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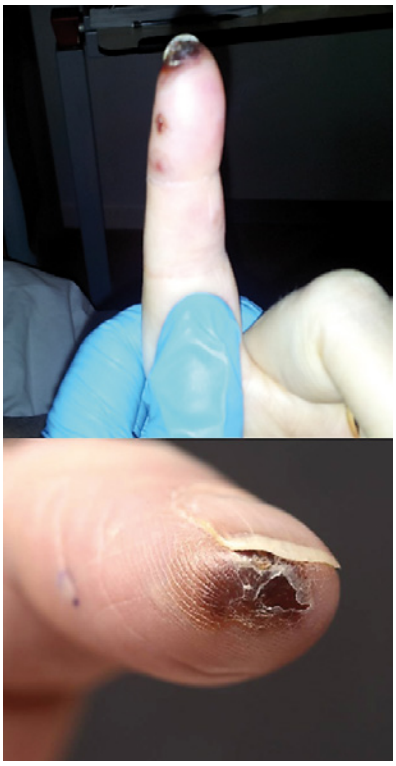

A purple finger after kidney transplantation; what is your diagnosis?

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Contents

EDITORIAL

From exposome to microbiome to infectome – pathogens vs. 'sanogens'

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In this issue of the journal, two reviews of major importance are published. Adriani *et al.*[1] give an extensive overview of the risk factors involved in community-acquired meningitis, and provide clinically useful recommendations for prevention and treatment of this often life-threatening disease. The authors identify four high-risk populations, defined by age, medical conditions leading to immunodeficiency, genetic susceptibility or anatomical defects. Lankelma *et al.*[2] report on the recent developments concerning the microbiome, describing the ongoing shift from association studies to intervention studies and clinical trials. In their review, the human gut microbiota is discussed as an organ, involved both in homeostasis and the maintenance of health, and in disease states.

During the previous two decades, our appreciation of microbes has changed dramatically. Microbes were viewed as either directly causing disease, as in the case of bacterial meningitis, or as mere bystanders, like our skin flora, only sporadically causing disease in specific situations, as in a catheter-related sepsis with a coagulase negative staphylococcus. It is only recently that the active role of microbes – not only in causing disease, but also in preventing it – has been recognised and has led to a completely new research field.

Nowadays it becomes clear that direct causality is something uncommon in medicine. In genetic analysis, it has become apparent that monogenic disorders are rare and most diseases are the result of a complex genetic interplay involving many genes. Likewise, the old concept of one pathogen causing one disease is shifting towards a more systems biology based approach. Health and disease can be viewed as the outcome of the interaction between all environmental triggers, both exogenous and endogenous, which we are exposed to in a lifetime, and our genetic make-up. This total of environmental triggers was termed the 'exposome'.[3] Quantification of exposures in time in relation to health or disease is challenging – just as the influence of single genes is difficult to determine in a genome-wide association study (GWAS). However, it has already been proven possible, with modelling and analysis in a manner similar to GWAS in a study focusing on diabetes mellitus.[4]

In a recent review paper, Bogdanos *et al.*[5] introduce the concept of the 'infectome', referring to the part of the exposome reflecting the infectious triggers an individual encounters during life. The authors propose that autoimmunity is generated by a cascade of infectious triggers. It all starts with inflammation, and through cell destruction, autoantigen release, molecular mimicry and activation of autoreactive lymphocytes, it eventually leads to autoimmune disease. In this view, the microbiome is the definition of the collection of microbial genes in a particular region of the body, such as the gut or the oral cavity. The infectome relates to the total of infectious organisms associated with the disease in question, in all body sites. By studying the infectome, and combining these results with the results from GWAS, we may start to understand the connection between genome and exposome. This will enable us to gain insight into the development of disease, which is the first step needed for prevention. Furthermore, as in the microbiome research field, we might be able to identify protective factors, microbes that are working with us against the formation of autoimmunity or severe sepsis. As opposed to pathogens, we might find evidence for 'sanogens', and in the end may even want to use those sanogens in treatment or prevention.

How will this change medical practice? In the coming years, systems biology and modelling will become more integrated in our professional lives, with an increasing need for bio-mathematicians, translating the vast amount of data into clinically useful conclusions. Modelling of infectious diseases as described by Bogdanos *et al.* will greatly influence public health and health politics. Preventive measures will become more personalised. The

public health field will rely more and more on *big data* and the output of systems biology models.

In the end, however, despite all preventive possibilities, people will still get sick. In the age of computational medicine, we will hopefully be able to further understand the totality of determinants of health and disease and to use this knowledge to prevent disease in a more effective way. But for that one patient in your Emergency Department room, presenting with fever and meningism, knowledge of risk factors and treatment options will remain life-saving.

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REVIEW

Risk factors for community-acquired bacterial meningitis in adults

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ABSTRACT

Bacterial meningitis is a life-threatening infectious disease with high mortality and disability rates, despite availability of antibiotics and adjunctive therapy with dexamethasone. Several risk factors and predisposing conditions have been identified that increase susceptibility for bacterial meningitis. Such risk factors can consist of medical conditions resulting in immunodeficiency, host genetic factors or anatomical defects of the natural barriers of the central nervous system. These factors can increase the risk of meningitis in general or result in a specific risk of meningococcal or pneumococcal meningitis, the two most important causes of bacterial meningitis, which are characterised by distinct host-pathogen interactions. In this review we describe several risk factors for community-acquired bacterial meningitis in adults and discuss what preventive measurements can be taken in these populations.

KEYWORDS

Bacterial meningitis, *S. pneumoniae*, risk factors, predisposing factors

INTRODUCTION

Bacterial meningitis is a life-threatening infection of the central nervous system.[1] *Streptococcus pneumoniae* and *Neisseria meningitidis* are the leading causative organisms, with the first being responsible for two-thirds of the cases in Western Europe and the United States.[2,3] Mortality is approximately 20% in high-income countries despite available treatment with antibiotics and dexamethasone, and is several times higher in low-income countries.[4,5] Approximately half of the survivors have neurological sequelae such as hearing impairment, focal motor deficits or cognitive impairment.[6]

Invasive disease by pneumococci and meningococci is preceded by nasopharyngeal colonisation. Asymptomatic colonisation of pneumococci and meningococci occurs in up to 100% and 18%, respectively, of the normal population.[7] In some persons these common colonisers are able to breach the mucosal barrier, survive in the blood stream and cross the blood-brain barrier to cause meningitis. Several factors have been identified that increase the susceptibility for bacterial meningitis. These can consist of specific age groups, medical conditions that cause immunodeficiency, host genetic factors and anatomical defects of the natural barriers of the central nervous system.[2] Patients with underlying conditions generally have a higher risk of having a poor outcome, and therefore identification of these groups and preventive measures such as vaccination are of the utmost importance (*table 1*).

Pneumococcal disease including meningitis is seen mostly in children below 2 years of age or adults over 50 years. Furthermore, susceptibility is increased in individuals with underlying conditions such as splenectomy or asplenic states and in children with cochlear implants. The use of immunosuppressive drugs, the presence of diabetes mellitus, a history of splenectomy, infection with HIV or alcoholism is found in 20% of adults with pneumococcal meningitis.[2] HIV infection affects the aetiology of acute meningitis, and is especially important in lower-income countries.[4] The meningococcus is the leading pathogen of meningitis in young children beyond the neonatal period and in young adults. Meningococcal disease has been associated with smoking, living in the same household as a patient (including students), and meningococcal disease in proxies.[8] Defects in innate immunity have been described to be associated with susceptibility to both pneumococcal and meningococcal infections within families.[2] In this

Table 1. *Acquired risk factors for bacterial meningitis and most common causative organisms*

Risk factor	Prevalence or incidence in Dutch population	Relative frequency[a]	Most common causative organism	Mortality
Elderly > 65 years	Prevalence 1,108,000[61]	37%[14]	*S. pneumoniae* *L. monocytogenes*	34%[14]
Splenectomy / hyposplenic state	Incidence 1000 spleen removals per year[62] Functional asplenia unknown	3%[39]	*S. pneumoniae*	25%[39]
Alcoholism	Prevalence 78,400 (adults between 18-65 year)[63]	4%-18%[22,26]	*S. pneumoniae* *L. monocytogenes*	33%[26]
HIV/AIDS	Prevalence 22,231[64]	In Western world: 1%[3]	*S. pneumoniae,* *Salmonella* spp.	24%[43]
Diabetes mellitus	Prevalence 110,880[65]	7-10%[22,23]	*S. pneumoniae,* *L. monocytogenes*	Unknown
Cancer	Incidence 101,000 per year[66]	Unknown	*S. pneumoniae,* *L. monocytogenes*	Unknown
Anatomical defect	Unknown	5%[22,56]	*S. pneumoniae*	Prone to recurrent meningitis, mortality in case of recurrent meningitis 15%[56]
Organ transplant recipients	Incidence 1200 per year[67]	5-10% of the patients CNS infections[38]	*S. pneumonia,* *L. monocytogenes,* *Nocardia*	Unknown

[a]Frequency: Percent of cases in adult patients with community-acquired bacterial meningitis.

review we describe risk factors for community-acquired bacterial meningitis, preventive measurements and treatment options.

AGE

The incidence of meningitis is highest in young children and the elderly.[9] In infants the waning passive immunity acquired from the mother and immature own immune system results in an increased susceptibility to bacterial infections. Due to the introduction of conjugated vaccines against *Haemophilus influenzae* type B and *S. pneumonia*, infants have become protected against these infections, and the age distribution of bacterial meningitis patients subsequently shifted. Whereas previously bacterial meningitis was mostly a disease of children and adolescents, currently most patients are elderly with an average age of 50.[3] In children, the common causative organisms have changed as a result of vaccinations, but clinical characteristics have not. Young infants with bacterial meningitis can present with nonspecific signs such as lethargy, poor feeding or irritability. Older children are more likely to present with typical signs and symptoms of bacterial meningitis.[3] The most common causative organisms are *S. pneumoniae* and *N. meningitidis* in infants older than one month (80% of cases in the Netherlands).[10] The remainder of cases is caused by group B streptococci, *E. coli, H. influenzae*, other Gram-negative bacilli, *L. monocytogenes* and group A streptococci. Recommended treatment consists of a third-generation cephalosporin (cefotaxime or ceftriaxone).[10] A meta-analysis of randomised trials showed a beneficial effect of dexamethasone in children in high-income countries in respect to hearing loss; in low-income counties this was not established.[2]

In the elderly, the function of the immune system declines, which has also been referred to as 'immunosenescence'.[11] Immunosenescence leads to increased susceptibility to infections in elderly patients, but also to reduced vaccine efficacy, and possibly autoimmune disease and cancer.[12,13] Elderly patients also frequently have comorbid conditions that affect the immune system. Symptoms can be atypical, and neck stiffness and headache are less frequently present, while focal neurological abnormalities occur more often.[2] The spectrum of causative bacteria is slightly different from younger adults, as *L. monocytogenes* is more frequently identified.[14] Vaccination of elderly patients with pneumococcal vaccines is currently not recommended in Dutch national guidelines because of a lack of evidence of effectiveness in the elderly.[15] Recommended treatment consists of ceftriaxone and amoxicillin (to cover for *L. monocytogenes*) in combination with adjunctive dexamethasone (*table 2*).

Table 2. *Treatment and preventive measures of bacterial meningitis in adult patients with a risk factor*[a]

Risk group	Treatment	Preventive measures
Splenectomy/hyposplenic states	Amoxicillin combined with a third-generation cephalosporin (ceftriaxone or cefotaxime) Dexamethasone 10 mg 4 times a day, 4 days	Pneumococcal vaccination every 5 years (PVC-23), meningococcal vaccinations every 3-5 years and *Haemophilus influenzae* type b vaccination (Hib) once. Consider providing patients antibiotics on demand[68]
HIV/AIDS	Amoxicillin combined with a third-generation cephalosporin (ceftriaxone or cefotaxime) If CD_4 count >200 dexamethasone 10 mg 4 times a day, 4 days If CD_4 count <200 no dexamethasone	Pneumococcal vaccination (controversial)
Diabetes mellitus, alcoholism, cancer	Amoxicillin combined with a third generation cephalosporin (ceftriaxone or cefotaxime) Dexamethasone 10 mg 4 times a day, 4 days	In case of diabetes regulation of blood glucose levels
Anatomical defect	Antibiotics: Amoxicillin combined with a third-generation cephalosporin (ceftriaxone or cefotaxime). Dexamethasone 10 mg 4 times a day, 4 days	If possible: repair of the leakage Otherwise: pneumococcal vaccination every 5 years (PVC-23), meningococcal vaccinations every 3-5 years and *Haemophilus influenzae* type b vaccination (Hib) once
Organ transplant recipients	Antibiotics: Amoxicillin combined with a third-generation cephalosporin (ceftriaxone or cefotaxime) Dexamethasone 10 mg 4 times a day, 4 days (consider differential diagnosis, dexamethasone can not be safely administered in all cases)	Pneumococcal vaccinations preferably before transplantation, type of vaccination depends on organ and organ disease[69]

[a]These recommendations apply to areas with a low rate of penicillin/cephalosporin-resistance of pneumococci, such as the Netherlands.

IMMUNODEFICIENCY

Life expectancy has increased due to advances of medical science and technology, and diseases that were previously uniformly lethal have now become chronic illnesses.[16,17] As a result, the number of immunocompromised individuals has increased over the past decades. Half of the patients with bacterial meningitis have predisposing conditions, one-third of these patients have an immunodeficiency.[3] Splenectomy, alcoholism, HIV, diabetes mellitus, cancer or the use of immunosuppressive medications have been recognised as risk factors for invasive bacterial infections including meningitis.[2] The most common causative organism of bacterial meningitis in patients with an immunocompromised state is *S. pneumoniae*, although other causative organisms may also be encountered in patients with specific risk factors.[2,3,18]

Diabetes mellitus

Diabetes mellitus has been reported as a risk factor for bacterial infections, and infection is an important cause of mortality in diabetic patients.[19-21] In reports of community-acquired bacterial meningitis in adults, diabetes was present before admission in 7-10%.[22,23] Common pathogens in diabetic patients with meningitis are *S. pneumoniae* and *L. monocytogenes*.[18,22,23] As diabetes is a risk factor for invasive pneumococcal disease in general, physicians in the United States and some European countries are advised to vaccinate diabetic patients with the 23-valent polysaccharide pneumococcal vaccine.[24] A large proportion of patients with bacterial meningitis are hyperglycaemic on admission, also of those without diabetes.[23] In these patients, high blood glucose levels are probably an epiphenomenon of severe illness. Blood glucose levels should be managed, although treatment-related hypoglycaemia should be avoided. Hypoglycaemia in bacterial meningitis is associated with unfavourable outcome and increased risk of major complications such as seizures and brain damage.[23] Adjunctive treatment with dexamethasone can cause further dysregulation of blood glucose levels,[25] so follow-up of blood glucose concentrations in all bacterial meningitis patients is warranted.

Alcoholism

Excessive alcohol use results in increased susceptibility to infections, probably due to a decreased innate and adaptive immune response.[26] It is unclear if alcoholism is an independent risk factor for bacterial meningitis. Patients who suffer from alcoholism have worse general

health, are more prone to head injury and more often have chronic liver disease,[26-28] all factors that have been associated with increased risk of bacterial meningitis. In a prospective nationwide observational cohort study of 696 episodes of bacterial meningitis, 4% of the patients were alcoholics.[26] Twenty-five patients with alcoholism suffered from a second episode of meningitis during this study, suggesting an association between alcoholism and bacterial meningitis, although a causal relationship remains unclear due to comorbid conditions. The most common causative organisms of bacterial meningitis in alcoholic patients were *S. pneumoniae* (70%) and *Listeria monocytogenes* (19%).[26] Alcoholics had a higher rate of complications compared with non-alcoholic patients (81% vs. 62%) and a higher rate of unfavourable outcome (67% vs. 33%). Complications most often consisted of cardiorespiratory failure due to underlying pneumonia or endocarditis. In a recent study, patients with bacterial meningitis and endocarditis were more often found to be alcoholics (17% vs. 4% in bacterial meningitis patients without endocarditis) and sometimes presented with the Austrian syndrome, consisting of pneumococcal meningitis, pneumonia and endocarditis.[29] Prognosis in these patients is generally poor.[29] Treatment should consist of amoxicillin and a third-generation cephalosporin combined with dexamethasone (*table 2*).

