain follow-up data on functional decline, patients were contacted by telephone 3 and 12 months after acute hospitalisation. In cases of no response after three attempts, the general practitioner was contacted to verify phone number and living arrangements. Finally, a letter was sent requesting a written response from those patients who could not be contacted. Data on mortality was obtained from municipal records. Patients who had not died and could not be reached at follow-up were considered as having no functional decline.

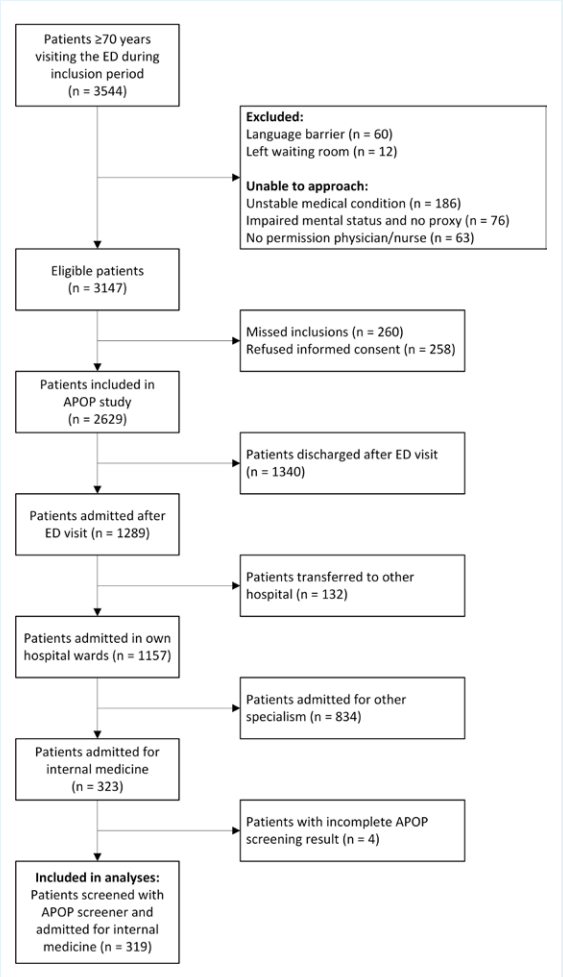
Sample size estimation

The required sample size to determine differences in 12-month mortality was calculated for the present study. Taking a difference of 20% in the mortality rate as relevant, 93 patients per group were needed to detect a difference between 'APOP high risk' and 'APOP low risk' patients with 80% power and a 5% significance level.

Data analyses

Continuous data are presented as means (standard deviation: SD) if normally distributed, and as medians (interquartile range: IQR) if skewed. Categorical data are presented as numbers (n, %). Differences in patient characteristics and outcomes between the APOP 'high risk' and 'low risk' patients were assessed using the independent samples t-test for normally-distributed data, the Mann-Whitney U test for skewed data, and the χ^2 test for categorical data. For categorical data, we present outcomes with 95% confidence intervals (95% CI). Differences in risks for adverse outcomes at 3 and 12 months between the APOP 'high risk' and 'low risk' patients were calculated using relative risk (RR; 95% CI). Survival was calculated by using Kaplan Meier survival curves for the population stratified by APOP screening result. We also conducted sensitivity analyses which led to the exclusion of patients with a maximum Katz ADL score at baseline, institutionalisation at baseline, and those lost to follow-up. A p-value < 0.05 was considered as statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 23.

Figure 1. *Flowchart of study population*



APOP = Acutely Presenting Older Patient (APOP) screening tool; ED = emergency department

Table 1. *Patient characteristics of older patients acutely hospitalised for internal medicine*

		APOP screening result		
	All (n = 319)	**'Low risk' (n = 225)**	**'High risk' (n = 94)**	**p-value***
Demographics				
Age (years), median (IQR)	80 (74-85)	78 (73-83)	84 (81-89)	< 0.001
Male, n (%)	152 (47.6%)	111 (49.3%)	41 (43.6%)	0.351
Living independently, n (%)	294 (91.0%)	219 (97.3%)	71 (75.5%)	< 0.001
Highly educated, n (%)	64 (20.2%)	47 (21.0%)	17 (18.3%)	0.585
Severity of disease indicators				
Arrival by ambulance, n (%)	202 (63.3%)	121 (53.8%)	81 (86.2%)	< 0.001
Triage urgency, n (%)				0.768
> 1 hour (green)	42 (13.2%)	30 (13.3%)	12 (12.8%)	
< 1 hour (yellow)	226 (70.8%)	157 (69.8%)	69 (73.4%)	
< 10 min (orange)	51 (16.0%)	38 (16.9%)	13 (13.8%)	
Chief complaint, n (%)				0.139
Minor trauma	18 (5.6%)	9 (4.0%)	9 (9.6%)	
Malaise	137 (42.9%)	93 (41.3%)	44 (46.8%)	
Chest pain	14 (4.4%)	11 (4.9%)	3 (3.2%)	
Dyspnoea	48 (15.0%)	34 (15.1%)	14 (14.9%)	
Abdominal pain	67 (21.0%)	55 (24.4%)	12 (12.8%)	
Loss of consciousness	8 (2.5%)	6 (2.7%)	2 (2.1%)	
Other	27 (8.5%)	17 (7.6%)	10 (10.6%)	
Fall prior to ED visit, n (%)	28 (8.8%)	9 (4.0%)	19 (20.2%)	< 0.001
Geriatric measurements				
Polypharmacy, n (%)	213 (66.8%)	152 (67.6%)	61 (64.9%)	0.645
Use of walking device, n (%)	177 (55.7%)	94 (41.8%)	83 (89.2%)	< 0.001
Katz ADL score, median (IQR)	1 (0-2)	0 (0-1)	3 (2-5)	< 0.001
6-CIT score, median (IQR)	6 (2-13)	4 (2-8)	14 (6-18)	< 0.001
Diagnosis of dementia, n (%)	18 (5.6%)	5 (2.2%)	13 (13.8%)	< 0.001

ADL = activities of daily living; ED = emergency department; IQR = interquartile range; n = number; 6-CIT = Six-item Cognitive Impairment Test.
* p-value between groups measured by χ^2 for categorical values and Mann-Whitney U test for non-parametric variables.
Missing information for 'low risk' patients: education level (1), Katz ADL (1), 6-CIT scores (21)
Missing information for 'high risk' patients: education level (1), walking device (1), Katz ADL (1), 6-CIT scores (26)

RESULTS

The APOP study included 2629 individual ED patients aged 70 years and older from four hospitals, of whom, 1157 (44.0%) patients were admitted to various hospital wards of the participating hospitals. A subset of 323 (27.9%) of the 1157 patients were acutely hospitalised and allocated to internal medicine. After excluding four patients due to an incomplete APOP screening result, a total of 319 patients could be included in the present study (figure 1).

Patient characteristics

Table 1 presents the patient characteristics of the study population in total and stratified per APOP screening result. In the total study population of 319 patients, the median age was 80 years (IQR 74-85), 152 (47.6%) patients

Table 2. *Short-term clinical outcomes in older patients acutely hospitalised for internal medicine*

		APOP screening result		
	All (n = 319)	**'Low risk' (n = 225)**	**'High risk' (n = 94)**	**p-value***
Hospital LOS in days (median; IQR)	4 (1-8)	3 (1-7)	5 (3-10)	0.006
In-hospital mortality, n (% (95%CI))	21 (6.6 (4.4-9.9))	13 (5.8 (3.4-9.7))	8 (8.5 (4.4-15.9))	0.381
Discharge	**(n = 296)[a]**	**(n = 210)[a]**	**(n = 86)[a]**	
Discharge to former place of residence, n (% (95%CI))				< 0.001
(Semi) Independent at home	220 (74.3 (69.1-79.0))	173 (82.4 (76.7-86.9))	47 (54.7 (44.2-64.8))	
Nursing home	24 (8.1 (5.5-11.8))	6 (2.9 (1.3-6.1))	18 (20.9 (13.7-30.7))	
New institutionalisation at discharge, n (% (95%CI))				
Other hospital	19 (6.4 (4.2-9.8))	17 (8.1 (5.1-12.6))	2 (2.3 (0.6-8.1))	
Nursing home	17 (5.7 (3.6-9.0))	7 (3.3 (1.6-6.7))	10 (11.6 (6.4-20.1))	
Rehabilitation	8 (2.7 (1.4-5.2))	2 (1.0 (0.3-3.4))	6 (7.0 (3.2-14.4))	
Hospice	6 (2.0 (0.9-4.4))	4 (1.9 (0.7-4.8)	2 (2.3 (0.6-8.1))	
Other	2 (0.7 (0.2-2.4))	1 (0.5 (0.1-2.7))	1 (1.2 (0.2-6.3))	