Cancer

Patients with cancer are more susceptible to infections for several reasons;[30] the disease itself can predispose to infections (especially haematological malignancies such as multiple myeloma) as does treatment with immunosuppressive chemotherapy, malnutrition and presence of indwelling venous catheters.[30-32] Patients with cancer were also found to have an attenuated inflammatory response in the central nervous system.[33]

Susceptibility for meningitis is particularly increased in patients with leukaemia and lymphoma; one-fourth of all central nervous system infections in cancer patients are found in this group. Patients with cancer who recently underwent neurosurgery are also at risk for meningitis; these patients account for 75% of the meningitis cases in cancer patients.[34] In a retrospective study in 77 patients with cancer, *S. pneumoniae* was the most common causative organism; other relatively frequent causative organisms included *L. monocytogenes* and *Cryptococcus neoformans*. Only 57% of these patients had cerebrospinal fluid (CSF) pleocytosis, reflecting the immunocompromised state of cancer patients.[33] Infections in these patients may go undetected because signs and symptoms can be atypical, resulting in a possible delay to diagnosis and treatment.[31,34] Another cause of diagnostic delay is deferral of lumbar puncture because of thrombocytopenia or intracranial space occupying lesions.[35] In high-risk cancer patients who are severely immunocompromised, vaccination or even prophylactic antibiotic treatment may be warranted.

Organ transplant recipients

Invasive pneumococcal infections occur more frequently in organ transplant recipients because of treatment with immunosuppressive therapy to prevent and treat rejection of the graft.[36] Liver transplant recipients have the highest incidence (354 per 100,000 persons per year), possibly due to a higher incidence of additional malfunction of the spleen. Time of onset of invasive pneumococcal disease after organ transplant is variable and the risk persists over time.[37] Other causative organisms include *Nocardia* (especially in case of multiple brain abscesses) and *Listeria monocytogenes*.[38] In organ transplant receivers with suspected bacterial meningitis, the differential diagnosis may include cryptococcal, tuberculous or other uncommon types of meningitis, in which adjunctive dexamethasone may be harmful without proper antibiotic coverage. Therefore, when bacterial meningitis is suspected in transplant patients, the potential benefit of adjunctive dexamethasone should be weighed against the risk of deterioration when it is caused by uncommon pathogens. The risk of invasive pneumococcal infections can be decreased by pneumococcal vaccination prior to transplantation. Administration of prophylactic antibiotics in these patients remains controversial, as there is a risk of resistance and recommendations in current guidelines vary.[37]

Splenectomy or hyposplenic state

Dysfunction or absence of the spleen predisposes to infections with encapsulated bacteria such as *S. pneumoniae* and *Haemophilus influenzae*.[39] A hyposplenic state can be congenital or acquired after surgical removal of the spleen (splenectomy). Furthermore, acquired functional hyposplenism occurs in 15-40% of the cases after allogeneic bone marrow transplantation and other causes of hyposplenism are graft-versus-host disease, sickle-cell anaemia, celiac disease and HIV infection.[40] The spleen is the only site in the human body where poorly opsonised bacteria such as *S. pneumoniae* can be cleared from the bloodstream and therefore hyposplenic patients are at risk for overwhelming sepsis caused by these bacteria.[39] Although this risk has been recognised for almost a century,[43] patients still do not receive adequate vaccination or information from their physicians about their risk of infection and when to seek medical attention.[39] Splenectomy or functional hyposplenia is an uncommon risk factor for bacterial meningitis and was identified in 24 of 965 cases (2.5%) of community-acquired meningitis in a prospective nationwide cohort study, and was associated with a high rate of mortality

(25%) and unfavourable outcome (58%). In this cohort study, all cases in patients with hyposplenia or asplenia were caused by *S. pneumoniae*. The increased risk of infections by encapsulated bacteria is life-long, and the median time between splenectomy or diagnosis of functional hyposplenia and the bacterial meningitis episode was found to be 16 years, with a range from 1-54 years.[39] In the majority of cases current guidelines for prevention of infection were not adhered to.[39,42] Preventive measurements such as sufficient and repeated vaccinations, patient education on how to recognise meningitis, and prescription of prophylactic antibiotics could be used to reduce the number of cases of bacterial meningitis in these individuals.[39,42]

HIV/AIDS

HIV-infected individuals have a 6- to 324-fold increased susceptibility for invasive pneumococcal disease.[2] The increased susceptibility is caused by a decreased humoral immunity: B-cell activation is impaired, resulting in decreased IgM synthesis and both T-cell dependent and T-cell independent differentiation of B-cells is impaired.[43] This results in a decreased humoral response to bacteria. In more advanced stages of HIV these disturbances are more pronounced and therefore most episodes of bacterial meningitis occur in individuals with a CD4 cell count of less than 200 cells per cubic millimetre.[43] Most of these patients are severely immunocompromised and do not take antiviral therapy. The most common causative bacterial agent of bacterial meningitis in HIV-infected individuals is *S. pneumoniae*, but *Salmonella* meningitis also occurs more frequently compared with HIV-negative controls.[2] In case of low CD4 counts, the differential diagnosis of bacterial meningitis is broad; tuberculous meningitis, cryptococcal meningitis and toxoplasma meningoencephalitis should be considered.

Ninety percent of the worldwide HIV-infected individuals live in low-income countries and bacterial meningitis is at least ten times more common in these regions. Diagnosis of bacterial meningitis in patients with HIV can be extremely difficult in resource-poor settings, with no imaging available and limited laboratory facilities.[4] Furthermore, antibiotic resistance and partially treated bacterial meningitis is an increasing problem in Africa, where antibiotics are often sold 'over the counter'; approximately 5% of the *S. pneumoniae* isolates have reduced susceptibility to ceftriaxone.[4]

A prospective cohort study in Spain performed in 2009 showed that there are several differences between HIV-infected and HIV-negative meningitis patients in high resource settings.[43] In this study 32 episodes of bacterial meningitis in HIV-infected patients were compared with 267 episodes in HIV-negative patients. HIV-infected patients with bacterial meningitis presented with more severe disease, reflected by a higher rate of altered mental state, focal neurological deficits and concomitant pneumonia. Leukocyte counts in the CSF were lower in HIV-infected patients, probably reflecting an impaired immune response.[43]

In the United States national guidelines advise pneumococcal vaccinations in HIV-infected individuals.[44] Because of a presumed low prevalence of pneumococcal infections in patients with HIV in the Netherlands, Dutch national guidelines only advise pneumococcal vaccination in case of concomitant use of intravenous drugs and state that in other HIV-infected individuals vaccination can be considered.[15] Studies that assessed vaccine efficacy of pneumococcal vaccinations in case of HIV infection have varying and conflicting results.[45] HIV-infected patients with CD4+ T-lymphocyte counts less than 500 cells/ul often have lower responses to pneumococcal vaccination.[44] Despite these uncertainties, vaccination is considered safe in this risk group and as the potential benefit is substantial, vaccination is advised.[46,47]

HOST GENETIC SUSCEPTIBILITY

The risk of acquiring bacterial infections is largely heritable, as shown by family, twin and adoption studies.[7,48] Several genetic traits for developing meningococcal and pneumococcal disease have previously been described in extreme phenotype studies, of which most concerned genetic deficits in genes coding for complement system proteins.[7] An increased incidence of invasive meningococcal meningitis was found in patients with a properdin deficiency and with deficiencies in the complement system (C5, C6, C7, C8 and, possibly, C9) in extreme phenotype studies.[2] A review and meta-analysis of genetic risk factors for invasive pneumococcal and meningococcal disease showed genetic variation in *NFKBIA*, *NFKBIE*, and *TIRAP* genes was protective, whereas *PTPN22* and MBL2 variants were associated with increased susceptibility for pneumococcal disease.[7] Increased susceptibility to invasive meningococcal disease was associated with genetic variants in the CFH, SFTPA2, CEACAM3, and CEACAM6 genes, whereas other CEACAM6 and SFTPA2 variants showed a protective effect.[7]

Recently, studies have addressed specific genetic risk factors for bacterial meningitis.[25,49-51] A genetic association study in adult bacterial meningitis patients showed that a common single nucleotide polymorphism (SNP), the complement factor 3 gene (C3), was associated with susceptibility for bacterial meningitis (OR 4.50, 95% CI 1.62-12.50).[49] Interestingly, the protective genotype of this SNP was previously shown to increase the risk of age-related macular degeneration, showing that a more readily activated complement system may protect

against bacterial infection while simultaneously increasing the risk of autoimmune disease. In the same cohort, polymorphisms in mannose binding lectin (MBL2) resulting in MBL deficiency were shown to increase the risk of pneumococcal meningitis.[52] Patients homozygous for variant alleles in exon1 of *MBL2* had eightfold (OR 8.21, 95% CI 1.05-64.1) increased susceptibility for pneumococcal meningitis. Also, high CSF MBL levels were associated with poor disease outcome and increased disease severity, suggesting that the lectin pathway is a possible target for adjunctive therapy in pneumococcal meningitis.[52]

After an experimental meningitis model showed *N. meningitidis* passes the blood-brain barrier by using the beta2-adrenoceptor,[53] genetic variation in the gene coding for this receptor (*ADRB2*) was shown to influence the risk of bacterial meningitis (OR 1.35, 95% CI 1.04-1.76).[54] Non-selective beta-blockers down-regulate this receptor, and therefore could be tested as a target in experimental studies to see whether it may decrease susceptibility to bacterial meningitis.[54]

Two studies from a Dutch paediatric cohort showed that susceptibility to bacterial meningitis was associated with genetic polymorphisms in Toll-like receptor (TLR) 2, 4, and 9 and nucleotide oligomerisation domain-like receptor 2.[50,51] TLRs and nucleotide oligomerisation domain-like receptors (NODs) are pathogen-recognising receptors and SNPs in genes coding for these receptors are thought to influence susceptibility, but also disease severity and outcome of bacterial meningitis by altering the inflammatory response.[50,51] TLR4 and NOD2 SNPs were significantly associated with susceptibility to meningococcal meningitis in children, and carriage of a combination of the risk alleles in TLR2/TLR4 and TLR4/NOD2 SNPs showed an even stronger association.[51] TLR9 SNPs were associated with a reduced susceptibility to meningococcal meningitis in this study.[50] Identification of genetic risk factors for bacterial meningitis may be used in future studies to identify pathophysiological mechanisms and could change vaccination policy in patients with established genetic risk factors.

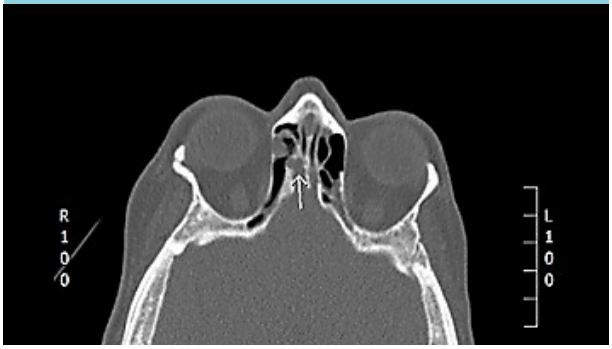
ANATOMICAL DEFECTS

The blood-brain barrier separates the central nervous system from the circulation and is an important defence mechanism against bacteria invading the central nervous system. It also maintains a stable environment inside the nervous system and potentiates molecular transport between blood and brain. Disruption of this barrier can occur following trauma, surgery, congenital defects or ear and sinus infection and provides an entry for bacteria.[55] When a patient has recurrent episodes of bacterial meningitis a disruption of the blood-brain barrier should be suspected, especially when the causative organism is *S. pneumonia*, but also *N. meningitides*, *S. aureus* and *H. influenzae* are possible causative organisms in case of a CSF leak.[56] With atypical causative organisms, or recurrent meningococcal meningitis, an immunodeficiency (e.g. complement or properdin deficiency) should be suspected. When no apparent cause of recurrent meningitis is identified on clinical grounds, diagnostic procedures should be performed to identify anatomical defects because of the high prevalence of this cause of recurrent meningitis (*figure 1*).[56] Optimal imaging consists of thin-slice CT of the skull base combined with MRI-3D constructive interference in steady-state imaging. It is important to take into account that small bone defects on CT do not prove CSF leakage.[56] Overt CSF leakage is seldom identified on presentation and most leaks resolve spontaneously within the first 24-48 hours. In case of a persistent CSF leak 7-30% of the patients develop meningitis, and this risk increases with the duration of the leakage.[55] There can be a delayed onset of several years between trauma and bacterial meningitis.[58-60] With an anatomical defect and/or CSF leakage present, a neurosurgeon or otolaryngologist should be consulted to evaluate the possibility of repairing the defect. Furthermore, vaccinations should be administered in case of persistent CSF leakage. Treatment with prophylactic antibiotics in these patients remains controversial but may be considered in patients with recurrent meningitis despite vaccination.[56]

VACCINATION AFTER BACTERIAL MENINGITIS

The risk of a recurrence of bacterial meningitis in patients with no anatomical defect or known immunodeficiency is estimated to be 1-3%.[56] Most of these cases are caused by

Figure 1. *Computed tomographic imaging: Anatomical defect of the fovea ethmoidalis on the right side in a 57-year-old woman, with a history of three episodes of pneumococcal meningitis and prior sinus surgery*



S. pneumoniae. The risk of a second meningitis episode is substantially increased compared with the risk of a first episode in the general population. Although the efficacy of pneumococcal vaccination in this population has not been studied, it appears logical to vaccinate all patients following an episode of pneumococcal meningitis, considering the substantially increased risk and lack of side effects of vaccination. This paradigm shift needs follow-up evaluation in cohorts of bacterial meningitis patients to determine whether the risk of recurrent pneumococcal disease is reduced.

CONCLUSION

Bacterial meningitis is a potentially life-threatening disease and several conditions that increase susceptibility are identified. Awareness of risk factors for meningitis may facilitate early recognition and treatment of the disease, and vaccinations can prevent cases of bacterial meningitis in various risk groups. Sufficient preventive measures in selected groups of individuals with increased risk for bacterial meningitis may reduce the number of cases.

DISCLOSURES

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REVIEW

The gut microbiota in internal medicine: implications for health and disease

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ABSTRACT

The human gut microbiota may be viewed as an organ, executing numerous functions in metabolism, development of the immune system and host defence against pathogens. It may therefore be involved in the development of a range of diseases such as gastrointestinal infections, inflammatory bowel disease, allergy and diabetes mellitus. Reversely, certain therapies that are often used, such as antibiotics and chemotherapy, may negatively affect the composition and function of the gut microbiota and thereby the wellbeing of patients. As the microbiota research field is currently moving from association studies to intervention studies and even clinical trials, implementation of this new knowledge into clinical practice is coming near. Several therapeutic interventions that target the gut microbiota are being evaluated, ranging from supplementation of food components to transplantation of faecal microbiota. In this review we provide an overview of current literature on the gut microbiota in both a healthy state and a range of diseases that are relevant for internal medicine. In anticipation of gut microbiota-targeted therapies, it is important to realise the key function of the gut microbiota in physiological processes and the collateral damage that may be caused when disrupting this ecosystem within us.

KEYWORDS

Antibiotics, diabetes, gut microbiota, inflammatory bowel disease, internal medicine

INTRODUCTION

The field of microbiota research has exploded in recent years. In the first six months of 2014, over 100 peer-reviewed articles were published on this topic on each consecutive day. The awareness that commensal microorganisms are not simple bystanders in our bodies, but instead play key roles in physiology and pathology, has excited scientists and clinicians in almost every discipline of medicine.[1-3] The unprecedented success of faecal transplantation as a potential cure for recurrent *Clostridium difficile* infection has become the gem of the microbiota research field;[4] still, the gut microbiota is suspected of having an important role in a whole range of diseases.[5]

'The last human organ' plays a major role in the development of the immune system, the defence against pathogens and the metabolism of fatty acids, glucose and bile acids (*figure 1*).[6] Of special interest is the degradation of otherwise non-fermentable dietary fibre such as resistant starch into short-chain fatty acids (SCFA), mainly by bacteria from the Bacteroidetes phylum.[7] These SCFA – particularly acetic, propionic and butyric acid – have anti-inflammatory and immune-signalling properties and are an energy source for epithelial cells.[8]

Our gut contains ten times more bacteria than our bodies contain cells of our own.[9] Moreover, the collective microbiome is 150 times larger than the human genome, indicating the astonishing number of processes that the intestinal microbes are involved in.[9] It remains to be determined, however, if this new wealth of knowledge will affect clinical medical practice. In this review we provide an overview of the current literature on the role of the gut microbiota in health and disease from the perspective of the internist.