LOS = length of stay; n = number; 95% CI = 95% confidence interval
*: p-value between groups measured by χ^2 for categorical values and Mann-Whitney U test for non-parametric variables.
[a]: Numbers of survivors being discharged after admission
Missing information for 'low risk' patients: hospital LOS (1), in-hospital mortality (2), discharge destination after admission (2)
Missing information for 'high risk' patients: hospital LOS (1)

were male, and 202 (63.3%) patients arrived at the ED by ambulance. Of the total study population, 29.5% (n = 94) were identified as 'high risk' by the APOP screener. These 'high risk' patients, when compared with 'low-risk' patients, were older (median 84 years vs. median 78 years, respectively; p < 0.001) and less likely to live independently (75.5% vs. 97.3%, respectively; p < 0.001). 'High risk' patients were also more likely to have had a fall-related visit (20.2% 'high risk' vs. 4.0% 'low risk'; p < 0.001) and had more geriatric-related impairments, including greater use of a walking device (89.2% vs. 41.8%, respectively; p < 0.001), a higher Katz ADL score (median 3 vs. median 0, respectively; p < 0.001) and a higher 6-CIT score (median 14 vs. median 4, respectively; p < 0.001).

Outcomes at hospitalisation

The median hospital LOS for the entire study population was four days (IQR 1-8) (table 2). When stratified by APOP risk group, the 'high risk' group had a median hospital LOS that was two days longer than the 'low risk' patient group (5 (IQR 3-10) vs. 3 (IQR 1-7) days, respectively; p = 0.006). In total, 21 (6.6%) patients died during hospitalisation, with numbers similar in both groups (p = 0.381). Following hospital admission, the discharge destination was significantly different between 'high risk' and 'low risk' patients, with 'high risk' patients more often newly institutionalised to a nursing home compared to 'low risk' patients (11.6% (6.4-20.1) vs. 3.3% (1.6-6.7), respectively; p < 0.001).

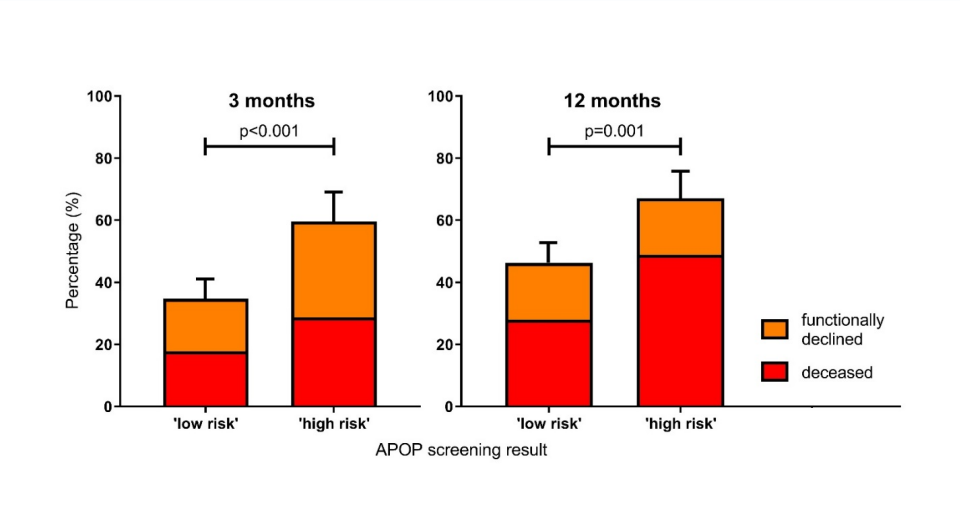
Outcomes at three months

At three months, 134 (42.0%) patients had an adverse outcome, including 67 (21.0%) who had died and 67 (21.0%) who experienced functional decline compared to their level of functioning two weeks before hospitalisation. Outcomes stratified per APOP screening result are shown in figure 2. Of the 94 'high risk' patients, 27 (28.7%) patients had died and an additional 29 (30.9%) patients showed functional decline within three months. Of the 225 'low risk' patients, 40 (17.8%) patients had died and an additional 38 (16.9%) patients had functional decline. 'High risk' patients showed an adverse outcome (deceased or functional decline) more often compared to 'low risk' patients (59.6% (49.5-68.9) vs. 34.7% (28.8-41.1), respectively; p < 0.001). 'High risk' patients showed a 1.7-fold higher relative risk (95%CI 1.3-2.2) for an adverse outcome at three months compared to 'low risk' patients.

Outcomes at 12 months

At 12 months, a total of 167 (52.4%) patients had an adverse outcome, of whom 109 (34.2%) had died and 58 (18.2%) experienced functional decline compared to their level of functioning two weeks before hospitalisation. Of the 94 'high risk' patients, 46 (48.9%) patients had died and an

Figure 2. *Functional decline and mortality, 3 and 12 months after acute hospitalisation stratified by APOP screening result*



Percentage of patients deceased or with decline in functioning compared to the level of functioning at baseline (2 weeks before hospitalisation), at 3 months and at 12 months after acute hospitalisation. Percentages are stratified by the APOP screening result in the ED. Absolute numbers at 3-month follow-up: 'Low risk' patients, n = 40 deceased, n = 38 functional declined. 'High risk' patients, n = 27 deceased, n = 29 functional decline. Absolute numbers at 12-month follow-up: 'Low risk' patients, n = 63 deceased, n = 41 functional decline. 'High risk' patients, n = 46 deceased, n = 17 functional decline.
APOP = Acutely Presenting Older Patient screener

additional 17 (18.1%) patients showed functional decline within 12 months. Of the 225 'low risk' patients, 63 (28.0%) had died and an additional 41 (18.2%) patients had functional decline. More 'high risk' patients had an adverse outcome compared to 'low risk' patients (67.0% (57.0-75.7) vs. 46.2% (39.8-52.7), respectively; p = 0.001). 'High risk' patients also showed a 1.5-fold higher relative risk (95%CI 1.2-1.8) for an adverse outcome at 12 months compared to 'low risk' patients. Supplementary figure 1 shows survival plots for 12-month mortality stratified per APOP screening result. Significantly more 'high risk' patients died within 12 months compared to 'low risk' patients (48.9% vs. 28.0%, respectively; p < 0.001).

We found similar differences between APOP 'high risk' and 'low risk' patients in the sensitivity analyses of outcomes at 3 and 12 months, from which we first excluded those patients who were lost to follow-up for the outcome functional decline and patients who by definition could not show a decline in function (supplementary table 1).

DISCUSSION

'High risk' acutely hospitalised older patients with indications related to internal medicine had a longer hospital LOS and were more often discharged to a nursing home compared to 'low risk' patients. One year after admission, two-thirds of this patient group was deceased or showed a decline in function, showing an overall 1.5-fold higher risk compared to 'low risk' patients.