COMPOSITION OF THE GUT MICROBIOTA

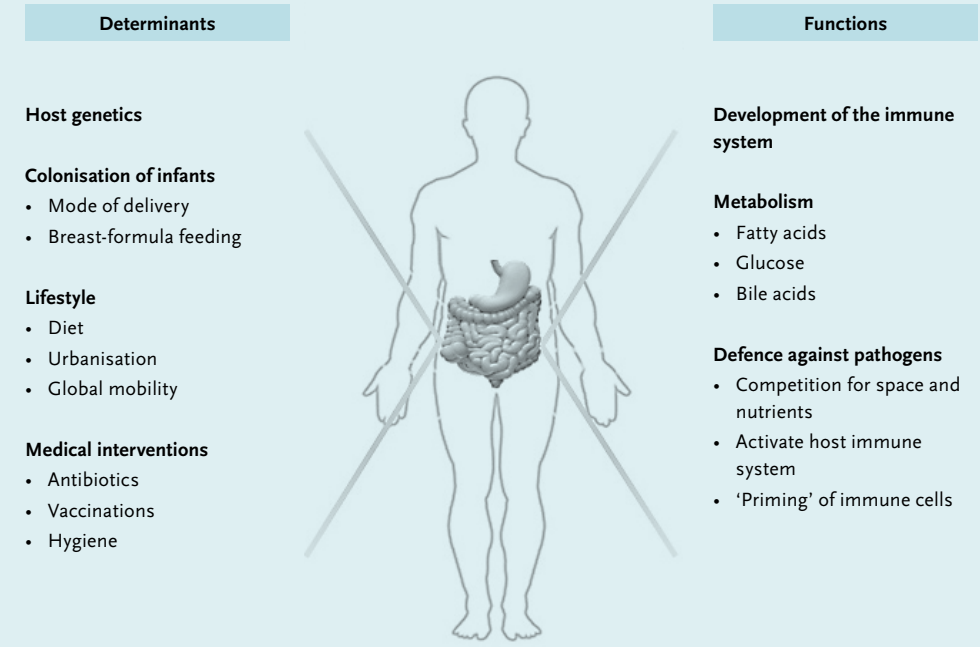
Determining the exact composition of the gut microbiota has been difficult due to the limited success of culturing (often anaerobic) bacteria. With the development of specialised microarrays and high-throughput sequencing techniques, our knowledge on this subject has started to expand quickly (*textbox*).[9,10] The new insights gathered with these new techniques start right at birth: we now know that we are not born sterile, as low levels of bacteria are detected in meconium and umbilical cord blood.[11] Major colonisation, however, starts after birth and is influenced by many factors such as the genetics of the host, mode of delivery, breast or formula feeding, nutrition and antibiotics. The diversity of the gut microbiota increases in the first years of life,[11] after which the core composition appears to remain relatively constant during adult life:[12,13] 60% of all gut microbiota strains in healthy adults remain detectable over a period of five years, following a power law that suggests that this core is present for a much longer period.[13,14]

Each individual has their own unique microbiota. Even between healthy persons the composition may vary strongly as was shown in over 1000 adults.[10,15] However, when looking at a functional level, most people carry equal amounts of bacterial genes involved in metabolic pathways. This suggests that the microbiota as a functional organ is similar among human beings.[10] Diet is one of the most important determinants of the microbial composition.[16] A study in healthy adults showed that an extremely fat-rich diet is capable of changing the microbiota in just a few days.[17] Still, pinpointing causal relationships between the absence or overabundance of bacterial strains and clinical observations is difficult. Few prospective and intervention studies have been performed hitherto.[13,17,18] With older age, inter-individual differences in gut microbiota composition become larger. For instance, long-term care residency is associated with lower bacterial diversity.[19] Still, the largest and most direct effects on microbial composition are achieved by antibiotics.

INFLUENCE OF ANTIBIOTICS

A simple antibiotic course, prescribed to kill just one or a few pathogens, will quickly result in major disturbances of the gut microbiota (*table 1*).[20] Recovery and stabilisation of its diversity level may take months, and the new composition of bacteria may significantly differ from pre-treatment. Not only bacteria that fall within the spectrum of the antibiotic are affected; species that are dependent on the ones being killed may disappear as well. Vancomycin, for example, does not only lead to the disappearance of Gram-positive species, but also Gram-negative species and results in more dramatic shifts

Figure 1. *Determinants and functions of the intestinal microbiota*



Investigating the gut microbiota

Techniques

Classic microbiological culturing techniques have proved inadequate in characterising the gut microbiota as a whole; sequencing techniques are therefore essential. High throughput methods have been introduced, sequencing either total DNA ('shotgun metagenomics') or 16S ribosomal RNA- genes.[9,10] 16S rRNA contains nine sequences that are highly variable between species and have been well conserved during evolution; therefore it is suitable as a key identifier of bacteria.

Two major reference databases serve as the basis of our knowledge of the structure and function of the human gut microbiota. The US Human Microbiome Project (HMP; www.hmpdacc.org) is a consortium that has reported the structure and function of the human microbiome in 242 healthy individuals at 18 body sites from a single time point, using 16S ribosomal RNA gene pyrosequencing.[10] Similarly, the European Metagenomics of the Human Intestinal Tract (MetaHIT; www.metahit.eu) project has studied faecal samples from 124 healthy, overweight and obese Europeans, as well as IBD patients, by total DNA metagenomic sequencing.[9]

Analysis

One of the major outcome measurements is species diversity, which is defined as the effective number of different species that are represented in a dataset. Results are often expressed as:

1. Species richness and species evenness, which together make up diversity (also termed alpha diversity – see below)
   - Species richness: number of different species represented in an ecological community
   - Species evenness: the relative abundance with which each species is represented in the community. An ecosystem where all the species are represented by the same number of individuals has high species evenness
2. Alpha and beta diversity
   - Alpha diversity : within-sample species diversity
   - Beta diversity: similarity between samples (from one individual at different time points or between samples from different subjects)
3. Different indexes, such as:
   - Shannon index: quantifies the degree of uncertainty when predicting an individual's type within a particular dataset
   - Simpson index: quantifies the probability that two entities taken at random from the dataset represent the same type

than amoxicillin.[21,22] Antibiotics may thus have negative effects on the health of patients, for example on their metabolic state. Use of antibiotics has been associated with the development of obesity, diabetes mellitus and asthma.[2]

THE ROLE OF THE GUT MICROBIOTA IN DISEASE

Gastrointestinal infections

A healthy gut microbiota protects the host directly against pathogens such as *C. difficile*, by competition for nutrients, space and binding spots on the epithelium and production of bacteriocins, but also indirectly, by activation of the host immune system resulting in release of IgA, cytokines and antimicrobial peptides.[23] Depletion of the microbiota by antibiotics therefore creates an opportunity for *C. difficile* to proliferate.

Multi-drug resistant organisms are a rapidly increasing problem around the world. Vancomycin-resistant enterococci (VRE) are now a leading cause of bloodstream infections in haematopoietic stem cell transplant recipients.[24] In both mice and men, domination by VRE (being at least 30% of the microbiota) correlates with subsequent development of a bloodstream infection[25] and in mice can be reversed by faecal microbiota transplantation (FMT).[24] This suggests that faecal transplantation therapy might be effective in both prevention and treatment of VRE infections. Assessment of faecal microbiota may be used to identify those patients at highest risk for bloodstream infection.[25]

The first randomised clinical trial for FMT in recurrent *C. difficile* infection was stopped prematurely because of a clear advantage in the FMT arm: 15 of 16 patients had complete resolution of disease, compared with four of 13 patients in the vancomycin arm and three of 13 patients in the vancomycin + bowel lavage arm.[4] European recommendations now include FMT for patients with multiple relapses of *C. difficile* infection.[26]

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is hypothesised to be either an aberrant immune response against normal

Table 1. *Overview of studies on the effect of antibiotics on the human intestinal microbiota*

Author	Antibiotic regimen	Subjects	Short-term results (days, weeks)	Long-term results (months, years)
De la Cochetiere et al., 2005[56]	Amoxicillin, orally, 5 days	6 adult healthy volunteers	Major shift in dominant species after 24 hours Average similarity (compared with pre-treatment) 74% after 4 days	1-2 month: average similarity 88-89%
Penders et al., 2006[57]	Mostly amoxicillin	28 paediatric patients (1 month old)	Decreased counts of *Bifidobacteria* and *B. fragilis* species compared with non-treated children	None reported
Jernberg et al., 2007[58]	Clindamycin, orally, 7 days	4 adult healthy volunteers (vs. 4 controls)	Day 7, 21: large and persistent shift in composition	3, 6, 9, 12, 18 and 24 months: large and persistent shift in composition
Dethlefsen et al., 2008[59]	Ciprofloxacin, orally, 5 days	3 adult healthy volunteers	Abundance of about a third of the bacterial taxa in the gut affected; decreased taxonomic richness, diversity, and evenness of the community	1 and 6 months: richness, diversity and evenness comparable to pre-antibiotic state, some long-term losses
Dethlefsen et al., 2010[20]	Ciprofloxacin, orally, 5 days; after 6 months again 5 days	3 adult healthy volunteers	Loss of diversity and shift in composition within 3–4 days; 7 days after end of a course, communities start returning to initial state; often incomplete	10 months: composition stabilised but altered; long-term losses of some taxa
Jakobsson et al., 2010[60]	Metronidazole + clarithromycin, orally, 7 days	3 adult healthy volunteers (vs. 3 controls)	Dramatic decline in diversity, especially loss of Actinobacteria, in both throat and faeces	1 year: diversity levels recovered to pre-treatment states
Fouhy et al., 2012[61]	Parenteral antibiotic treatment with ampicillin and gentamicin (within 48 h of birth)	9 paediatric patients (plus 9 untreated infants)	4 weeks after treatment, antibiotic-treated infants had higher proportions of Proteobacteria and lower proportions of Actinobacteria as well as the genus *Lactobacillus*	2 months: Proteobacteria levels remained higher, but Actinobacteria and *Lactobacillus* levels had recovered
Panda et al., 2014[62]	Different broad-spectrum antibiotic regimens, orally	21 adult patients	Fluoroquinolones and b-lactams decreased microbial diversity by 25% and reduced the number of taxa from 29 to 12; Increase in proportion Bacteroidetes taxa B-lactams increased the average microbial load two-fold	None reported

commensal bacteria, or a normal immune response against abnormal gut microbiota, both driven by an autoimmune genetic background.[27] Indeed, IBD patients have a significantly lower microbial diversity compared with healthy controls[28] as well as a lower abundance of immune-modulating bacteria such as *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*.[5,29] *F. prausnitzii* and *A. muciniphila* produce butyrate and propionate, respectively, both SCFA that are thought to protect the mucosal barrier. A meta-analysis of 41 cases in which FMT was used for ulcerative colitis found that 63% of patients entered remission and 76% could stop medications.[28] Obviously, these numbers may suffer from publication bias. Thirteen clinical trials are currently ongoing to evaluate the effect of FMT in IBD (*table 2*).

Transplant immunology

Intestinal inflammation secondary to graft-versus-host disease (GVHD) is likely to be influenced by the gut microbiota. A mouse model of GVHD caused by allogeneic stem cell transplantation (SCT) showed that mice developing intestinal GVHD have a decreased bacterial diversity compared with those that do not.[30] A greater proportion of bacteria consisted of Lactobacillales, while Clostridiales were decreased. The same pattern was found in 31 patients receiving allogeneic SCT, probably

Table 2. *Current status of microbiota-related therapies on clinicaltrials.gov and trialregister.nl (September 2014)*

Gastrointestinal infections	• First randomised trials in *C. difficile* completed, with promising results for FMT in severe recurrent infections • 21 clinical trials for FMT in *C. difficile* infections registered • First case report describing FMT clearing extended spectrum beta-lactamase producing *Escherichia coli* (ESBL) colonisation in end-stage renal disease patient[64]
Inflammatory bowel disease	• Thirteen clinical trials currently registered for FMT in IBD (both Crohn's disease and ulcerative colitis)
Transplant immunology	• No microbiota-related clinical trials being conducted yet
Colorectal cancer	• No microbiota-related clinical trials being conducted yet
Allergic airway diseases	• > 50 trials registered for prebiotics or probiotics for treatment or prevention of allergic airway disease, mostly in infants
Metabolic disorders	• Two randomised trials for FMT in metabolic syndrome registered, one completed • One randomised trial for FMT in recently diagnosed diabetes mellitus type 1 registered • One randomised trial for FMT in diabetes mellitus type 2 registered

FMT = faecal microbiota transplantation; IBD = inflammatory bowel disease.

due to chemotherapy, irradiation and antibiotics. The percentage of *Enterococcus faecium* and *Enterococcus faecalis* (of the order Lactobacillales) in the microbiota was 21% in patients who did not develop intestinal GVHD, 46% in those that subsequently developed GVHD and 74% at the time of actual GVHD.[31] The diversity level at the time of engraftment seems to predict outcome: after classifying 80 SCT patients into low, intermediate, and high diversity groups, overall three-year survival was 36%, 60%, and 67% respectively.[32]

Colorectal cancer and response to chemotherapy

The intestinal microbiota play a key role in mucosal immune responses, epithelial cell homeostasis, barrier function and metabolism, all of which are important for tumour formation. For example, the microbiota produce (precursors of) carcinogenic substances, such as nitrate.[5] A strictly animal-based diet (meats, eggs and cheeses) of only five days in healthy volunteers increased bile acids produced by the microbiota, including deoxycholic acid which promotes DNA damage and hepatic carcinoma.[17] Accordingly, bile-tolerant bacteria such as *Bilophila wadsworthia* were increased, which in mice have been described to induce IBD by producing hydrogen sulphide.[17] This supports the association between diets that are rich in red meat and the development of colorectal cancer.[33] Faecal transplantation from colon tumour-developing mice to germ-free mice resulted in a higher incidence of colon tumours in the latter, compared with transplantation from control mice – most likely by inducing inflammation prior to induction of tumorigenesis by dextran sodium sulphate in drinking water.[34]

The gut microbiota also influences the effectiveness of anticancer therapy.[35-37] In different cancer mouse models, it was shown that in the absence of a healthy microbiota, tumour-infiltrating myeloid cells are less capable of producing cytokines and inducing cytotoxicity upon immunochemotherapy or platinum chemotherapy, resulting in decreased necrosis of tumours.[35] Both cytotoxic drugs and irradiation may cause translocation of bacteria or bacterial particles to lymph nodes, thereby enhancing immune responses directed against malignant cells.[36,37] Destruction of the microbiota by antibiotics and other drugs may therefore reduce the efficacy of these treatments.[35-37]

Allergic and infectious airway diseases

Low diversity of the gut microbiota, whether caused by low exposure to environmental microbes or (prenatal or postnatal) use of antibiotics, is associated with increased risk of allergic airway disease.[38,39] The intestinal microbiota is postulated to enhance the haematopoiesis of dendritic cell precursors in bone marrow by production of SCFAs.[40] Intriguingly, dendritic cells from mice that have received propionate exhibit an impaired ability to activate T-helper 2 cells in the lung, thus preventing allergic airway

inflammation.[40] Supplementation of dietary fibre or SCFA may therefore prevent or reverse allergic airway disease.
A similar 'priming' effect by healthy gut microbiota has been observed in murine models of pneumonia and peritonitis.[41-43] Depletion of the gut microbiota by antibiotics in neonatal mice appears to hamper neutrophil production by the bone marrow, thus leading to increased counts of pathogenic bacteria and decreased survival.[43] It is suggested that lipopolysaccharide from gut bacteria is the priming component, although another role for SCFA is not excluded. For now, evidence for this 'gut-lung axis' in humans is lacking.