In the present study, the APOP screener was used as a risk stratification instrument to identify risk of adverse outcomes in older patients. APOP 'high risk' patients could be considered 'frail', although no consensus on the definition of frailty exists. The present study shows how the APOP screener can be used to operationalise the concept of frailty in the ED, by showing the implications of the screener for acutely hospitalised older internal medicine patients.
Over the short term, APOP 'high risk' patients had a 2-day longer median hospital LOS and ~4 times higher risk for new institutionalisation to a nursing home, compared to 'low risk' patients. These results are aligned with existing literature, in which frailty was found to be a good predictor of various short-term adverse outcomes such as hospital length of stay, in-hospital mortality, and institutionalisation.[6,16,17] A recent review concerning acutely admitted general medicine patients reported that frailty was predictive of LOS in 57% of studies and of institutionalisation in 100% of studies.[6] Using frailty/risk-stratification tools at the beginning of an acute care episode may therefore have additional value because it

facilitates the identification of those internal medicine patients who will be hospitalised for a longer period and are likely to be subsequently discharged to a new living environment.

At three months, around one-third of 'high risk' hospitalised internal medicine patients had died and almost half of the survivors exhibited functional decline. These proportions are very comparable to previous Dutch studies in this patient group.[5,18] More importantly, we showed that early risk stratification at admission can also predict long-term adverse outcomes at one year. Despite the fact that the APOP screener was originally designed to predict outcomes at three months, we found that higher risks for mortality or functional decline were still statistically significant at one year; our results align with another Dutch study by Buurman et al., which also reported a significant association between one-year mortality and various geriatric conditions.[19]

The present study has a number of implications for clinical practice. The routine use of the APOP screener upon arrival in the ED can help to identify vulnerable patients at the very beginning of an acute episode. This risk stratification could allow better targeted assessment (i.e., comprehensive geriatric assessment) in patients who need it most and could avoid unnecessary assessment of severely frail/high-risk patients. If risk stratification is not used, care providers may be unaware of differences in frailty amongst older patients, leading to a risk of generalisation of treatment advice. On the one hand, generalisation might lead to overtreatment of frail older patients. This is especially problematic as frail patients are often underrepresented in clinical studies and thus the impact of treatment is often unclear or not focused on the outcomes of interest for these patients.[20,21] On the other hand, there is also a risk of undertreatment of frail older patients. Some of the effects of hospitalisation, such as immobility resulting in functional decline, might be preventable by initiating assessments immediately during hospital admission.[22] Despite the fact that it is unclear why the 'high risk' patients in our study had a longer LOS, the extra 2 days of hospitalisation could be used as a window of opportunity. In some hospitals, these patients could be admitted to specific geriatric departments, but if this is not possible, an internist ought to be aware of opportunities to improve patient outcomes. Perhaps the most important opportunity would be first, to use a comprehensive geriatric assessment, which has known positive effects on prevention of institutionalisation, death, and deterioration in older patients.[23,24] Second, the use of advance care planning would help to establish goals and preferences for future care.[25] And finally, safe transitions between care settings should be ensured, for example, by the use of transitional care.[26] In addition, it is also worth considering that the interventions described above could be of benefit to patients screened as 'low risk'. An important clinical impact of the use of frailty/risk-stratification tools is increased awareness of the risk of poor outcomes, which in turn, may help clinicians tailor approaches to the individual patient. The specific details of how clinicians can do this to improve outcomes or to prevent further decline should be addressed in future research.

Our study has several strengths. First, an unselected group of acutely hospitalised older internal medicine patients was included from four separate Dutch hospitals. Second, although the APOP screener is not technically a frailty screening instrument, it is validated to identify adverse outcomes. As it can be used directly after patient arrival in the ED and requires only two minutes to complete, it is clearly suitable for large-scale use in clinical practice.

Our study also has several limitations. First, we did not have reliable data on the medical reason or diagnosis at hospitalisation, which may have influenced the risk of adverse outcomes. However, a novel aspect of the present study was the risk stratification of patients at the very beginning of an acute care episode to predict outcomes even before the final diagnosis was clear. Second, for the present study we used the development and validation cohort of the APOP study and calculated the APOP screener retrospectively. Nevertheless, we consider the degree of selection or information bias due to the retrospective design to be minimal due to the prospective follow-up design of the study and the inclusion of all consecutive older ED patients. A retrospective design could also be considered an advantage, as clinicians were unaware of the screening results and it therefore could not have influenced course and clinic. In view of the ongoing implementation of the APOP screener in several Dutch hospitals, it would be of value to repeat these analyses in different populations in the future.

In conclusion, the APOP screener identifies acutely hospitalised internal medicine patients at high risk of short and long-term poor outcomes. Early risk stratification at admission could aid in individualising treatment decisions and therefore facilitate optimised outcomes for acutely hospitalised older patients with internal medicine-related indications.

DISCLOSURES

Prior presentations

This paper formed the basis of an oral presentation given at the Dutch Internist Days (Internistendagen), Maastricht, April 24th, 2019.

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Conflict of interest statement

The authors declare that they have no conflicts of interest.

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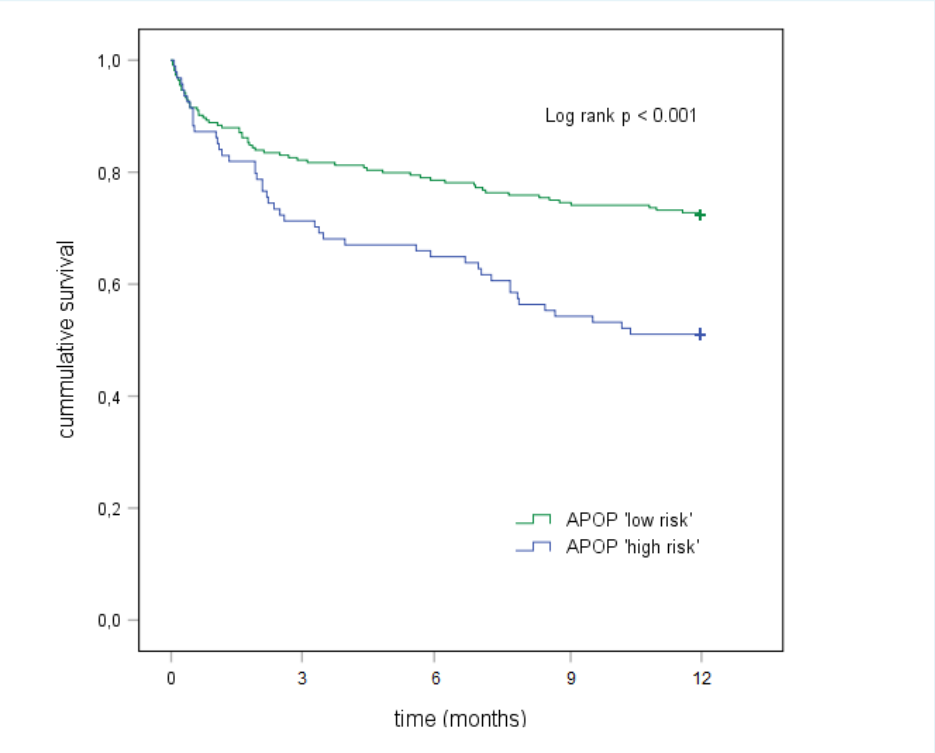
APPENDIX

Supplementary figure 1. *Survival of older internal medicine patients after acute hospitalisation, stratified by APOP screening result*



Kaplan Meier survival curves stratified by APOP screening result. After 12-month follow-up, 109 patients had died, consisting of 46 'high risk' patients and 63 'low risk' patients. There was an association between 'high risk' as determined by the APOP screener and mortality (Hazard Ratio 1.97 (95%CI 1.35-2.89), p < 0.001).
APOP = Acutely Presenting Older Patient screener

Supplementary table 1. *Sensitivity analysis – adverse health outcomes in older patients acutely hospitalised for internal medicine, excluding patients who were lost to follow-up for functional decline or who could not decline (categorised as 'no functional decline') because of a maximum Katz ADL or institutionalisation at baseline*

	All		APOP low risk		APOP high risk		p-value
3 months							
Mortality, n (%)	n = 319	67 (21.0%)	n = 225	40 (17.8%)	n = 94	27 (28.7%)	
Functional decline, n (%)	n = 295	67 (22.7%)	n = 214	38 (17.8%)	n = 81	29 (35.8%)	
Composite outcome, n (%)	n = 295	134 (45.4%)	n = 214	78 (36.4%)	n = 81	56 (69.1%)	< 0.001
12 months							
Mortality, n (%)	n = 319	109 (34.2%)	n = 225	63 (28.0%)	n = 94	46 (48.9%)	
Functional decline, n (%)	n = 301	58 (19.3%)	n = 217	41 (18.9%)	n = 84	17 (20.3%)	
Composite outcome, n (%)	n = 301	167 (55.5%)	n = 217	104 (47.9%)	n = 84	63 (75.0%)	< 0.001

APOP = Acutely Presenting Older Patient screener; n = number
Exclusion at 3 months: 13 patients lost to follow-up and 11 patients who could not decline in function (categorised as 'no functional decline').
Exclusion at 12 months: 10 patients lost to follow-up and 8 patients who could not decline in function (categorised as 'no functional decline').