Metabolic disorders

Obesity has been associated with a lower microbial diversity in the intestine.[44,45] Studies have reported an association between obesity and increased levels of Firmicutes, combined with decreased Bacteroidetes (the two most dominant phyla), although this could not be replicated in all cohorts.[5,46] Overweight and obese individuals have higher levels of SCFA, without a different intake of dietary fibre, reflecting either an increased production or decreased absorption by the gut and utilisation by bacteria.[46]
In both mice and men, there is evidence that the obese microbiota is more effective at harvesting energy from food, as suggested by enriched biochemical pathways.[5,47] Accordingly, transplantation of gut microbiota from obese to germ-free mice leads to more weight gain than transplantation of microbiota from control mice.[47] Transplantation of faeces from lean human donors into patients with metabolic syndrome decreases peripheral insulin resistance six weeks after transplantation. This is potentially caused by increased levels of butyrate-producing bacteria (*Roseburia intestinalis*).[48] By contrast, among 20 children of 3-5 years of age, levels of butyrate-producing bacteria were also found to be higher in those that subsequently developed type 1 diabetes.[49] Of interest, seven days of vancomycin therapy resulted in decreased peripheral insulin sensitivity in ten males with metabolic syndrome.[22] This was associated with a decrease in Firmicutes bacteria and decreased metabolism of bile acids.

From theory to therapy

Although the role for the gut microbiota in the pathophysiology of disease may be easy to imagine, translation of this knowledge into new therapeutic strategies represents a tremendous challenge. Changing one's intake of specific nutrients would be the most natural way to prevent diseases. Consumption of dietary fibre seems one of the best solutions, since they are fermented into SCFA that may have beneficial effects. Both extreme diets[17] and the use of various fibres were shown to modify the human intestinal microbiota, although the latter not in all subjects.[50]
Probiotics that include a variety of lactic acid bacteria are by now commonplace in both the supermarket and the clinic, but their effectiveness is highly debatable.[51] Moreover, higher mortality in patients treated with lactic acid bacteria during severe pancreatitis compared with placebo treatment has led to increased caution regarding these bacteria, notably in a clinical setting.[52]
The successful trial on severe *C. difficile* infection has put the spotlight on FMT.[28] However, we currently do not know how to select a microbiota that is best to transplant. In addition, the possibility of transplanting pathogenic microorganisms, or the possible risk of metabolic and autoimmune diseases, might be a reason for caution. So far, no serious (long-term) adverse events have been reported.
Transplanting a cocktail of bacteria (the synthetic microbiota) that can be cultured or isolated easily, may be a key objective the coming years.[53] The first steps were already taken in 1989, when ten selected intestinal strains that showed inhibitory effects against *C. difficile* cured five patients with *C. difficile* infection.[53,54] More recently, two *C. difficile* infected patients were transplanted with 33 cultured bacterial species, isolated from healthy donor faeces – both resulting in resolution of disease and no relapse in 24 months.[23] These results encourage the development of synthetic, standardised microbiota.

CONCLUSION

The booming popularity of the gut microbiota in biomedical research seems justified; in fact, the interest in host-microbiota interactions and their potential value as a therapeutic target seems rather belated in view of our ancient commensal relationship. Obviously, results from animal studies cannot be translated directly to human health and disease. Still, some of the possible microbiota-disease interactions will prove to be relevant and applicable in everyday clinical practice. The great possibilities offered by this new field of research provide hope for new, relatively straightforward and inexpensive therapies. However, most research is currently in a preclinical phase, and causative relationships remain to be established. Moving from correlation to causality will be particularly difficult for phenotypically and/or genetically heterogeneous disorders, such as diabetes, IBD and GVHD.[55] In anticipation of gut microbiota-targeted therapies, it seems important to realise the key function of the gut microbiota in physiological processes and the potential collateral damage we cause when disrupting this well-balanced ecosystem.

DISCLOSURES

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ORIGINAL ARTICLE

The validity of national hospital discharge register data on dementia: a comparative analysis using clinical data from a university medical centre

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ABSTRACT

Background: Most information on the incidence and prognosis of dementia comes from small studies with limited precision and generalisability. Nationwide registers can be an alternative source of information, but only when the diagnosis is validly recorded. We assessed the validity of the Dutch Hospital Discharge Register (HDR).

Methods: HDR data on dementia diagnoses (ICD-9 codes 290.0; 290.1; 290.3; 290.4; 294.1; 331.0; 331.1; 331.82) in a university medical centre in the Netherlands were collected. Diagnoses were verified by using hospital medical records. Positive predictive values (PPVs) were calculated. Multivariate logistic regression models were used to evaluate determinants of inaccuracy in discharge diagnoses.

Results: A sample of the HDR data was used for this study (n = 340). PPV was 93.2% for overall dementia, indicating confirmation of 93.2% of HDR dementia diagnoses by the medical records. The accuracy of the diagnosis of overall dementia in patients aged ≥ 65 years was significantly higher compared with younger patients (PPV 95.5 % vs. 67.9%; p = 0.0001). There was no difference in the accuracy of the diagnosis between men and women and accuracy was not influenced by type of admission, comorbidity and polypharmacy.

Conclusion: The results of this study show a high validity of the diagnosis of overall dementia in the HDR, making this register of great value for further nationwide research on dementia.

KEYWORDS

Dementia, ICD codes, hospital register, prognosis, validity

INTRODUCTION

Dementia is one of the major causes of morbidity and mortality among older people. There is a great need for reliable methods to elucidate mechanisms and risk factors underlying the poor prognosis of dementia and to give insights into morbidity and mortality risks to reduce this burden. As a national dementia registry is not available in most countries, evidence on morbidity and mortality risks is only available from small specific population studies.[1,2] These studies have limited precision, though, and generalisability may be questioned. Therefore, confirmation from large long-term population-based studies is needed. However, those studies are complex, expensive and time consuming, especially in this population where loss to follow-up is common as a result from accelerated cognitive decline and mortality.[3,4]

The potential of using linkage methods to create large disease-specific cohort studies is increasingly being recognised. Existing nationwide administrative registries and databases are linked which enables estimation of, for example, age-sex specific mortality rates in an efficient and relatively inexpensive way. The validity of the outcomes from studies using these data, however, depends on the completeness and accuracy of the data (both disease status as well as disease outcome event (cause of hospitalisation and cause of death)) in the national registers and the accuracy of the linkages.

The validity of the diagnosis of dementia in national registries is largely unknown. Therefore, we aimed to assess the validity of dementia diagnoses in the Dutch nationwide Hospital Discharge Register (HDR). In the HDR, medical and administrative data of inpatients and day clinic patients visiting Dutch hospitals are routinely

recorded.[5] The results from this study will provide information about the usefulness of the HDR in future nationwide research on the prognosis of dementia.

METHODS

Dutch Hospital Discharge Register

Since the 1960s, medical and administrative data of admitted and day clinic patients visiting a Dutch hospital are recorded in the HDR; no information on outpatient visits is available. Circa 100 hospitals participate in the register. The HDR contains information on patient demographics, principle and secondary diagnoses, and other admitting and discharge data. At the medical administration department of each participating hospital, a new record is created after each new hospital admission or day clinic visit by a professional clinical coder based on admission data and the discharge letter. The principle and secondary diagnoses are determined and coded using the ninth revision of the International Classification of Diseases – Clinical Modification (ICD-9-CM).[6]

Although investigators might consider ICD coding of causes for hospitalisation as inferior, there is sufficient evidence to suggest that the coding in the Netherlands is of a high level. It has been shown that 99% of the personal, admission and discharge data, and 84% of the principal discharge diagnoses (validated through medical record review by medical specialists) were correctly registered in a random sample of all hospital admissions registered in the HDR.[7] Positive predictive values (PPVs) of registration in the HDR have shown to be 97% for acute myocardial infarction, 95% for subarachnoid haemorrhage, 91% for intracerebral haemorrhage, 98% for non-ruptured abdominal aortic aneurysm (AAA), 97% for ruptured AAA and 80% for congestive heart failure.[8-10]

Cohort enrolment

We used a random sample of 340 patients aged 40 years and older registered with a principal or secondary diagnosis of dementia (ICD-9 code 290.0; 290.1; 290.3; 290.4; 294.1; 331.0; 331.1; 331.82) in the HDR of the University Medical Center Utrecht between 1 January 2006 and 31 December 2010. The University Medical Center Utrecht is one of the largest healthcare facilities in the Netherlands. The day/memory clinic serves as a secondary and tertiary care referral centre. Patients are referred to the day/memory clinic either in case of memory-related disorders (memory clinic) or with multi-morbidity which might also include memory-related disorders (day clinic). The study was conducted in accordance with privacy legislation in the Netherlands.

Data collection

Information on each patient acquired from the HDR included: patient hospital number, age, gender, admission date, type of hospital contact (admission vs. day clinic) and the principal and secondary diagnoses according to the ICD-9 code.

Medical records of the hospital wards (admissions) and memory/day clinic (day clinic visits) that belong to the selected patients were reviewed. Information on each patient acquired from these medical records included: diagnosis made by the physician, medical history/comorbidity and medication use/polypharmacy. To obtain an overview of the medical history of all patients, presence of comorbidity according to the Charlson Comorbidity Index (CCI) was collected from the medical records using Deyo's coding algorithm.[11] This index does not completely cover comorbidity, but contains 17 major comorbidities. All comorbidities were defined dichotomously (yes or no). Use of medication was evaluated to assess the presence of polypharmacy. Polypharmacy was defined as the use of five or more regular drugs, excluding temporary drugs (e.g. antibiotics).[12]

Validation

Diagnosis of dementia reported in the medical records by the treating physician was considered the reference diagnosis. Routine clinical care in all patients who visited the day clinic comprised a standardised diagnostic work-up including a comprehensive geriatric assessment, neurological and physical examination, blood tests and on indication neuropsychological testing. Patients who visited the memory clinic also underwent a standardised extensive neuropsychological assessment. If there was an indication for neuroimaging, patients underwent a computed tomography or magnetic resonance imaging scan. Diagnosis of dementia, and its subtypes, was made at a multidisciplinary consensus meeting based on internationally accepted criteria.[13-17] All patients admitted to the geriatric ward received a similar comprehensive geriatric assessment. Patients underwent neuroimaging and a standardised extensive neuropsychological assessment on indication. Patients admitted to other departments where formal cognitive testing was not a routine did not receive a comprehensive geriatric assessment. The majority of these patients had a history of dementia and were consequently coded with a secondary diagnosis of dementia. It was not possible to determine whether these patients had previously undergone formal cognitive testing.

Statistical analysis

Continuous data were summarised as mean and standard deviation or as median and interquartile range where

appropriate. Categorical data were summarised as proportions.

First, we determined whether patients were correctly classified in the HDR as having dementia (overall dementia; any dementia disorder). Positive predictive values (PPVs) were calculated with 95% confidence intervals (CIs) and defined as the number of patients diagnosed with dementia based on the medical records, divided by the total number of patients coded with dementia in the HDR. Differences between PPVs were analysed by the chi-square test or Fisher's exact test where appropriate. Multivariate logistic regression models were used to evaluate determinants of inaccuracy in diagnoses of dementia in the HDR. The determinants included in the multivariate models were age, gender, and comorbidity (CCI). In a second multivariate model, comorbidity was replaced by polypharmacy (comorbidity and polypharmacy were not included simultaneously, because these variables were highly correlated).

Secondly, we evaluated the accuracy of the two most common dementia subtypes, Alzheimer's disease (AD) and vascular dementia (VaD), in the HDR. An ICD-9 code for mixed-type dementia (most common is a combination of AD and VaD) does not exist; therefore, patients diagnosed with mixed-type dementia at the hospital ward or memory/day clinic were considered correctly classified in the HDR if they received the following codes: 331.0 (AD), 290.40 (VaD) and 290.0 (senile dementia).

During the validation procedure, we noticed that a large number of the patients registered with ICD-9 code 290.0 (senile dementia) in the HDR were diagnosed with AD according to the treating physician. Therefore, we additionally studied whether patients registered with ICD-9 code 290.0 in the HDR were representative for patients with AD. We calculated the PPV of ICD-9 code 290.0 for the diagnosis of AD. As in future the ICD-9 code 290.0 in the HDR might be used (in addition to ICD-9 code 331.0) to answer prognostic research questions concerning AD, we performed multivariate logistic regression analysis to assess whether there were differences with regard to prognostic determinants between patients registered with ICD-9 code 290.0 in the HDR with and without the reference diagnosis of AD (or mixed-type dementia) according to the treating physician, in a similar approach as described before. Since AD and VaD are the most common subtypes of dementia and since numbers of other dementia subtypes were rather low we only evaluated the validity of AD and VaD.

Data were analysed using the SPSS 20.0 statistical package (SPSS Inc, Chicago, Illinois, USA). A two-sided p value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

In total 340 medical records of patients admitted between 2006 and 2010 were used in this study. Patient characteristics are shown in *table 1*.

Validation procedure

Overall dementia

Overall dementia was present in 317 patients (PPV 93.2%; 95% CI 90.0-95.5) based on the reference diagnoses of the treating physicians (*table 2*). There was no significant difference in PPV for men vs. women: 91.6% (95% CI 85.7-95.2) and 94.4% (95% CI 90.2-97.0) respectively (p = 0.29). The PPV significantly increased with age:

Table 1. *Baseline characteristics*

	Total *N = 340*
Age, years (median and IQR)*	80 (74-84)
• < 65 years (n)	28
• ≥ 65 years (n)	312
Gender	
• Female (%)	58.2
Type of admission (%)	
• Outpatients (%)	46.5
- Memory clinic	95.6
- Day clinic	4.4
• Inpatients (%)	53.5
• Admitted at (%)	57.7
- Geriatric ward	14.3
- Neurology ward	12.6
- Internal medicine ward	10.4
- Surgical ward	4.9
- Psychiatry ward	
ICD-9 code†	
• 290 (senile dementia, uncomplicated)	66.2
• 290.1 (presenile dementia)	10.8
• 290.3 (senile dementia, with delirium)	0.6
• 290.4 (VaD)	6.8
• 294.0 (dementia classified elsewhere)	14.4
• 331.0 (AD)	0.9
• 331.1 (FTD)	0.3
• 331.82 (DLB)	0
Polypharmacy (%)	
• No drugs	6.8
• 1-4 drugs	37.5
• ≥ 5 drugs	55.2
Comorbidity (sum of categories CCI)‡ (%)	
• No comorbidities	35.1
• 1 comorbidity	39.2
• 2 comorbidities	17.4
• ≥ 3 comorbidities	8.3

*IQR = interquartile range; †AD = Alzheimer's disease; VaD = vascular dementia; DLB = dementia with Lewy bodies; FTD= frontotemporal dementia; ‡CCI = Charlson Comorbidity Index.

Table 2. *Validity of dementia diagnosis in the HDR using diagnosis made by the treating physician as reference*

	TP N	FP n	PPV %	95% CI	P
Dementia, overall					
• All	317	23	93.2	90.0 - 95.5	0.29
• Men	130	12	91.6	85.7 - 95.2	
• Women	187	11	94.4	90.2 - 97.0	
Age					
• < 65 years	19	9	67.9	49.2 - 82.2	<0.01
• ≥ 65 years	298	14	95.5	92.6 - 97.4	
Type of admission					
• Inpatient	173	9	95.1	90.7 - 97.5	0.15
• Day/memory clinic	144	14	91.1	85.6 - 94.8	
Polypharmacy					
• < 5 drugs	141	11	92.8	87.4 - 96.0	0.62
• ≥ 5 drugs	176	11	94.1	89.7 - 96.8	
Comorbidity					
• No comorbidity	112	7	94.1	88.2 - 97.3	0.82
• 1 comorbidity	122	11	91.7	85.7 - 95.5	
• 2 comorbidities	56	3	94.9	85.5 - 98.8	
• ≥ 3 comorbidities	26	2	92.9	76.3 - 99.1	

HDR = Hospital Discharge Register; TP = true positive; FP = false positive; PPV = positive predictive value; CI = confidence interval.