ORIGINAL ARTICLE

Efficacy of intravenous iron therapy in non-anaemic iron-deficient patients with fatigue

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ABSTRACT

Iron deficiency, without anaemia, is common in the general population and induces various symptoms. Its management consists of oral and intravenous supplementation for cases of inefficacy of or intolerance to oral iron. We assessed the efficacy of intravenous iron therapy in non-anaemic iron-deficient patients with fatigue.

We prospectively evaluated the level of fatigue, using the Fatigue Severity Scale (FSS), in patients suffering from iron deficiency without anaemia, treated by intravenous iron at the moment of the perfusion (W0), after 4 weeks (W4), and 12 weeks (W12).

Of 25 patients, at W0, the mean FFS was 49.3+/-13.7. There was a significant improvement in FSS at W4 (44+/-15; p = 0.01) and a sustained response at W12 with an FFS of 35.8+/-17.1 (p < 0,0001). There was no correlation between FSS and serum ferritin level at W12 (p=0.54) or between serum ferritin at W12 and difference between FSS at W0 and W12 (p=0.58). There were six mild adverse events (24%): asthenia (8%), nausea (8%), headache (4%), local pain (4%); and no serious adverse events.

Our results suggest the rapid efficacy of intravenous iron in improving fatigue in iron deficiency without anaemia with a good profile of tolerance.

KEYWORDS

Fatigue, ferritins, iron, iron deficiency

Iron deficiency (ID) is commonly reported in the general population, in particular, in post-menopausal females. In a cohort of 29 million American patients, there is a 31.7% prevalence of ID.[1] Iron is an essential micronutrient with various functions in haematopoiesis, oxygen transport, DNA synthesis, and numerous physiological metabolic mechanisms.[2] ID can cause various symptoms in the absence of anaemia, such as asthenia,[3] depression,[4] and chronic heart failure aggravation.[5] Iron supplementation in this population (with iron deficiency) improved these symptoms.[6,7] Standard therapy for ID treatment is based on oral iron supplementation.[3] Nevertheless, oral iron leads to frequent (20-35%) adverse events (nausea, abdominal pain, constipation, diarrhoea).[8] These gastrointestinal side effects can impair adherence to treatment (up to 33% of treatment discontinuance).[9] While oral iron supplementation is well studied in ID without anaemia, data are lacking for intravenous iron supplementation. We investigated the efficacy of intravenous iron for the treatment of fatigue due to ID in non-anaemic patients.

We conducted a prospective observational single-institution study on subjects with ID as defined by serum ferritin < 30 ng/mL; they did not have anaemia (haemoglobinaemia > 13 g/dL in men and > 12 g/dL in women) but did have fatigue. They received intravenous iron supplementation from November 2016 to November 2017 in an internal medicine department. We evaluated their fatigue on the Fatigue Severity Scale (FSS) at the moment of iron infusion. The FSS is a self-assessment questionnaire with nine items to assess fatigue.[10] The patients scored all the items on a scale between 0 and 7 (range of cumulative score: 0-63). The patients completed the FSS again via a phone call at 4 and 12 weeks after infusion; the phone call

at 4 weeks also included questions about infusion side effects. We asked them about their serum ferritin levels at 12 weeks after infusion. The objective was to test the efficacy of intravenous iron in improving FSS at 4 weeks after infusion. Quantitative variables were described using means and range or standard deviations; categorical variables were described using numbers and percentages. Comparison of FSS and serum ferritin at the baseline and during the follow-up was performed using a paired sample t-test. The tests were two-sided. P-values < 0.05 were considered significant. All analyses were performed with R software.

Table 1. *Baseline clinical and laboratory characteristics*

Variables	Patients (n = 25)
Age (years)	
Mean	42.5
Range	12-86
Gender (n, %)	
Female	24 (96)
Male	1 (4)
Serum ferritin level (µg/L)	
Mean	14
Range	2.9-30
Haemoglobin level (g/dL)	
Mean	12.7
Range	12-14.6
Iron infusion indications (n, %)	
Idiopathic	9 (36)
Gynaecological loss	8 (32)
Pernicious anaemia	4 (16)
Iron poor-alimentation	3 (12)
Chronic diarrhoea	2 (8)
Gastric ulcer	1 (4)
Helicobacter Pylori gastritis	1 (4)
Indication for intravenous iron therapy (n, %)	
Gastrointestinal side effects due to oral iron	18 (72)
Poor response to oral iron	7 (28)
Type of intravenous iron (n, %)	
Iron (III)-hydroxide sucrose	14 (56)
Ferric carboxymaltose	11 (44)
Dosage (n, %)	
Iron (III)-hydroxide sucrose 200 mg	12 (48)
Iron (III)-hydroxide sucrose 300 mg	2 (8)
Ferric carboxymaltose 500 mg	5 (20)
Ferric carboxymaltose 1000 mg	6 (24)

We enrolled 25 patients, 24 females and one male. Baseline clinical and laboratory characteristics are presented in table 1. All patients reported a disabling fatigue at the day of infusion (W0) with a mean FSS of 49.3+/-13.7. There was a significant improvement in FSS at the 4-week time-point (W4) with an FSS of 44+/-15 (p = 0.01) and sustained measurements at the 12-week time-point (W12) with a FSS of 35.8+/-17.1 (p < 0.0001) (table 2). In a linear regression analysis, age, sex, type or dosage of treatment, the cause of ID, serum ferritin at W0, and haemoglobin were not associated with the decrease of FSS at W4 or W12. The serum ferritin level was measured in 17 patients at W12 (8 patients did not follow the biological prescription); the mean serum ferritin at W12 was 55.8 µg/l (range: 12-138). No serious adverse events were reported due to intravenous iron infusion. Drug-related side effects were reported in six patients (24%): asthenia (2 patients, 8%), nausea (2 patients 8%), headache (1 patient, 4%), and local pain at the infusion point (1 patient, 4%).

One randomised, placebo-controlled study on 250 iron-deficient non-anaemic women highlighted the efficacy of a single dose of 1000 mg ferric carboxymaltose on fatigue eight weeks after infusion.[11] Another randomised, placebo-controlled study evaluated the efficacy of iron (III)–hydroxide sucrose versus placebo on 90 iron-deficient non-anaemic women with a serum ferritin concentration < 50 ng/ml. There was no significant difference on a FSS at 6 and 12 weeks.[12] However, there was significant efficacy of this treatment at 6 weeks in the subgroup with serum ferritin < 15 ng/ml. This suggests, similar to our results, that intravenous iron could be more effective in a population with a serum ferritin concentration below 30 µg/l.

This report is one of the rare studies evaluating efficacy of intravenous iron supplementation on fatigue in a real-life setting. The main concern of this study is the absence of a blinded placebo-controlled arm. The effect of iron on fatigue could be in part, due to a placebo effect. With this in mind, the prolonged impact of intravenous iron at W12

Table 2. *FSS and serum ferritin at baseline and during follow-up*

	W0: mean +/- SD	W4: mean +/- SD (mean difference, p-value)	W12: mean +/- SD (mean difference, p-value)
Fatigue Severity Scale	49.3 +/- 13.7	44.0 +/- 15.0 (5.3, p = 0.01)	35.8 +/- 17.1 (13.5, p < 0.0001)
Serum ferritin (µg/l)	14.0 +/- 6.7		55.8 +/- 40.4 (p = 0.003)

SD = standard deviation; W = week (W0 = week 0; W4 = week 4, W12 = week 12)

may not favour a placebo effect but could be related to the effect of intravenous iron.

This fatigue-decreasing effect reflects the non-haematologic effects of iron. An evaluation of the treatment schedules for future re-infusion of intravenous iron is needed to establish clinical protocols.

DISCLOSURE

The authors have no conflicts of interest to declare.

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CASE REPORT

Viral encephalitis associated with rituximab maintenance therapy: two cases and a review of literature

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SUMMARY

Rituximab is increasingly used in the treatment of CD20-positive B-cell-mediated disease. Prolonged use may cause B-cell dysfunction, dose-dependent T-cell dysfunction, and hypogammaglobulinaemia and result in severe non-neutropenic infections. We present two cases of viral encephalitis in patients treated with rituximab maintenance therapy: one patient presented with deafness; the other patient with paroxysmal light flashes, apraxia, and weakness.

What was known on this topic?
Prolonged use of rituximab may result in hypogammaglobulinaemia which can result in severe non-neutropenic infections.

What does this add?
In patients treated with rituximab maintenance therapy, viral encephalitis should be considered if neurological symptoms are present.

KEYWORDS

Encephalitis, lymphoma, rituximab

INTRODUCTION

Rituximab is an anti-CD20 monoclonal antibody which is widely used in CD20-positive B-cell-mediated diseases, including several auto-immune diseases and B-cell malignancies. In B-cell non-Hodgkin's lymphomas (B-NHL), rituximab is the backbone of the therapy and in some types of B-NHL (low grade NHL and mantle cell lymphoma), maintenance treatment for 2-3 years is standard care. We present two patients treated with rituximab maintenance therapy who were diagnosed with a viral encephalitis.

CASE 1

The first case is a 66-year-old Caucasian female with a stage IV mantle cell lymphoma (MCL) diagnosed in 2008, with a relapse in 2017. Initial treatment consisted of three cycles of R-CHOP (rituximab, cyclophosphamide, adriamycin, vincristine, and prednisone), followed by two cycles of rituximab and cytarabine and consolidated with an autologous stem cell transplantation after BEAM (carmustine, etoposide, cytarabine, and melphalan) conditioning. The relapse nine years later was treated with rituximab-bendamustine. After six courses of rituximab-bendamustine, a complete remission was achieved. Afterwards, the patient was scheduled for rituximab maintenance therapy (375 mg/m^2) every three months.

After the first cycle of rituximab, she developed a headache and severe perceptual hearing loss in both ears resulting in deafness within two weeks. A course of prednisone did not improve her hearing. The neurological examination was unremarkable expect for an unsteady gait. On cerebral magnetic resonance imaging (MRI), there was neither evidence of lymphoma localisation nor other pathology in the cerebellopontine angle or trochlear nerve. The cerebrospinal fluid (CSF) was clear with a slightly elevated leucocyte count and protein level (table 1). The polymerase chain reaction (PCR) of the CSF was positive for enterovirus DNA; C-reactive

Table 1. *Results of blood and cerebrospinal laboratory tests for case 1*

Case 1	Reported value	Unit	Normal range
Blood results			
Albumin	22	g/l	35-50
Glucose	6.8	mmol/l	4.0-11.0
C-reactive protein	228	mg/l	< 5
IgG	6.1	g/l	7.0-15.5
Haemoglobin	6.8	mmol/l	7.5-10.0
Mean cell volume	95	fl	80-100
Thrombocytes	120	x 10^9/l	150-400
Segments	51	%	45-75
Lymphocytes	35	%	20-50
Monocytes	7	%	2-10
Rods	7	%	< 5
Cerebrospinal fluid			
Leucocyte count	7*	/ul	< 5
Protein	0.30	g/l	0.26-0.79
Glucose	2.5	mmol/l	2.5-3.7

* 100% mononuclear cells, immunology: no monoclonal B lymphocytes

protein (CRP) was elevated; there were a leucopoenia and thrombocytopenia; and IgG was lowered (6.1 g/l; normal range 7.0-15.5 g/l). Both a computed tomography (CT) scan of the neck, thorax, and abdomen and a bone marrow biopsy demonstrated no evidence of residual MCL. There were not enough B cells present in the CSF to determine monoclonality. At presentation, the patient also had a fever without shortness of breath. On chest-X ray, a consolidation was seen, suspicious for pneumonia. Blood cultures, obtained before the start of antibiotics, were negative. A consolidation in the right lower lobe was seen, suspicious for pneumonia. The patient was therefore treated with a 5-day course of amoxicillin. Because the patient remained neurologically stable, and because her IgG was only slightly lowered and neutropenia was absent, no intravenous immunoglobulin (IVIG) was administered. The patient was scheduled for cochlear implants.

Before cochlear implantation, the patient developed a new fever, atrial fibrillation, pleural effusions, peripheral edema, and diarrhoea with submucosal thickening of the ileum on CT. No evidence for MCL was found in the pleural effusion. Treatment consisted of broad-spectrum antibiotics and a 5-day course of IVIG (400 mg/kg). Despite these interventions and paramedical support by the physiotherapist and feeding by an enteral feeding tube, the physical condition of the patient gradually deteriorated. She developed rapidly changing blood pressures, became respiratory insufficient, and died several days later in the intensive care unit. No apparent cause was found for this sudden deterioration. Repeated blood, faeces, and urine cultures were negative. Pulmonary embolism was ruled out. After the patient died, the family did not give permission for autopsy.

CASE 2

The second case is a 73-year-old Caucasian male with a stage IVa low grade non-Hodgkin lymphoma diagnosed in 2016 with encasement of the thoracic aorta and mediastinal vessels. Treatment consisted of eight cycles of R-CVP (rituximab, cyclophosphamide, vincristine, and prednisone), after which, a partial response was achieved, followed by rituximab maintenance therapy every three months (375 mg/m^2). After five cycles of rituximab maintenance therapy, the patient developed paroxysmal complaints consisting of nausea and light flashes in both eyes during several minutes, followed by apraxia and weakness in the right leg for half an hour with spontaneous resolution. The patient experienced several of these episodes during the week, often at the end of the day. Other complaints were a postural

Table 2. *Results of blood, cerebrospinal, and urine laboratory tests for case 2*

Case 2	Reported value	Unit	Normal range
Blood results			
Glucose	6.3	mmol/l	4.0-11.0
C-reactive protein	5	mg/l	< 5
Haemoglobin	8.9	mmol/l	7.5-10.0
Mean cell volume	87	fl	80-100
Thrombocytes	359	x 10^9/l	150-400
Leucocytes	12.6	x 10^9/l	4.0-10.0
Total gamma globulins	5.7	g/l	6.0-13.7
Cerebrospinal fluid			
Leucocyte count	505+	/uL	< 5
Protein	1.73	g/l	0.26-0.79
Glucose	1.3	mmol/l	2.5-3.7

+ 66% mononuclear cells, 44% polynuclear cells

headache. Neurological examination was normal except for symmetrical hyporeflexia of the upper extremities and an intentional tremor. Cerebral MRI did not show structural abnormalities.

Because the complaints declined spontaneously, rituximab was continued. After the next cycle of rituximab, the paroxysmal complaints increased in frequency and severity. The headache increased and ataxia of the lower extremities was observed with a wide-based gait. The patient was mildly confused. A fever was also measured (38.5°C). Total gamma globulins were slightly lowered (5.7 g/l, normal range 6.0-13.7 g/l). Laboratory results are shown in table 2. In the CSF, the leucocyte count was elevated with decreased glucose levels and elevated protein. There was no monoclonal cell population detected. CT and MRI of the brain did not reveal abnormalities expect for atrophy. Antibiotic treatment for bacterial meningitis was initiated. The CSF PCR was positive for parechovirus DNA, resulting in the final diagnosis of a viral meningoencephalitis, after which the antibiotics were stopped and a three-day course of IVIG (400 mg/kg) was started. The patient's walking ability recovered completely and the he no longer needed a walking aid. The last two cycles of rituximab were cancelled. We consider vincristine to be an unlikely cause for the complaints because they started several months after the last dose of vincristine. A side effect of rituximab cannot be ruled out as the complaints increased after another dose of rituximab, however this treatment may also exacerbate a parechovirus encephalitis. Rituximab has also been associated with progressive multifocal leukoencephalopathy (PML); however, both a CT and MRI of the brain did not reveal abnormalities indicative of PML.