67.9% (95% CI 49.2-82.2) for patients < 65 years (n = 28) vs. 95.5% (95% CI 92.6-97.4) for patients ≥ 65 years (n = 312) (p < 0.01). Furthermore, analyses showed no difference in PPV regarding the setting of diagnosis (admission versus memory/day clinic visit), number of comorbidities and presence of polypharmacy. Multivariate analysis showed similar results with a significantly higher probability of an accurate diagnosis for patients ≥ 65 years compared with patients < 65 years (adjusted odds ratio (OR) 10.5; 95% CI 3.7-30.3).

Alzheimer's disease

In total 228 patients were registered with either ICD-9 code 290.0 (senile dementia) or 331.0 (AD) in the HDR. Three patients were diagnosed with ICD-9 code 331.0, all correctly classified as AD according to the reference diagnosis reported by the treating physician (PPV 100%). Of the 225 patients registered with ICD-9 code 290.0, 141 patients were diagnosed with AD according to the reference diagnosis reported by the treating physician (PPV 62.7%; 95% CI 56.2-68.7). In total 144 of the 228 patients with either ICD-9 code 290.0 or 331.0 were correctly classified as AD, which resulted in a PPV of 63.2% (95% CI 56.7-69.2%) (table 3). Diagnoses in the HDR from memory/day clinic patients were more accurate compared with diagnoses in the HDR from admitted patients (PPV 78.2%; 95% CI 69.8-84.7% vs. 46.8%; 95% CI 37.7-56.1% (p < 0.01)). There were no significant differences in accuracy between the wards (geriatric versus other wards). Other variables did not significantly affect the accuracy.

In total 84 of the 225 patients (37.3%) registered in the HDR with ICD-9 code 290.0 did not have AD according to the reference diagnoses reported by the treating physicians. Of these 84 patients, 36 (42.9%) were diagnosed with dementia not otherwise specified according to the treating physicians, 22 patients (26.2%) with VaD, ten patients (11.9%) with dementia with Lewy bodies, five patients (6.0%) with frontotemporal dementia, five patients (6.0%) with Parkinson's dementia, one patient (1.2%) with mild cognitive impairment and five patients (6.0%) were not demented.

As in future research the ICD-9 code 290.0 in the HDR might be used (in addition to ICD-9 code 331.0) to answer questions on prognosis concerning AD, prognostic determinants (age, gender, comorbidity) were assessed to see whether there were differences between patients registered in the HDR with ICD-9 code 290.0 with versus without the reference diagnosis of AD (or mixed-type dementia), according to the treating physician. This multivariate analysis showed no statistically significant differences in age, sex and comorbidity between patients with the reference diagnosis of AD (or mixed-type dementia) and patients without this reference diagnosis.

Similarly, a multivariate analysis was performed using polypharmacy as a covariate instead of number of comorbidities. This analysis showed that patients with the reference diagnosis of AD (or mixed-type dementia) were less likely to have polypharmacy compared with patients without this reference diagnosis (adjusted OR 0.5; 95% CI 0.3-0.9). Polypharmacy was present in 47.5% of the

patients with the reference diagnosis of AD compared with 61.9% of the patients without the reference diagnosis of AD.

Vascular dementia

In total 23 patients were registered with VaD in the HDR (ICD-9 code 290.40). According to the reference diagnoses reported by the treating physicians, two patients were improperly classified as VaD patients, resulting in a PPV of 91.3% (95% CI 72.0-98.8%) (*table 3*). There were no significant differences in PPV according to age, gender, setting of diagnosis, and comorbidity. All patients had polypharmacy.

DISCUSSION

The results in this study indicate that the validity of using HDR codes to identify patients with dementia is high. Overall PPV was 93.2%. The accuracy was neither

Table 3. *Validity of AD and VaD diagnosis in the HDR using diagnosis made by the treating physician as reference*

	TP n	FP n	PPV %	95% CI	*p*
AD (ICD-9 code 290.0 + 331.0)					
All	144	84	63.2	56.7 - 69.2	0.99
• Men	55	32	63.2	52.7 - 72.6	
• Women	89	52	63.1	54.9 - 70.6	
Age					0.30
• < 65 years	4	5	44.4	18.8 - 73.4	
• ≥ 65 years	140	79	63.9	57.4 - 70.0	
Type of admission					<0.01
• Inpatient	51	58	46.8	37.7 - 56.1	
• Day/memory clinic	93	26	78.2	69.8 – 84.7	
Polypharmacy					0.041
• < 5	75	32	70.1	60.8 - 78.0	
• ≥ 5	69	52	57.0	48.1 - 65.5	
Comorbidity					0.47
• No comorbidity	58	26	69.1	58.5 - 78.0	
• 1 comorbidity	53	36	59.6	49.2 - 69.2	
• 2 comorbidities	19	15	55.9	39.4 - 71.1	
• ≥3 comorbidities	13	7	65.0	43.2 - 82.0	
VaD (ICD-9 code 290.4)					
All	21	2	91.3	72.0 - 98.8	0.12
• Men	12	0	100		
• Women	9	2	81.8	51.2 - 96.0	
Age					0.75
• < 65 years	1	0	100		
• ≥ 65 years	20	2	90.9	72.0 - 98.7	
Type of admission					0.44
• Inpatient	16	2	88.9	66.0 - 98.1	
• Day/memory clinic	5	0	100		
Polypharmacy					n.a.
• < 5	0	0	0		
• ≥ 5	21	2	91.3	72.0 - 98.8	
Comorbidity					0.73
• No comorbidity	2	0	100		
• 1 comorbidity	5	1	83.3	41.8 - 98.9	
• 2 comorbidities	8	1	88.9	54.3 - 100	
• ≥3 comorbidities	6	0	100		

AD = Alzheimer's disease; VaD = vascular dementia; ICD-9 code 290.0 = uncomplicated senile dementia; ICD-9 code 331.0 = Alzheimer's disease; ICD-9 code 290.4= vascular dementia; HDR = hospital discharge register; TP = true positive; FP = false positive; PPV = positive predictive value; CI = confidence interval; n.a. = not applicable.

influenced by gender and setting of diagnosis (admission or day/memory clinic) nor by number of comorbidities and polypharmacy. Multivariate analysis showed a significantly lower validity in patients younger than 65 years versus those older than 65 years, which is in line with a previously performed study reporting on over-registration of dementia in relatively young patients.[18] Overestimation might result from a broader differential diagnosis of dementia in younger patients. Often, extensive diagnostic strategies and much longer time are needed before definite confirmation of diagnosis of dementia is possible. Consequently, in younger patients who are registered with dementia in the HDR, the medical files from the doctor more often reveal conversion of dementia diagnosis to another diagnosis. Our results are consistent with two previously performed studies in Northern Europe, showing PPVs of dementia discharge diagnosis close to and more than 90%.[19,20]

Accuracy regarding registered dementia subtype diagnoses was also high, but less reliable. Although PPV for code 290.4 (VaD) and code 331.0 (AD) was 91.3% and 100% respectively, the numbers of patients within these groups were unexpectedly low (23 and 3 respectively), while AD and VaD contribute to the two most common causes of dementia worldwide.[21] During the validation procedure, we noticed that a large number of the patients diagnosed with AD according to the treating physician were registered as senile dementia (ICD-9 code 290.0). Similar findings were reported by Phung et al.[19] This could be explained by the fact that the specific subtype of dementia is often diagnosed in a two-step procedure:[22] 1) identification of dementia (syndrome) during the first visit and 2) identification of the underlying disease (i.e. subtype of dementia) during follow-up visits, usually at the outpatient clinic after additional investigations, such as neuropsychological testing and imaging. In many cases the final diagnosis, including an underlying disease, has therefore not been made at the first visit/discharge. As a consequence, the discharge diagnosis is coded as senile dementia (ICD 290.0). Since follow-up data of outpatient visits are not available in the HDR, the ICD-9 code will not be adjusted after the conclusive diagnosis is reached. Furthermore, in traditional literature senile dementia is often used when referring to AD.[23] For this reason, clinical coders might choose to register AD diagnoses with ICD-code 290.0. Both explanations might contribute to the high number of diagnoses registered with ICD-9 code 290.0.

We additionally studied whether patients registered with ICD-9 code 290.0 in the HDR were representative for patients with AD. PPV was modest at 63.2% but overall comparability with respect to prognostic determinants between patients registered in the HDR with ICD-9 code 290.0 with versus without the reference diagnosis of AD (or mixed-type dementia) was high. This implies that the ICD-9 code 290.0 (in addition to ICD-9 code 331.0) can be used to select a representative group of patients in further research with the focus on the prognosis of AD.

The validity of diagnosis of AD in the HDR (codes 290.0 and 331.0) from patients of the day/memory clinic was superior to the validity of diagnosis of AD in the HDR from admitted patients (p=0.0001). Admitted patients tend to have higher incidences of delirium and associated symptoms which could be (incorrectly) registered by clinical coders as ICD-9 code 290.0. Secondly, since hospital admissions are more often associated with multiple diagnoses and procedures, the primary and secondary diagnosis might be a matter of opinion of the clinical coder, creating the potential for inaccuracy.[24]

Strengths

This is one of the few validation studies about dementia registration in the HDR and is therefore of great value for further research on the prognosis of dementia. We had access to all medical journals of the included registered patients with dementia between 2006 and 2010 to validate the HDR. Furthermore, the distribution of dementia diagnoses in this study reflects the general distribution of dementia worldwide, which makes it a representative sample for further research.[21]

Limitations

The present study showed data from one university hospital in the Netherlands, which may impede nationwide generalisability. However, diagnoses are routinely registered by clinical coders at the medical administer department of a hospital (either academic or not academic) in accordance to a structured procedure using the predefined ICD-9 codes. A previous study that studied the general validity of the HDR using data from 55 hospitals (and coders) showed that 84% of registered diagnoses were correct.[7] Thus, the potential of problems with generalisability is less likely.

Furthermore, even in small hospitals without special geriatric or elderly care, generalisability is not jeopardised since we found no significant differences in validity between the different wards (geriatric, neurology, internal medicine, surgical, or psychiatric). Diagnosis of dementia in a small hospital will then also be made by other doctors such as neurologists and psychiatrists which will not influence the accuracy.

Clinical implications

This study underlines the potential use of HDR data in future research. Although the HDR does not contain data on outpatients, it is a valid and useful registry of inpatients and day clinic patients. ICD codes 290.0; 290.1; 290.3; 290.4; 294.1; 331.0; 331.1; 331.82 can be used for initial case finding to construct a nationwide cohort of dementia

patients, hospitalised and/or memory day clinic domain. Several important questions concerning the prognosis can be answered, since there is a great need to elucidate differences in prognosis of patients with dementia. With the use of a nationwide cohort of dementia patients, short- and long-term morbidity and mortality risks can be assessed and changes over time can be explored. We showed the potential to use the ICD code 290.0 in combination with ICD code 331.0 to identify AD patients although PPV was lower. Furthermore, PPV for VaD diagnosis was shown to be high.

CONCLUSION

In conclusion, we found that the validity of using HDR codes to identify patients with dementia is high. Furthermore, we showed the potential of using the ICD-9 code 290.0 (senile dementia) to select a representative group for AD patients although PPV was lower. Overall, the HDR constitutes a very useful starting point for nationwide research on the prognosis of dementia.

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DISCLOSURES

The authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Risk factors for gas-related complications of peroral endoscopic myotomy in achalasia

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ABSTRACT

Background: Peroral endoscopic myotomy (POEM) is a novel endoscopic technique for the treatment of achalasia. However, there are POEM-associated complications, the most common of which being gas-related. The aim of the current study was to determine the occurrence of and risk factors for gas-related complications of POEM in patients with achalasia.

Methods: Retrospective analyses were performed on the clinical data of 216 achalasia patients receiving POEM at our hospital during the period from August 2011 to November 2013. Univariate and multivariate analyses were conducted to look for potential risk factors for gas-related complications.

Results: The rate of gas-related complications was 10.2% (22/216). Univariate analyses indicated that simple longitudinal mucosal incision, tunnel width ≤ 3 cm, sigmoid-type oesophagus, myotomy depth and operative time were risk factors for gas-related complications ($p < 0.05$). Multivariate analyses indicated that simple longitudinal mucosal incision, tunnel width ≤ 3 cm and sigmoid-type oesophagus were risk factors for the complications ($p < 0.05$).

Conclusions: Simple longitudinal mucosal incision, tunnel width ≤ 3 cm and sigmoid-type oesophagus are independent risk factors for gas-related complications for achalasia during POEM, but not myotomy depth and operative time.

KEYWORDS

Achalasia, complications, peroral endoscopic myotomy, risk factors

INTRODUCTION

Since Inoue *et al.*[1] applied peroral endoscopic myotomy (POEM) to treat achalasia in 2010, its safety and efficacy have been evaluated in both animal studies and clinical trials.[2-6] However, various studies have demonstrated the presence of some POEM-associated complications, such as infections, pleural effusion, haemorrhage, mucosal perforation and gastrointestinal tract fistula. The most common complications are gas-related, such as subcutaneous emphysema, pneumothorax and pneumoperitoneum. The chance of occurrence varied greatly among different reports and could be as high as 100%.[7,8] Although many patients could obtain relief through conservative treatment, these complications might cause discomfort, extend the duration of the operation and even result in pulmonary atelectasis and respiratory failure in severe cases, when invasive interventions are needed to relieve the symptoms.[8] Thus, it is of vital importance to detect and prevent the occurrence of complications. Our objective was to determine the risk factors for gas-related complications so as to provide the rationale for preventing the occurrence of these complications.

MATERIALS AND METHODS

Patient clinical data

A total of 216 achalasia patients receiving POEM from August 2011 to November 2013 were recruited, comprising 109 males and 107 females with a mean age of 41.9 (14-74) years. Prior to POEM, the mean Eckardt score[9] and duration of symptoms were 6.2±1.7 and 4 (0.5-33) years, respectively. The Eckardt score grading was: II (n = 8), III (n = 127) and IV (n = 81). The grading of Henderson criteria[10] for the degree of oesophageal dilatation was: I (n = 21), II (n = 169) and III (n = 26). There were 11 cases of sigmoid-type

oesophagus. Those undergoing previous therapy with calcium antagonists or nitrates accounted for 1.9% (4/216), stenting 1.9% (4/216), balloon dilatation 9.3% (20/216) and laparoscopic Heller's myotomy 3.2% (7/216). Thirteen related risk factors were recorded: patient age, gender, symptom duration, previous therapies, Eckardt score, oesophageal dilatation degree, sigmoid-type oesophagus, simple longitudinal mucosal incision, tunnel length, tunnel width, myotomy depth, myotomy length and operative time. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008) as reflected in a priori approval by the institution's Human Research Committee. Informed consent was obtained from all patients before the procedure was performed. All patients were informed of the possible complications and other possible treatment options.

POEM procedures

POEM was performed under general anaesthesia via tracheal intubation using a single-channel endoscopy (GIF-Q260Z; Olympus, Tokyo, Japan) with a transparent cap (D-201-11802, Olympus) attached to the front. A carbon dioxide insufflator (UCR; Olympus) was used. Other equipment and accessories included a high-frequency generator (ICC 200; ERBE, Tübingen, Germany), an argon plasma coagulation unit (APC300; ERBE), a hybrid knife (ERBE, Erbe Elektromedizin GmbH) or a dual knife (KD-650Q; Olympus, Japan), an injection needle (NM-4L-1; Olympus), and haemostatic clips (HX-600-90; Olympus). The POEM procedure was performed as follows:

1. A submucosal injection (a mixed solution of 100 ml saline + 2 ml indigo carmine + 1 mg epinephrine) was made into right posterior oesophageal wall at a position about 10 cm above the oesophagogastric junction.
2. To create the tunnel entry, a longitudinal mucosal incision of over 2 cm, using a dual knife or a hybrid knife, was used in some patients while in the others submucosal dissection was performed with the range of at least 0.5 cm along both sides of the longitudinal incision. Then a submucosal tunnel was created, passing over the oesophagogastric junction, and about 3 cm into the proximal stomach.
3. The myotomy was performed starting from the position 2-3 cm distal to the mucosal entry point to the position 3 cm distal to the oesophagogastric junction in all patients. In some patients, selective myotomy of the circular muscle bundles was attempted. In this case, the longitudinal muscle bundles were carefully protected and left intact. In other patients, a full-thickness myotomy including the circular and longitudinal muscular layers was performed within a range of 6 cm from oesophagogastric junction.
4. After careful haemostasis, several metal clips were applied to close the mucosal entry site.