DISCUSSION

Rituximab is a CD20-specific antibody which targets B cells from the pre-B cell stage up to the pre-plasma cell. Immunoglobulin production is relatively unaffected if rituximab is applied shortly because plasma cells do not express CD20. Prolonged use of rituximab may however, result in decreased B-cell function that can contribute to the risk of non-neutropenic infections (e.g., viral encephalitis) other than bacterial sinopulmonary infections which are frequently seen in patients with a common variable immunodeficiency. However, gammaglobulins are a marker for B-cell function. Moreover, rituximab may result in a dose-dependent T-cell inactivation which may also increase the risk of other types of infection.[1]

In a systematic review, it was shown that the addition of rituximab to chemotherapy resulted in an improved survival without an increase in severe infections (relative risk of infections = 1.00; 95% confidence interval range 0.87 to 1.14).[2] In a more recent single centre retrospective study of 211 patients, 39% of patients developed hypogammaglobulinaemia after rituximab-based treatment for B-cell lymphoma.[3] In 15 patients, immunoglobulins were administered because of recurrent infections. Hypogammaglobulinaemia was

more frequently seen in patients receiving rituximab maintenance therapy compared to rituximab-based (chemo)immunotherapy (54.2% vas 32.8%, p = 0.015). In another series, especially the combination of rituximab and fludarabine resulted in non-neutropenic infections due to hypogammaglobulinaemia.[4]

Parechovirus and enterovirus belong to the group of picornaviruses, small RNA viruses without an envelope. Enterovirus infections may present with a wide range of symptoms from an undifferentiated febrile illness to severe meningoencephalitis.[5] Enteroviruses are responsible for 72% of the viral central nervous system (CNS) infections in adults and are generally associated with a relatively favourable outcome.[6,7] Both the innate and adaptive immune system are activated following an enterovirus infection.[8] However, when B-cell function is compromised, enterovirus infections may cause more severe disease. In patients with primary immunodeficiency, 50% mortality rates of non-polio enterovirus CNS meningoencephalitis have been described.[9] Commonly, patients with enterovirus CNS infections are treated with IVIG. Grisiariu and colleagues report on 12 patients with viral encephalitis during rituximab treatment who received IVIG, of whom 5 died.[10] However, other studies stated that there is currently no effective treatment for enterovirus CNS infection.[9,11]

CONCLUSION

Rituximab maintenance therapy improves the event-free survival of B-cell lymphoma. However, prolonged use of rituximab can result in severe non-neutropenic infections, which may be due to B-cell dysfunction, dose-dependent T-cell dysfunction and hypogammaglobulinaemia. In cases of neurologic complaints, one must be aware of viral encephalitis.

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CASE REPORT

A diphtheria case in Indonesia: a future foe to Europe?

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KEY WORDS

Corynebacterium, diphtheria, Indonesia, vaccination

What was known on this topic?
Following the introduction of a vaccine against diphtheria, the number of cases dropped drastically. The formation of a pseudo membrane in the pharynx region is considered a hallmark symptom of respiratory diphtheria. Diphtheria complications include respiratory insufficiency, myocarditis, and neurologic toxicity. Treatment consists of antibiotics and diphtheria antitoxin.

What does this case add?
A declining vaccination coverage could result in emergence of diphtheria cases, which is illustrated by a case that originates from Indonesia. Recent data from the European Centre for Disease Prevention and Control shows an increase of diphtheria cases in Europe. International travel, lack of routine re-vaccination against diphtheria, and declining diphtheria-specific antibodies may put those living in diphtheria-free countries at risk.

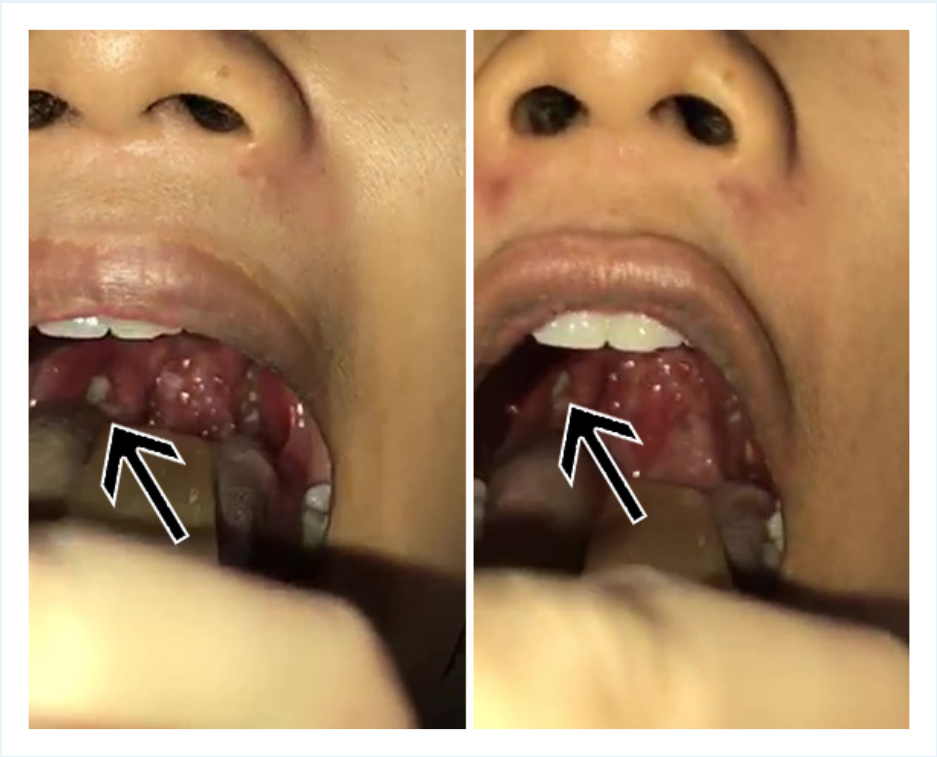
BACKGROUND

Corynebacterium diphtheria, a non-encapsulated gram-positive rod-shaped bacterium, can cause severe disease in humans. Physicians in European countries are usually familiar with this pathogen for its part in childhood vaccination programs. Following the introduction of vaccinations in the mid-twentieth century, the number of diphtheria cases dropped drastically. Therefore, it is not unlikely for a young doctor to not directly recognise the hallmark symptoms of classical (respiratory) diphtheria. In recent years, multiple cases and outbreaks were reported on ProMED-mail (Program for Monitoring Emerging Diseases)[1] and in literature[2-4] and concerns about vaccination coverage were raised.[5] Since 2017, an increasing number of diphtheria cases[6,7] have been recorded in the Republic of Indonesia (population: 266 million), which was followed by an immunisation effort to vaccinate two million children.[8] This outbreak is not only of importance for local healthcare workers, but can be of international significance due to the popularity of Indonesia as a travel destination. We therefore present the following case.

CASE

In January 2019, a 22-year-old woman, without medical history, visited the emergency department of the Universitas Airlangga Hospital in Surabaya (a university teaching hospital), East Java, Indonesia with complaints of cough and dysphagia. Symptoms started four days prior to admission. In addition, she reported a fever for the past six days. There was no stridor or dyspnoea. She lived in the city of Surabaya, but went camping in the West Java province one week before onset of the first symptoms. None of her family members nor anyone in her close proximity suffered from similar symptoms. She received her childhood vaccinations 20 years ago, of which no registration was available. Physical examination showed isolated white spots on her both tonsils and pharynx (figure 1), but none on her uvula. A gram stain of the tonsillar swab showed gram-positive rod-shaped cells with metachromatic (beaded) staining. Due to restricted laboratory resources, no further determination

Figure 1. *Image of the patient's oral cavity. The arrow indicates the most pronounced spot of grey and white exudate on one of the tonsils; it is surrounded by pharyngeal erythema. Image published with permission of the patient.*



was performed and the case was classified as 'clinically compatible' with diphtheria (see Discussion). She received anti-diphtheria serum 40,000 IU intravenously and was prescribed erythromycin 500 mg four times a day (QID) for five days. The patient recovered and the lesions disappeared on the third day of treatment. She was discharged after 10 days in the hospital. During this period, we screened first-degree family members and performed nasal swabs, which were sent to a regional reference lab. All first-degree family members were given prophylactic erythromycin 250 mg QID for seven days per governmental guidelines, regardless of test results.

DISCUSSION

Diphtheria is a highly contagious disease that is usually recognised in patients upon the existence of a tough pharyngeal pseudo membrane. Infection with *C. diphtheria* may either lead to respiratory or cutaneous symptoms or to an asymptomatic carrier state. After a two to five-day incubation period, the respiratory infection manifests with a gradual increase of symptoms of a sore throat, malaise, and lymphadenopathy. Pharyngeal erythema progresses to spots of grey and white exudate (shown in figure 1). The formation of a pseudo membrane that adheres tightly to the underlying tissue is considered pathognomonic and occurs subsequently, in one-third of all cases. Complications occur by the spread of the pseudo membrane in the pharynx, resulting in stridor and eventually leading to respiratory insufficiency with case fatality rates reported up to 20%.[9] Furthermore, absorption and dissemination of toxins can lead to myocarditis, renal failure, and neurologic toxicity. A suspected case should be confirmed with clinical specimens (i.e., swabs) by culture and toxin detection. Primary culture is done on blood tellurite medium, followed by selective culture on cystinase medium (Tinsdale). Screening and biochemical tests can identify the species. Confirmation is based on the phenotypic detection of the toxin (Elek test).[10] Treatment consists of antibiotic therapy and those infected with toxin-producing strains benefit from prompt administration of antitoxin (hyperimmune antiserum). Airway management is important to prevent airway obstruction and cardiac function should be monitored. In case of respiratory diphtheria, droplet isolation could prevent further spread.

Early vaccine research against diphtheria started in the 1920s and today, childhood vaccination against this

pathogen is common around the world.[11] High vaccination coverage (80-85% on the population level) is considered a cornerstone in the prevention of new infections.[12] The recent outbreak response only targets children and adolescents, and the case presented here is illustrative of the susceptibility of those with possible impaired antibody titres due to non-repeated vaccination.

The European Perspective

Over the past decades, although limited numbers of diphtheria have been reported in European countries, clinicians do treat diphtheria infections: The European Centre for Disease Prevention and Control (ECDC) reported 17 diphtheria cases in 2017 in Europe, of which four were reported as classical respiratory diphtheria. Three out of the 17 were considered from indigenous transmission.[13] These illustrate both the necessity to be adequately protected by means of a repeated vaccination, as well as the need for healthcare providers to be aware of the clinical landscape. Interestingly, routine vaccination programs of European Union countries are not uniform in their recommendations for repeated diphtheria vaccination throughout adolescence, early adulthood, and middle age.[14] For some countries, repeated vaccination (for example, during late adolescence) is not included and only suggested by travel medicine guidelines or disease-specific guidelines. Travel to Saudi-Arabia for the Hadj/Umrah gatherings, for example, does not require a recent vaccination against diphtheria. On the other hand, the country was able to contain an ongoing outbreak of *Neisseria meningitides* by demanding a mandatory vaccination when entering the country.[15] This highlights the possible importance of routinely administering repeated vaccination as it is known that diphtheria-specific antibodies can remain below levels of protection and that long-term protection is frequently not achieved.[16,17] This impaired protection against *C. diphtheria* might thus put those living in diphtheria disease-free countries at risk.

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PHOTO QUIZ

Fatal Spanish Souvenir

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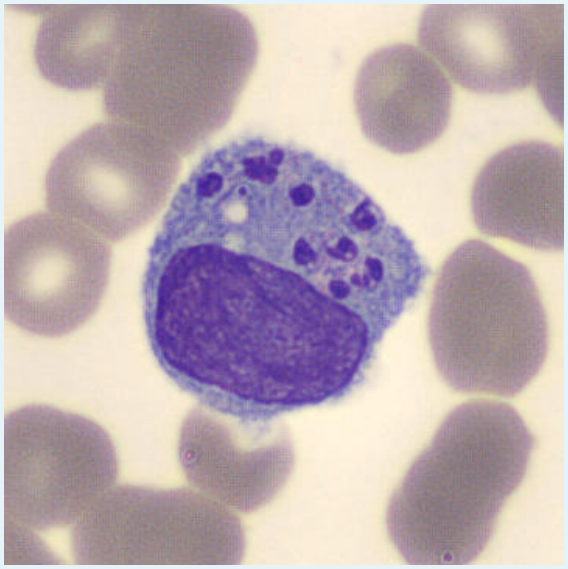
Figure 1. *Monocyte in peripheral blood smear*



CASE REPORT

An 83-year-old Dutch woman with a history of atrial fibrillation, bronchitis, and methotrexate use for rheumatoid arthritis and psoriasis, presented to the outpatient clinic of internal medicine with progressive fatigue and pancytopenia. She had been living in Spain for the past three years in Sant Jordi and Vinaros, and was visiting the Netherlands for the holidays. Blood results showed: haemoglobin 6.0 mmol/l, mean corpuscular volume 94 fl, thrombocytes 66 x 10^9/l, leucocytes 1.0 x 10^9/l, neutrophils 0.5 x 10^9/l (with 2% myelocytes), lymphocytes 0.3 x 10^9/l, and haptoglobin concentration of 1.31 g/l. Vitamin B12 and folic acid levels were normal. Normal renal and liver function tests were found, whereas a splenomegaly of 14 cm was noted on ultrasound examination. Methotrexate was stopped, as this was the presumed cause of her pancytopenia. One month later, the patient was admitted to the hospital with fever and persistent pancytopenia. Peripheral blood smear showed one particular monocyte with cytoplasmic inclusions (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 45 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 44)

FATAL SPANISH SOUVENIR

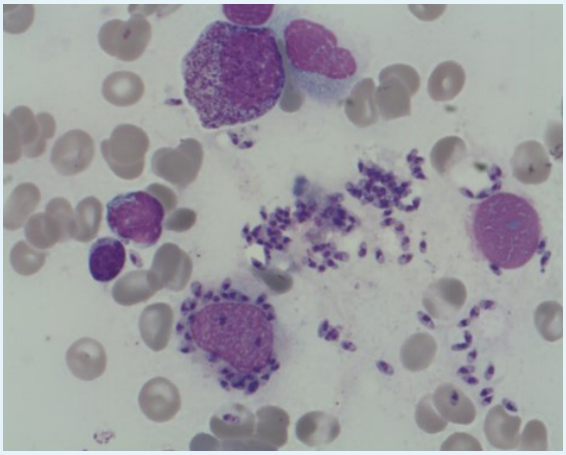
DIAGNOSIS

The peripheral blood smear revealed one monocyte with intracellular micro-organisms: *Leishmania amastigotes*. Amastigotes are small spherical non-flagellated cells ranging from 2-4 µm in diameter. The nucleus and kinetoplast are surrounded by a small ring of vacuolated cytoplasm (figure 1). Detection of *Leishmania amastigotes* in bone marrow aspirate (figure 2) and Leishmania DNA by polymerase chain reaction (PCR) using whole blood confirmed the diagnosis of visceral leishmaniasis. Discrimination between *Leishmania infantum* or *Leishmania donovani* was not possible, but epidemiologically, *Leishmania infantum* is the most likely causative micro-organism in this case.