Figures 1 and *2* depict an example of the peroral endoscopic full-thickness myotomy procedure.

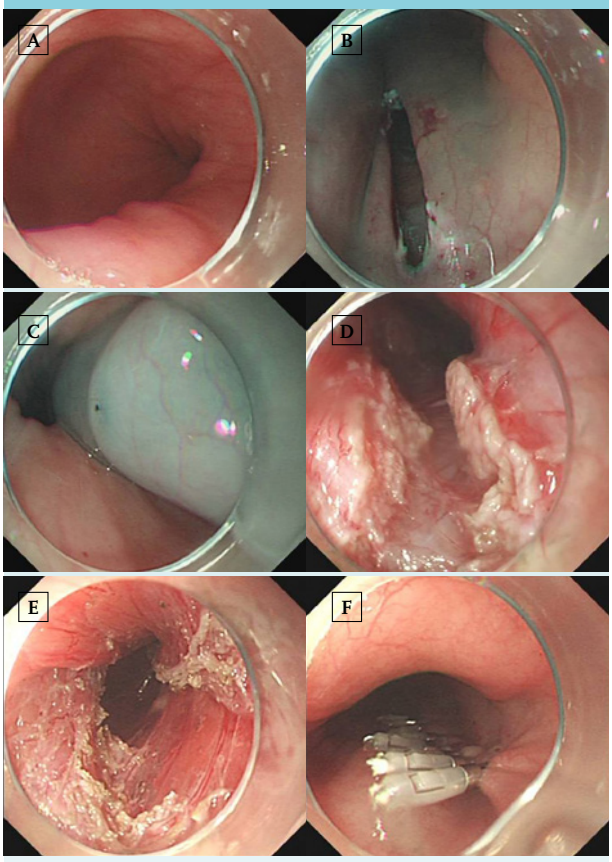
Postoperative measures

Patients received nothing by mouth for 24 hours and a liquid diet for three days, returning gradually to a normal diet within two weeks. Intravenous proton pump inhibitors (PPI) and antibiotics were used for three days. At Day 2 after operation, thoracoabdominal X-ray, or sometimes a chest CT, was performed to check for the occurrence of emphysema, pneumothorax, pneumoperitoneum and pleural effusion.

Follow-up

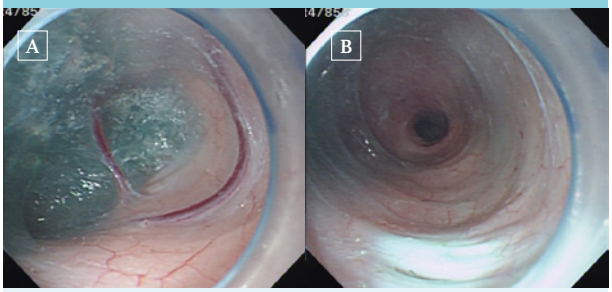
Patients were scheduled for a follow-up visit at 1, 3, 6 and 12 months after POEM and then annually for oesophagogastroduodenoscopy to observe the healing of the wound

Figure 1. *Procedure of peroral endoscopic myotomy (POEM)*



A. Oesophageal cleaning, a dilated and distorted oesophagus lumen was noted
B. Mucosal entry, submucosal dissection following a longitudinal incision was made
C. Endoluminal injection to guide the tunnel direction
D. Circular myotomy initiated 2-3 cm below tunnel entry
E. Full-thickness myotomy was performed within a range of 6 cm from the oesophagogastric junction
F. Several metal clips were applied to close mucosal entry

Figure 2. *Tunnel creation during POEM*



A. Tunnel width was larger than 3 cm, submucosal vessels can be visualised
B. Endoscopic view of cardia within submucosal tunnel

and check for any signs of reflux oesophagitis. They also underwent barium swallow to measure the oesophageal diameter. Patients were contacted by telephone to assess the occurrence of complications and obtain a current Eckardt score.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation. All data were analysed with SPSS17.0 statistical software. Chi-square or Fisher's test was used for univariate analyses and logistic regression for multivariate analyses.

RESULTS

Clinical efficacies

Of the 216 patients, 86 underwent a simple longitudinal incision, while the others underwent additional submucosal dissection of at least 0.5 cm along both sides of the longitudinal incision. The width of the submucosal tunnel was > 3 cm in 100 cases and ≤ 3 cm in another 116. Simple circular myotomy was performed in 83 cases, while full-thickness myotomy in 133 cases. The overall treatment success rate (Eckardt score ≤ 3) was 99.1% (214/216). The average operative time was 59.1 (38-120) min, and average follow-up period 16.8 (6-33) months. As compared with pre-operation, the Eckardt score decreased markedly at 12 and 24 months after the operation (0.54 vs. 6.1, 0.56 vs. 6.0, $p < 0.001$); the average oesophageal diameter decreased significantly at six months' post-operation (53.7 vs. 30.8 mm, $p < 0.001$). There was no recurrence during the follow-up period (recurrence was defined as an Eckardt score ≥ 4 at > 6 months post-operation).

Complications

Of the total patients, 28 (13.0%) had complications, namely gas-related complications (n = 22, 10.2%), tunnel mucosal perforation (n = 3, 1.39%), reflux oesophagitis (n = 2, 0.92%) and acute peritonitis (n = 1, 0.46%). The 22 cases of gas-related complications were subcutaneous emphysema (n = 21) and mediastinal emphysema plus subcutaneous emphysema (n = 1). For the 21 cases of simple subcutaneous emphysema, no special intervention was required, and all were absorbed spontaneously within 3-5 days. The patient with mediastinal emphysema plus subcutaneous emphysema had chest tightness, which disappeared after deflation through a subcutaneous puncture. For three cases of tunnel mucosal perforation, 1-2 clips were applied for closure. After an oral intake of PPI, the symptoms of patients with reflux oesophagitis were relieved. The patients with acute peritonitis had a postoperative onset of moderate fever (highest body temperature of 38.9°C), epigastric pain, epigastric tenderness and rebound tenderness. The symptoms subsided after a five-day course of antibiotics.

Risk factors for gas-related complications

Univariate analyses revealed that simple longitudinal mucosal incision, tunnel width, sigmoid-type oesophagus, myotomy depth and operative time were risk factors for gas-related complications (*table 1*). Multivariate analyses further indicated that simple longitudinal mucosal incision, tunnel width and sigmoid-type oesophagus were risk factors, but not myotomy depth and operative time. The occurrence of complications increased for those patients with a simple longitudinal mucosal incision, tunnel width ≤ 3 cm and sigmoid-type oesophagus (*table 2*).

DISCUSSION

Achalasia is an oesophageal motility disorder characterised by lack of peristalsis of the oesophageal body and impaired relaxation of the lower oesophageal sphincter with clinical symptoms including dysphagia, chest pain, regurgitation, and even coughing and aspiration pneumonia.[11] POEM is a novel technique for the treatment of achalasia with exciting results in both animal experiments and clinical studies.[2-6] As shown in an international prospective multicentre clinical trial, POEM has become an effective therapeutic modality for achalasia.[12]

As demonstrated by clinical trials, gas-related complications occurred frequently during POEM. Although the occurrence varies greatly in different reports, it may reach up to 100%.[7] The gas-related complications are known to include subcutaneous emphysema, pneumothorax, pneumoperitoneum and mediastinal emphysema. The cases with mild complications usually presented with merely a small amount of subcutaneous emphysema, which did not require any special intervention. In severe cases, however, vast gas accumulation may occur in the chest, abdominal cavity, mediastinum or under the skin. Sometimes acute respiratory and circulatory failure and

even death may occur. In such a setting, emergency invasive interventions of deflation via subcutaneous puncture and closed thoracic drainage should be taken for symptom relief.[8] In addition, severe emphysema in the patients may cause great suffering, increase the operative time and hospitalisation duration and increase the economic burden as well. Thus it is of vital importance to know how to prevent the occurrence of complications. As revealed by the results of multivariate analyses, simple longitudinal mucosal incision, tunnel width and sigmoid-type oesophagus are independent risk factors of gas-related complications during POEM. In the cases of simple longitudinal mucosal incision or tunnel width ≤ 3 cm, sigmoid-type oesophagus, the occurrence of gas-related complications increases dramatically.

Two methods, transverse and longitudinal incisions, are available for creating tunnel entry in POEM. Transverse incisions will not result in close contact between tunnel entry and the endoscope, facilitating gas discharge from the tunnel and lowering the occurrence of gas-related complications.[13] However, endoscopic closure of a transverse incision in the oesophagus is technically difficult or loose, which likely results in intra-tunnel infections and gastrointestinal tract fistula. In contrast,

Table 1. *Univariate analyses of risk factors for complications*

Risk factors	Classification	N	Complication (n)	Occurrence (%)	χ^2	P
Sex	Female Male	107 109	9 13	8.4 11.9	0.729	0.393
Age	~30 30~45 45~60 60~	41 93 61 21	3 9 7 3	7.3 9.7 11.5 14.3	0.892	0.827
Disease course	~1 1~5 5~10 10~	29 90 84 13	2 8 11 1	6.9 8.9 13.1 7.7	1.374	0.712
Previous therapies	Yes No	28 188	4 18	14.3 9.6	0.591	0.499
Eckardt score	I II III	8 127 81	0 13 9	0 10.2 11.1	0.983	0.612
Oesophageal dilatation depth	I II III	21 169 26	2 15 5	9.5 8.9 19.2	2.652	0.265
Sigmoid-type oesophagus	Yes No	11 205	7 15	63.6 7.3	36.198	0.000
Simple longitudinal mucosal incision	Yes No[a]	86 130	20 2	23.3 1.5	26.686	0.000
Tunnel length (cm)	~13 13~16 16~	148 61 7	17 4 1	11.5 6.6 14.3	1.280	0.527
Tunnel width (cm)	~3.0 3.0~	116 100	21 1	18.1 1.0	17.173	0.000
Myotomy depth	Circular Full-thickness	83 133	13 9	15.7 6.8	4.421	0.040
Myotomy length (cm)	~10 10~15 15~	80 133 3	9 13 0	11.3 9.8 0	0.464	0.793
Operative time (min)	<60 60-90 >90	122 86 8	7 12 3	5.7 14.0 37.5	10.498	0.005

[a]After longitudinal mucosal incision, bilateral transverse dissections ≥ 0.5 cm along submucosa.

Table 2. *Results of multivariate analyses for risk factors of complications*

Items	B	SE	Sig	OR	95%CI for OR
Constants	1.851	1.206	0.125	6.364	/
Simple longitudinal mucosal incision	-2.280	0.825	0.006	0.102	0.020,0.525
Tunnel width (cm)	-1.688	0.598	0.005	0.185	0.057,0.597
Sigmoid-type oesophagus	2.393	0.786	0.002	10.949	2.344,51.148

it is easy to close the entry site to the tunnel using longitudinal incisions. Nevertheless, close contact between endoscope and tunnel entry may ensue, which forms a high-tension status within the tunnel so that there were increased risks of gas-related complications. To effectively solve the above dilemma, we performed the subsequent submucosal dissection of at least 0.5 cm along both sides of the longitudinal incision. Our cohort study has shown that, when a simple longitudinal incision was used, the occurrence of gas-related complications was 23.

3% (20/86). However, the occurrence of gas-related complications was significantly reduced to 1.54% (2/130) when submucosal dissections for at least 0.5 cm were performed along both sides of the longitudinal incision. Therefore, we believe that subsequent submucosal dissection along both sides of the longitudinal incision while creating tunnel entry in POEM could effectively lower the occurrence of gas-related complications.

In addition, we found that tunnel width was an independent risk factor for gas-related complications. The occurrence was 18.1% (21/116) in the cases with a tunnel width ≤ 3 cm, but it decreased to 1% (1/100) in the cases with a tunnel width > 3 cm. These findings suggest that sufficient tunnel width might markedly reduce the risk of gas-related complications. Since the endoscope itself occupies a certain amount of space, the tunnel should be wide enough to ensure the presence of ample space between the instrument and tunnel during the procedure. In this way, sufficient space could not only reduce the difficulty in handling the endoscope within the tunnel, but also lessen the procedure-related injuries of tunnel mucosa, blood vessel, peri-oesophageal or gastric serous membrane. Moreover, this facilitates gas diffusion and prevents the formation of high pressure within the tunnel, particularly in an extremely twisting oesophagus. Inoue *et al.* recommended that tunnel width should be greater than or equal to one-third of the oesophageal circumference during POEM.[14] In our experience, to effectively lower the occurrence of gas-related complications during POEM, tunnel width should be > 3 cm for those patients without obvious oesophageal twisting.

Sigmoid-type oesophagus was another independent risk factor for the occurrence of gas-related complications. In the present cohort, the occurrence of gas-related complications for patients with sigmoid-type and non-sigmoid-type oesophagus was 63.6% (7/11) and 7.3% (15/205) respectively. Sigmoid-type oesophagus, especially type S2, was previously considered to be a relative contraindication of POEM.[15] Due to oesophageal twisting, it is difficult to create a submucosal tunnel in patients with sigmoid-type oesophagus. And the curvature interferes with gas discharging out of the tunnel, which might form a state of high pressure within the tunnel so as to cause such complications as subcutaneous emphysema, pneumothorax and pneumoperitoneum. In the present study, all the six patients with sigmoid-type oesophagus before March 2012 had an onset of gas-related complications with an occurrence of 100%. However, the occurrence of gas-related complications significantly dropped to 20% (1/5) in patients with sigmoid-type oesophagus thereafter. In our opinion, to reduce the occurrence of gas-related complications, more attention should be paid to the following points of caution:

1. A submucosal injection in the oesophageal cavity with a mix solution containing indigo was used to preset tunnel routes to ensure a straight tunnel into the proximal stomach, which helps avoid 'tunnel maze', and reduces gas accumulation within the tunnel caused by close contact between endoscope and tunnel;
2. During tunnelling, one should make great efforts to bypass the sites of the most serious oesophageal twisting or invagination so as to reduce the difficulty of tunnel creation and prevent tunnel obstruction;
3. The tunnel width of a sigmoid-type oesophagus should be broader than that of a non-sigmoid-type, even surpassing half of oesophageal circumference.

In addition, we also found that myotomy depth was not a risk factor for gas-related complications. During POEM, most researchers preferred to adopt a simple circular myotomy and preserve the longitudinal muscular

layer in order to reduce the chance of gas entry into the thoracic and abdominal cavity. However, some studies have demonstrated that the application of full-thickness myotomy did not increase the occurrence of complications. Moreover, full-thickness myotomy helps accelerate the functional recovery of oesophageal peristalsis and shorten operative duration.[7,16] Notably, the longitudinal oesophageal muscle layer is relatively thinner, and is closely connected with the circular muscle, but not with the peri-oesophageal membrane. Unintentional splitting of longitudinal muscle is likely to occur during simple circular myotomy, either due to intra-operative mechanical trauma or electrocautery damage. In our experience, to prevent tunnel perforation and gas-related complications during POEM, one of the most important steps is to maintain the integrity of peri-oesophageal or serous membrane. Yet the integrity of longitudinal muscle should not be overemphasized.

In conclusion, simple longitudinal mucosal incision, tunnel width and sigmoid-type oesophagus are independent risk factors for gas-related complications for achalasia during POEM, but not myotomy depth and operative time.

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DISCLOSURES

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CASE REPORT

A hypertensive emergency with acute visual impairment due to excessive liquorice consumption

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ABSTRACT

Hypokalaemic hypertension is the classical presentation of primary hyperaldosteronism but may also result from other mineralocorticoid activity, such as liquorice ingestion. Onset of hypertension as well as serum renin and aldosterone levels are central for the diagnosis. Liquorice ingestion has been reported to induce hypertension, hypokalaemia and metabolic alkalosis due to inhibition of the enzyme 11-β-hydroxy steroiddehydrogenase 2. Here, we report the case of a hypertensive emergency with acute visual impairment due to hypertensive retinopathy in clear conjunction with a considerable consumption of liquorice.