Visceral leishmaniasis is a chronic infectious disease with an incidence of approximately 0.2-0.4 million cases each year. More than 90% of cases occur in India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia. The case fatality rate is estimated at 10%.[1] Visceral leishmaniasis is transmitted from animals to humans by infected female sand flies. It is endemic in the Mediterranean countries and in southern Europe, it is responsible for 700 cases per year.[2] *Leishmania infantum* is the most frequent species in this region. Domestic dogs serve as the main reservoir, but other mammals such as red foxes, rats, and mice can also host this parasite *Leishmania donovani* also causes visceral leishmaniasis, but it is only reported in Cyprus and Turkey.[3]

Immunocompromised patients are at the highest risk of visceral leishmaniasis.[4] Typical symptoms of visceral leishmaniasis are intermittent fever, hepatosplenomegaly, and pancytopenia. The parasite proliferates in the mononuclear phagocytic system in the spleen, liver, and bone marrow. Diagnosis is confirmed by serological and molecular methods and by microscopy of bone marrow aspirate. Microscopic evaluation of peripheral blood is usually not diagnostic, but in immunosuppressed patients, amastigotes may sometimes be seen in peripheral mononuclear cells, a very specific finding for visceral leishmaniasis.[5]

Figure 2. *Bone marrow smear showing numerous loose and phagocytosed Leishmania amastigotes*



The patient received a therapy of intravenous liposomal amphotericin B (300 mg/day) for 10 consecutive days. After the fourth day, the dose interval was doubled because of her impaired renal function. During the eighth dose, the patient became dyspnoeic, hypotensive, and died suddenly. Probable cause of death was myocardial infarction or pulmonary embolism, but unfortunately no autopsy was performed.

DISCLOSURE

The authors declare no conflicts of interest.

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PHOTO QUIZ

Hips don't lie?

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Figure 1A. *Gram staining of blood culture samples, showing gram-positive cocci in chains.*

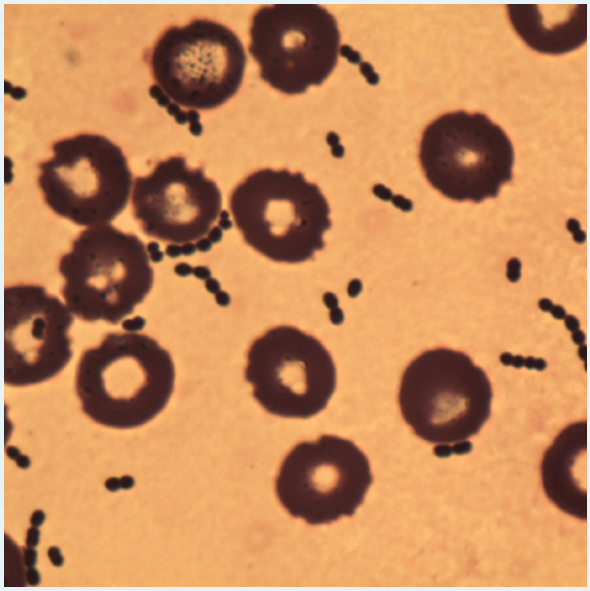
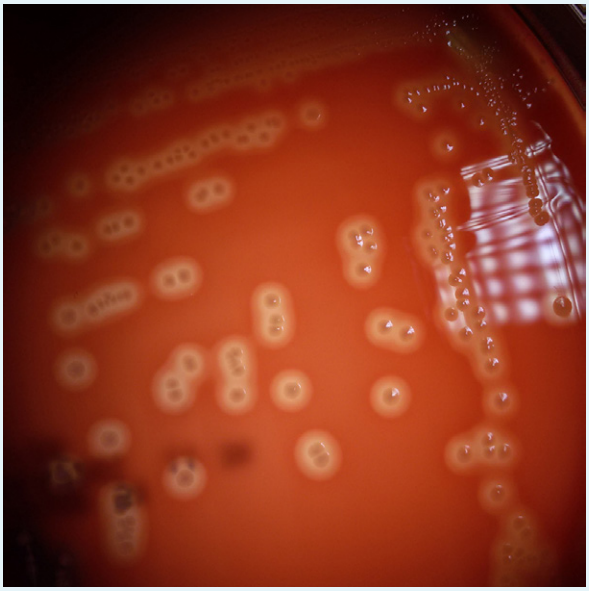


Figure 1B. *Cocci in chains on sheep blood agar plates, showing haemolysis.*



CASE REPORT

An 82-year-old woman presented at the Emergency Department (ED) with a suspected hip fracture after a fall at home. Despite gradual weight loss and malaise over the previous months, her medical history was uneventful. At the patient's request, a do-not-resuscitate/intubate policy was agreed upon at presentation.

Shortly after presentation, she developed hypotension (84/35 mmHg). Physical examination further revealed tachycardia (117 beats/minute), oxygen saturation 98% with 2 l/minute of oxygen supplementation, tachypnoea (22 breaths/minute), and a basal body temperature of 36.7°C. Laboratory results showed leucocytosis (20.8 x 10^9/l), elevated C-reactive protein (282 mg/l), elevated troponin-T (119 ng/l) and a haemoglobin concentration of 7.6 mmol/l. Electrocardiogram and chest X-ray revealed no abnormalities, however the hip X-ray confirmed a left femur fracture. The left thigh was clinically not suspected for internal haemorrhage. She responded positively to fluid resuscitation and showed no signs of organ failure.

Three hours after presentation, the patient unexpectedly deteriorated. At the request of the patient and her family, no life prolonging therapy was initiated. She died of refractory shock, most likely due to cardiac failure. To our surprise, four blood culture samples taken at the ED showed gram-positive cocci the next day (figure 1).

WHAT IS YOUR DIAGNOSIS?

See page 47 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 46)

HIPS DON'T LIE?

DIAGNOSIS

Fulminant sepsis in combination with gram-positive haemolytic cocci in blood culture samples often brings Group-A haemolytic streptococcus (GAS) to mind. In our case however, the gram-positive haemolytic cocci were identified as *Streptococcus agalactiae* (group-B haemolytic streptococcus/GBS) by matrix-assisted laser desorption/ionisation. GAS is notorious for causing severe disease (fasciitis, septic shock, and toxic shock syndrome) in a wide range of patients,[1] whereas GBS is mainly known for causing infections in newborns and pregnant women.[2] Nevertheless, over recent decades, the incidence of invasive GBS infections in non-pregnant adults has increased from 8.1 to 10.9 per 100,000 patients in 2008 and 2016 respectively, in the United States.

Common risk factors for invasive infection include increasing age, obesity, and diabetes.[2] Primary sites of infection in non-pregnant adults are skin and soft tissues, the urogenital tract, and the lungs. Bacteraemia without a clear focus however, occurs in 30% of all cases.[2,3] Overall mortality of invasive GBS infection in the non-pregnant adult population is 5.6%.[2] Ten different GBS serotypes are identified; community-dwelling healthy elderly in the United States are most frequently colonised with serotypes V (47.3%), Ia (22.8%), and III (12.3%).[3,4] These specific serotypes also account for two-thirds of cases with invasive disease.[4] In our case, our patient was infected with GBS serotype Ia. In light of increasing resistance to non-beta-lactam antibiotics (lincosamides, macrolides, and fluoroquinolones), the treatment of choice is benzylpenicillin.[3-5] Reported resistance to clindamycin and erythromycin are 15% and 32.8%, respectively.[5] In particular, serotype V is associated with a higher resistance to clindamycin (3.9 times) and erythromycin (2.9 times).[5] We speculate that our patient developed bacteraemia at home, with ensuing hypotension probably causing her to fall and break her hip. It seems plausible that the fracture initially masked the underlying infection and led to a relatively earlier presentation in the disease course. This would explain the atypical presentation and absence of organ failure typically associated with GBS sepsis. Fulminant GBS sepsis with sudden onset multiorgan failure, similar to streptococcal toxic shock syndrome has been reported in the literature.[6] This may be the cause of the acute deterioration seen in our patient.

It is important for clinicians to be reminded of the potentially unusual presentations of sepsis in order to start antibiotics and supportive care as soon as possible.

DISCLOSURES

The authors have no conflicts of interest to declare.

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