KEYWORDS

Hypertensive emergency, pseudohyperaldosteronism, mineralocorticoid excess, glycyrrhizin, liquorice

What was known on this topic?
Hypertensive emergencies require immediate therapy and a thorough diagnostic approach to identify the underlying cause. While hypokalaemic hypertension is the classical presentation of primary hyperaldosteronism, liquorice ingestion inhibiting the enzyme 11-β-hydroxysteroid dehydrogenase 2 and thereby inducing hypertension, is an important differential diagnosis.

What does this add?
Chronic regular liquorice consumption, even of a famous and frequently sold German sweet manufacturer, should be viewed as a potential culprit for the development of hypertension including hypertensive emergencies. Chronic liquorice intake should always be considered in adult hypokalaemic hypertensive patients.

CASE PRESENTATION

A 57-year-old Caucasian male patient with an unremarkable medical history presented to the Emergency Department with acute visual impairment. Initial examination revealed hypertensive retinopathy grade IV on ophthalmological fundoscopy and a blood pressure of 250/110 mmHg. The examination was otherwise normal (heart rate 88 bpm, temperature 36.8°C, weight 75 kg, height 1.75 m). Laboratory testing showed a severe hypokalaemia of 2.07 mmol/l (*table 1*). Potassium chloride was administered and antihypertensive treatment immediately started using intravenous urapidil and sublingual glyceryl trinitrate, which finally resulted in improved blood pressure levels. To identify a potential cause for the hypertensive emergency, further diagnostic procedures were initiated. Normal serum creatinine, urinalysis and an inconspicuous abdominal ultrasound and renovascular Doppler imaging ruled out renoparenchymous or renovascular hypertension. Endocrine testing (*table 1*) excluded hypertension due to hyperaldosteronism, hypercortisolism, pheochromocytoma and paragangliomas. However, suppressed plasma aldosterone and renin levels were suggesting pseudohyperaldosteronism. Further evaluation of the patient's habits disclosed a noticeable weekly consumption of at least three packs of liquorice (each 300 g) from a

Table 1. *Laboratory findings on admission and on discharge as well as one month later at follow-up*

Laboratory tests	On admission	On discharge	At follow-up	Reference values
Blood counts				
• Haemoglobin	14.4	13.5		12.0-16.0 g/dl
• Platelets	222	190		150-400 x 10^3/µl
• White blood cells	7.46	5.57		4.0-9.5 x 10^3/µl
Electrolytes and renal parameters				
• Sodium	144	140	139	133-146 mmol/l
• Potassium	**2.07**	4.19	4.11	3.5-5.1 mmol/l
• Chloride		108		133-146 mmol/l
• Bicarbonate	**29.4**			22-28 mmol/l
• Creatinine	99.3	81.3		55-110 mmol/l
Hormonal parameters (serum)				
• TSH	2.86			0.27-4.20 mIU/l
• Renin	**< 1***			3-16 ng/l
• Aldosterone	**< 11***			10-160 ng/l
• Cortisol	652 / 80 § *			171 - 535 nmol/l
• Adrenaline	**120 ***			< 85 pg/ml
• Noradrenaline	325 *			< 400 pg/ml
• Dopamine	< 30 *			< 50 pg/ml
• Metanephrine	40.9 *			< 90 pg/ml
• Normetanephrine	108			< 200 pg/ml
24-hour urinary excretion				
• Potassium	26			30-100 mmol/day
• Sodium	260			60-260 mmol/day
• Adrenaline	14.3 *			≤ 40 µg/day
• Norepinephrine	42.8 *			≤ 80 µg/day
• Metanephrine excretion	47.6 *			≤ 80 µg/day
• Normetanephrine	34.1 *			≤ 120 µg/day

Abnormal values are depicted in bold. *Endocrine tests in serum, plasma and urine were obtained following initial blood test on admission. §Cortisol obtained 1 hour after administration of 1 mg dexamethasone. TSH = thyroid-stimulating hormone.

German sweet manufacturer for the last three to four months. Since this nutritional behaviour was considered to be likely related to the patient's hypertension, the liquorice consumption was stopped. Initially, antihypertensive medication comprising seven different drugs was required to lower the blood pressure below 140/70 mmHg (*table 2*), finally resulting in substantial improvement of eye vision at the time of discharge. One month later, his blood pressure (160/80 mmHg) had already improved with the need for only two antihypertensive drugs (*table 2*). While the hypertension was not yet satisfactorily controlled, laboratory tests showed a normal potassium value at that time (*table 1*). After three months, with the patient still not eating any liquorice and having treatment with the same two antihypertensive drugs, a normal blood pressure was achieved (*table 2*).

DISCUSSION

Hypertension associated with hypokalaemia is the classical presentation of primary hyperaldosteronism. However, the present patient's laboratory testing showed suppressed aldosterone and renin levels with a normal aldosterone-to-renin-ratio. While the clinical picture resembled hyperaldosteronism, the hormonal profile showed hyporeninaemic hypoaldosteronism. Liddle's syndrome is a hereditary hypertension with hypokalaemia due to gain-of-function mutations in the gene encoding the renal epithelial sodium channel.[1] However, this disorder is rare and the age of onset is in childhood; thus, Liddle's syndrome was considered very unlikely to be present in this case. Another form of low-renin hypertension associated with low aldosterone and hypokalaemia is apparent mineralocorticoid excess (AME), a congenital defect in 11-β-hydroxysteroid dehydrogenase 2 (11-β-HSD2).[2,3] Since 11-β-HSD2 converts the biologically active cortisol to inactive cortisone, the defect allows cortisol to act as a ligand for the mineralocorticoid receptor, thereby resulting in sodium retention and volume expansion. However, as with Liddle's syndrome, AME is a disorder of the young, starting in early childhood. A much more common cause of hypertension due to pseudohyperaldosteronism in the adult is excessive liquorice consumption, with its ingredient glycyrrhizin being responsible for a mineralocorticoid effect. Previous reports showed that glycyrrhizin

Table 2. *Antihypertensive medication on admission, discharge and at follow-up after one and three months, respectively*

Antihypertensive drugs	On admission	On discharge	1 month later	3 months later
Metoprolol	ø	95 mg qd	ø	ø
Urapidil	ø	90 mg tid	ø	ø
Ramipril	ø	5 mg bid	5 mg qd	5 mg qd
Hydrochlorothiazide	ø	25 mg qd	ø	ø
Triamterene	ø	50 mg qd	ø	ø
Amlodipine	ø	5 mg bid	10 mg qd	10 mg qd
Spironolactone	ø	50 mg qd	ø	ø

qd = daily; bid = twice daily; tid = three time a day.

blocks 11-β-HSD2, thereby mimicking AME and resulting in hypertension, hypokalaemia and alkalosis at any age.[4] Due to the blockage of 11-β-HSD2 the active cortisol binds to the aldosterone receptor. Via this mechanism the serum concentration of renin and aldosterone is low in affected patients. In fact, consumption of a considerable amount of liquorice explained the hypertension which probably developed gradually in the present patient, finally resulting in a hypertensive emergency. In addition, the elevated bicarbonate level indicated alkalosis, which perfectly complements the laboratory findings. While liquorice-induced hypertension is a well-known entity and liquorice is coming more and more into discredit,[5-7] a hypertensive emergency with acute visual impairment in terms of retinopathy due to liquorice in sweets has not yet been reported. Liquorice ingestion has been described to be a possible cause of posterior reversible encephalopathy syndrome (PRES), which could induce visual disturbance.[8] MRI or at least CT scan of the brain might have been useful for differential diagnosis in our case. However, because of the hypertensive retinopathy proven by fundoscopy and the triad of hypertension, hypokalaemia and alkalosis PRES was unlikely to be the underlying reason. Further investigations in the present case revealed that the glycyrrhizin content per kg ingested liquorice was about four grams. Since the patient stated an average consumption of approximately 1 kg liquorice per week, his intake resulted in approximately 500 mg glycyrrhizin per day. Recently, a Swedish study with healthy volunteers revealed a linear dose-response relationship of liquorice ingestion and hypertension starting at a daily intake of 75 mg glycyrrhizin for two weeks.[9] In addition, in 2006, Sigurjonsdottir et al. described that the aldosterone effect of liquorice might be gender specific and males may be more susceptible.[10] This might have even increased the effect of the liquorice ingestion in the present case. Of note, the hypertensive effect may prevail for some time despite stopping ingestion of liquorice, as observed in the present case. Thus, patients should be monitored for some time after stopping liquorice intake, Sigurjonsdottir and colleagues recommend a period of up to four months until the renin suppression is overcome.[9] In the present case, effective control of blood pressure over time with only two antihypertensive drugs underlines that prior excessive liquorice consumption had been at least partly responsible for the hypertensive emergency in this patient. However, a potential effect of additional essential hypertension cannot be excluded. In conclusion, regular liquorice consumption should clearly be viewed as a culprit for the development of hypertension including hypertensive emergencies and liquorice intake should always be considered in hypokalaemic hypertensive patients.

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SPECIAL REPORT

Solving a cold case of haemolysis: Back to the basics

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ABSTRACT

Membrane disorders comprise an important group of inherited haemolytic anaemias. Diagnostic work-up starts with examination of the blood smear, followed by osmotic gradient ektacytometry. In special cases DNA analysis is performed to confirm the diagnosis. For this purpose a next-generation sequencing-based method has been developed. The combination of these techniques established the correct diagnosis in a case of haemolytic anaemia of unknown cause.

KEYWORDS

Hemolytic anemia, blood smear, next generation sequencing

What is known on this topic?
Haemolytic anaemias due to abnormalities of the erythrocyte membrane comprise an important group of inherited disorders. The 'gold' standard for the detection of various disorders of the red cell membrane is osmotic gradient ektacytometry.

What does this add?
Next-generation sequencing can be used in the diagnostic work-up of haemolytic anaemia after the blood smear and ektacytometry. This is the first report of a case of spherocytosis / stomatocytosis solved by next-generation sequencing.

INTRODUCTION

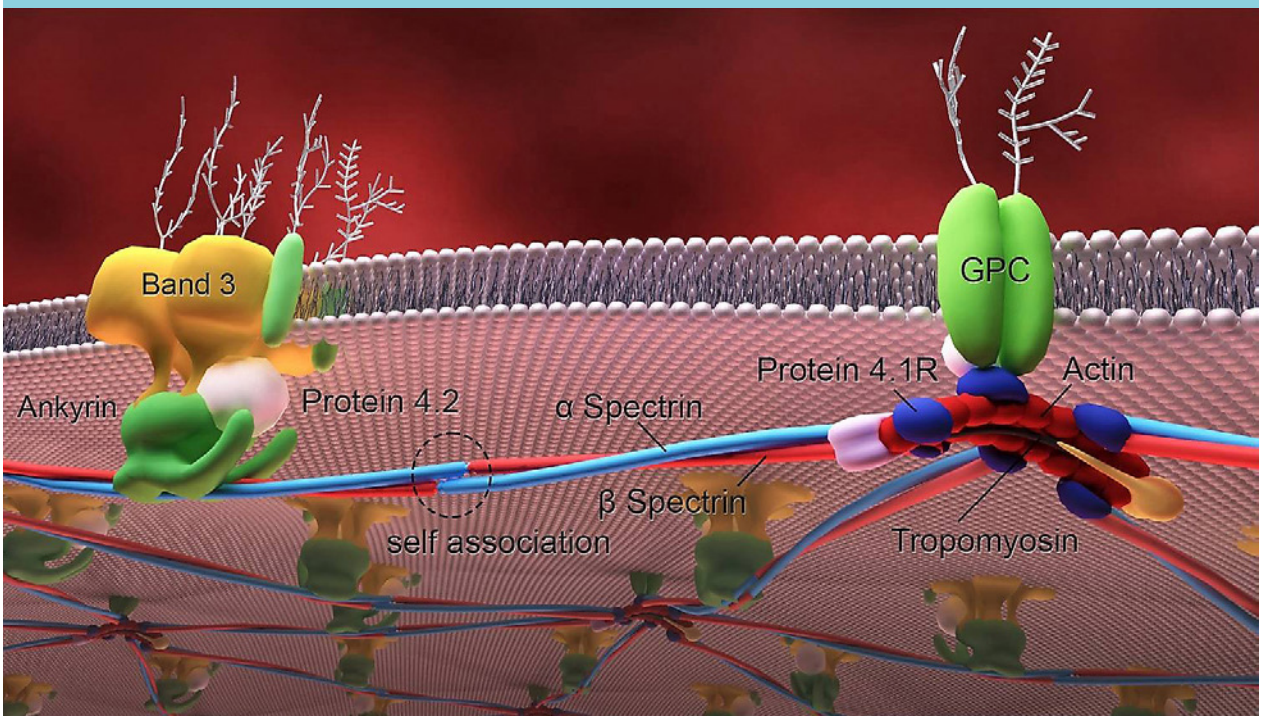
Many skills in jobs, art forms and sports are based on the application, repetition and training of elementary principles. The following case report reminds us to pursue the basic steps in elucidating the cause in a patient with haemolytic anaemia.

Erythrocyte membrane abnormalities are a well-known cause of hereditary haemolytic anaemia. The specific make-up of the red cell membrane accounts for its remarkable deformability and enables the cell to pass through small capillaries with a diameter of only one-third of its own. The membrane consists of a lipid bilayer in which transmembrane proteins are located which anchor the bilayer to an intracellular cytoskeleton. The latter forms a two-dimensional hexagonal filamentous meshwork, mainly consisting of spectrin tetramers (*figure 1*). Spectrin tetramers consist of alpha- and beta-spectrin protein, linked in a head-to-head arrangement, and encoded by the *SPTA1* and *SPTB* genes. Another prominent membrane protein is band 3, encoded by the *SLC4A1* gene. Band 3 is located in the erythrocyte plasma membrane and is part of a multiprotein complex that acts as an attachment site for the cytoskeleton. Abnormalities of each component of the cytoskeleton and anchoring proteins compromise their interactions, often leading to characteristic shape changes.[1]

CASE PRESENTATION

A 20-year-old Caucasian woman presented with jaundice. Her previous history included an episode of haemolytic anaemia three years earlier during a parvo B19 infection. At that time laboratory investigation revealed no haemoglobinopathy and no deficiencies of glucose-6-phosphate dehydrogenase or pyruvate kinase. Erythrocyte membrane protein analysis revealed 'normal'

Figure 1. *Artist impression of the erythrocyte cytoskeleton*



signals for spectrin (RIA) and Band 3 (EMA/FACS). One year later a second haemolytic episode occurred during an Epstein-Barr virus infection.

Five days before consultation she became acutely ill with nausea, vomiting and diarrhoea when travelling in Spain. Symptoms abated spontaneously, but she became jaundiced. On examination she was not acutely ill, but icteric. Results of laboratory investigations pointed to haemolysis as the cause of the jaundice (*table 1*).

A presumptive diagnosis of mild infectious enterocolitis with episodic nonimmune haemolysis was made. A new systematic review was performed. The persistently decreased level of haptoglobin, increased bilirubin, and reticulocytosis were indicative of mild chronic haemolysis. The blood smear disclosed abnormal erythrocyte morphology with prominent presence of spherocytes and stomatocytes (*figure 2*). Osmotic gradient ektacytometry showed decreased EI_{max} and increased O_{min}, indicative for spherocytosis (*figure 3*).

Based on these results a novel next-generation sequencing (NGS) technique was applied to detect mutations in the seven genes most commonly associated with erythrocyte membrane disorders (*SPTA1, SPTB, SLC4A1, ANK1, EPB41, EPB42,* and *RHAG*). This analysis revealed compound heterozygosity for two pathogenic *SPTA1* gene mutations: c.2806-13T>G (α-spectrin$^{St\ Claude}$) and c.4339-99C>T (α-spectrin^{LEPRA}). These mutations have previously been associated with autosomal recessively inherited poikilocytic anaemia and spherocytosis, respectively. In addition, the patient was found to be heterozygous for a c.118G>A, p.(Glu40Lys) missense mutation in *SLC4A1* which is associated with autosomal recessively inherited spherocytosis. The two α-spectrin mutations were present on separate alleles (*in trans*) as confirmed by DNA analysis of the parents.

Figure 2. *A May Grünwald Giemsa stained bloodsmear made after recovery of the patient showing abundant spherocytes (arrows) and stomatocytes (arrowheads)*

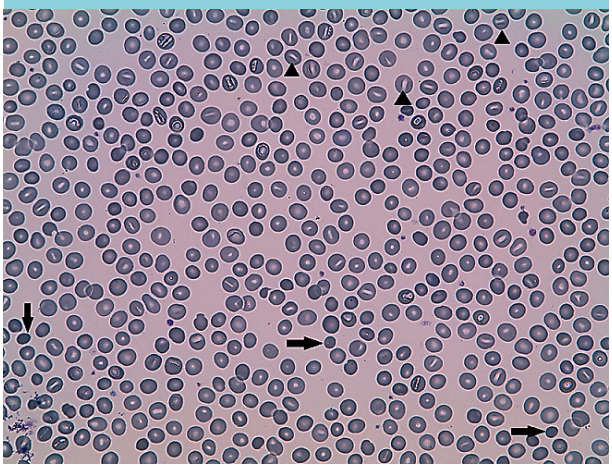
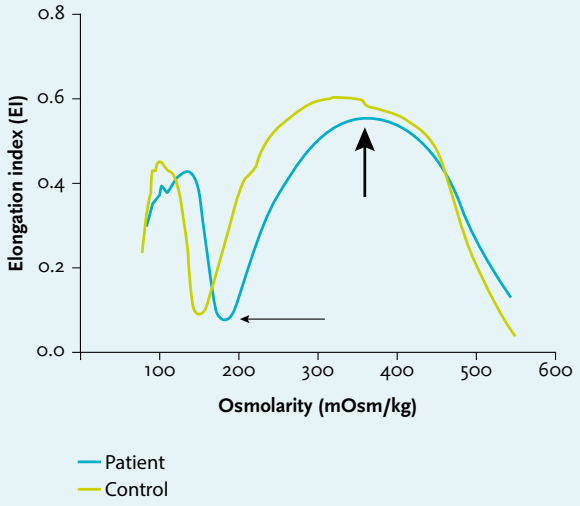


Table 1. *Laboratory results*

Haematology	Value	Reference range
ESR	15	<20 mm/h
Haemoglobin	7.1	7.5-10 mmol/l
Haematocritt	0.33	0.36-0.46 l/l
MCV	99	80-100 fl
RDW	66	39-52.3 fl
MCH	2.1	1.6-2.1 fmol/l
CHC	21.3	19-23 mmol/l
Erythrocytes	3.38	4.0-5.2 /pl
Reticulocytes	157	25-110 /nl
Leucocytes	6.7	4.0-10.0 /nl
Basophils	0.02	<0/2 /nl
Eosinophils	0.06	<0/4 /nl
Neutrophils	4.33	1.5-7.5 /nl
Lymphocytes	1.88	1.0-3.5 /nl
Monocytes	0.52	0.1-10.0 /nl
Immature granulocytes	0.9	<0.5 /nl
Thrombocytes	205	150-400 /nl
APTT	26	26-34 seconds
Prothrombin time	13	12-15 seconds
Chemistry		
Creatinine	61	50-90 umol/l
Albumin	43.1	35-55 g/l
C-reactive protein	2.4	<10 mg/l
Total bilirubin	114	<17 umol/l
Direct bilirubin	46	<5 umol/l
Alkaline phosphatase	77	35-105 U/l
γGT	54	<40 U/l
ASAT	129	<30 U/l
ALAT	129	<35 U/l
LDH	570	<250 U/l)
Haptoglobin	<0.1	0.4-2.0 g/l
DAT	Negative	Negative

ESR = erythrocyte sedimentation rate; MCV = mean corpuscular volume; RDC = red blood cell distribution width; MCH = mean corpuscular haemoglobin; CHC = cellular haemoglobin concentration; APTT = activated partial thromboplastin time; γGT = gamma glutamyltransferase; ASAT = aspartate aminotransferase; ALAT = alanine aminotransferase, LDH = lactate dehydrogenase; DAT = direct antiglobulin test.

Figure 3. *Osmotic gradient-ektacytometry by Laser-assisted Optical Rotational Cell Analyzer (LORCA) shows decreased maximal Elongation Index (Imax, thick arrow) and increased Omin (indicative of increased osmotic fragility, thin arrow). The pattern is typical for spherocytosis*



DISCUSSION

Examination of the peripheral blood smear was the key in establishing the correct diagnosis in this patient, despite normal results for red cell membrane disorder markers in previous investigations. In addition, osmotic gradient ektacytometry was performed which is the 'gold' standard for the detection of various disorders of the red cell membrane. By this method erythrocytes are exposed to both shear stress (by centrifugal force) and gradually changing osmotic conditions. These induced changes in the shape of the red cells are detected and render characteristic deformability profiles for elliptocytosis, spherocytosis, and stomatocytosis.[2]

Heterogeneity in the clinical severity and erythrocyte morphology of the hereditary haemolytic anaemias due to defective membranes is the rule as different proteins are involved in conjunction with a variety of genetic causes: deletions, nonsense, missense, null and low expression mutations and splicing variants.

The *SLC4A1* c.1178G>A mutation was first reported by Rybicki *et al.* and the protein harbouring the consequent p.(Glu40Lys) substitution was named band 3^{Montefiore}.[3] Their propositus was homozygous for this mutation and had hereditary spherocytosis. The defective band 3 protein probably resulted in faulty interactions with protein 4.2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Relatives who were heterozygous for the mutation were clinically unaffected. Whether the heterozygous state for this mutation is clinically relevant for the current case is uncertain, but an additive effect on the presentation of clinical features cannot be excluded.

Most mutations in the *SPTA1* gene affect the self-association site of the α- and β-spectrin molecules, leading to elliptocytosis. However, mutation at other sites can lead to pyropoikilocytosis and spherocytosis.

The *SPTA1*$^{\text{St Claude}}$ allele was first reported by Fournier *et al.*[4] Their homozygous propositus was symptomatic whereas the heterozygous parents were clinically and biochemically unaffected.

The *SPTA1*$^{\text{LEPRA}}$ allele was reported by Wichterle *et al.*[5] Their propositus was compound heterozygous for *SPTA1*$^{\text{LEPRA}}$ and *SPTA1*$^{\text{PRAGUE}}$ causing severe spherocytic haemolytic anaemia. The parents, heterozygous carriers of either of these mutations, were asymptomatic. Intriguingly, apart from spherocytosis our patient showed a large number of stomatocytes on the blood smear. This unique appearance may be related to co-inheritance of the band 3$^{\text{Montefiore}}$ allele.

It is highly likely that the mutations in spectrin and band 3 are responsible for the spherocytosis / stomatocytosis and the corresponding subclinical haemolysis. In patients with such membrane alterations intercurrent infections can exacerbate the haemolysis and provoke haemolytic crises. At the age of 15 and 16 years she had clinical jaundice which was attributed to haemolytic anaemia as part of serologically proven parvo B19 and Epstein-Barr virus infections, respectively, as the clinicians were unaware of the presence of structural changes in the red cell membrane. The last icteric episode occurred during an acute, self-limiting infectious enterocolitis (traveller's diarrhoea). It is, however, remarkable that she had no history of haemolytic crisis earlier in life since she must have suffered from other infectious childhood diseases.

Molecular analysis of the genes encoding red blood cell membrane proteins has long been hampered due to the size and number of the genes involved. With the advent of NGS techniques it has now become feasible to analyse many genes in parallel with high quality. For erythrocyte membrane disorders, the DNA diagnostics section of the UMC Utrecht has implemented NGS-based sequencing of seven genes known to be causally involved: *SPTA1, SPTB, ANK1, SLC4A1, EPB 41, EPB42* and *RHAG*. This gene panel analysis was validated and accredited under the ISO15189 standard and yields a mutation detection sensitivity of > 95% in the seven genes analysed. As such, a leap forward has been made in the facilitation of molecular genetic diagnostic analysis of patients with suspected erythrocyte membrane disorders. The fact that spectrin and expression of Band 3 protein on the erythrocyte membrane surface were previously determined as normal shows that a normal amount does not imply a normal structure and function of the protein.

CONCLUSION

This case presentation illustrates that a 'simple' examination of the blood smear, a basic laboratory test, is still a good starting point from which more sophisticated tests can be requested. The fact that the 'automated' haemocytometry report mentions results of a (leukocyte) blood cell 'differentiation' does not imply that the morphology of the red cells has been investigated. Moreover, most current automated cell counters do not recognise red cell shape alterations. Even if there has already been an extensive academic workup this should not discourage other physicians from performing a new systematic analysis in a cold case. Interaction with clinical chemists and the progress in DNA technology comes to aid the clinicians and patients at the bedside.

DISCLOSURES

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

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PHOTO QUIZ

Take a deep breath...

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CASE REPORT

A 57-year-old male with a history of chronic obstructive pulmonary disease presented to the emergency department (ED) after an episode of vigorous coughing resulting in severe chest pain and the sensation of a cough-dependent swelling on the right side of the thorax. The patient was recently discharged from the pulmonary department where he was treated for a spontaneous right-sided pneumothorax with chest tube placement.

Figure 1. *Chest X-ray (posterior-anterior) shows no striking abnormalities apart from subcutaneous emphysema*

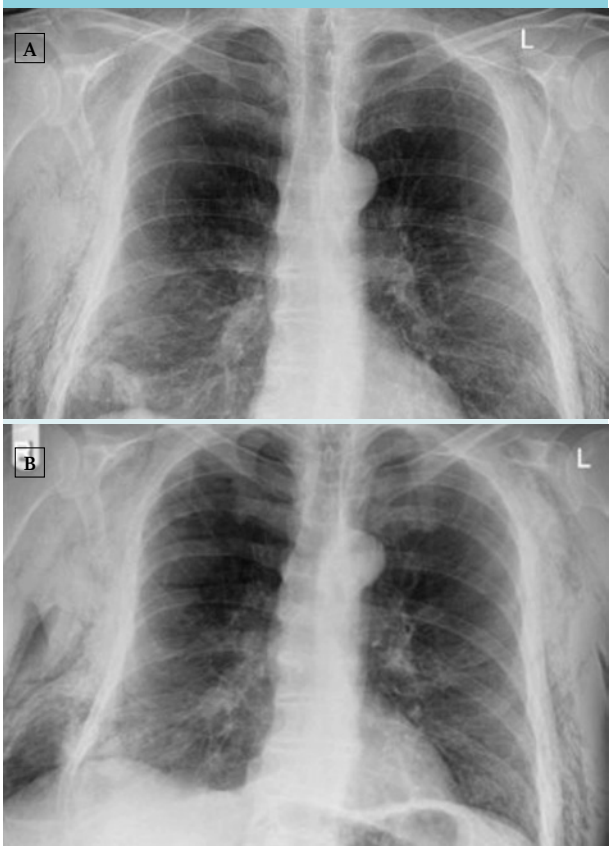
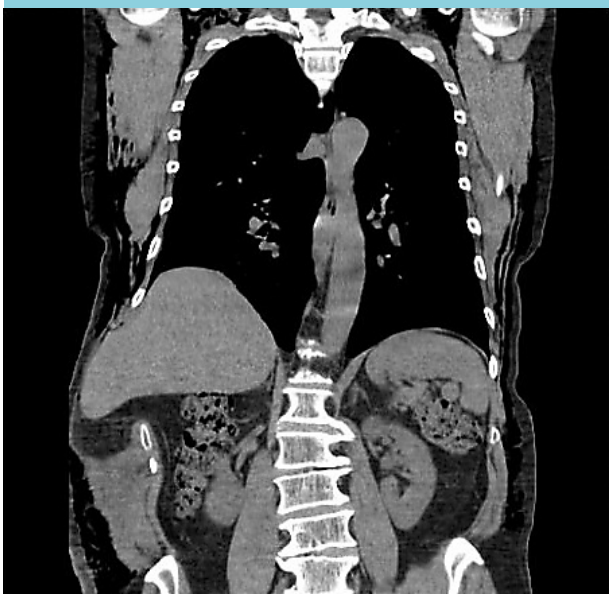


Figure 2. *Computed tomography demonstrating a large intercostal herniation of the liver with no sign of strangulation of lung tissue*



At presentation to the ED, a chest X-ray showed residual subcutaneous emphysema after his pneumothorax, but when the patient was asked to perform the Valsalva manoeuvre (*figure 1*), a region of air density became apparent beyond the confines of the thoracic cavity. Furthermore, during admission he also complained of abdominal pain and a computed tomography scan revealed intercostal herniation of the liver (*figure 2*).

WHAT IS YOUR DIAGNOSIS?

See page 93 for the answer to this photo quiz.

PHOTO QUIZ

A man with erythema and blisters on his forearms

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CASE REPORT

A 71-year-old man with a history of chronic ischaemic heart disease presented to the hospital with stinging and itchy blisters on both arms. The patient was a non-alcoholic and non-smoker. There was no relevant family history. His medications included enalapril, carvedilol and aspirin. He had been working in a garden three days ago when he got scratches from a bush there. After this episode the patient realised that he had some blisters and redness on his forearms and hands. The patient did not remember any insect bites. He denied taking any over-the-counter medication or other new medication.

Physical examination revealed confluent erythematous macules forming vesicles and tense bullae (*figure 1*) over some remains of scratches. Some of the lesions had linear patterns (*figure 2*). There were isolated meliceric crusts that showed impetiginisation. The Nikolsky sign (shearing of normal epidermis in response to lateral pressure) was negative. Oral mucosa and genitalia were respected. He had neither fever nor other symptoms.

Figure 1. *Confluent eritematous macules forming vesicles and blisters on photoexposed areas of hands.*

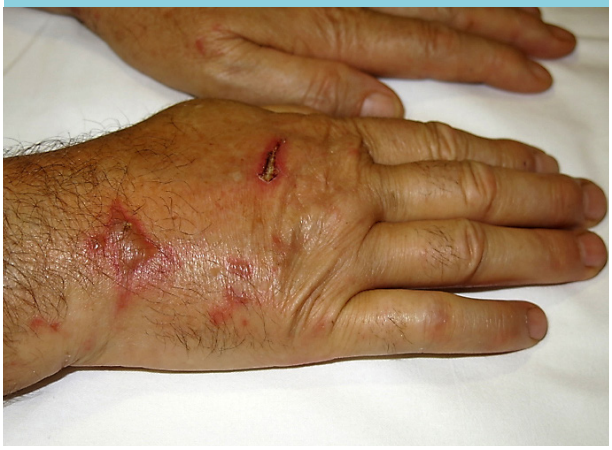
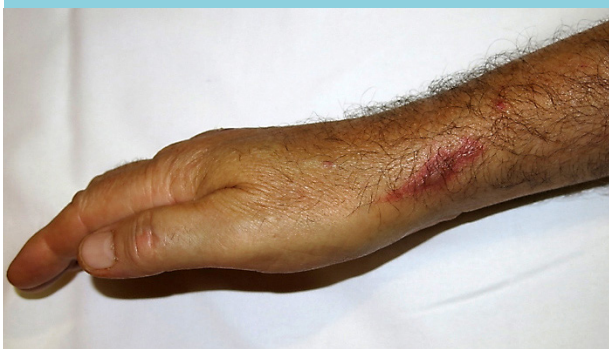


Figure 2. *Eritematous macules and a bulla with linear pattern.*



WHAT IS YOUR DIAGNOSIS?

See page 94 for the answer to this photo quiz.

PHOTO QUIZ

A purple finger two months after kidney transplantation

Y.R.P. de Waal[1]*, W.A.M. Blokx[2], J. M. Mommers[3], L.B. Hilbrands[1]

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CASE REPORT

A 68-year-old woman presented with a purple lesion on her finger. She had diabetes type 2 and kidney failure due to atherosclerotic vascular disease, and had undergone a kidney transplantation two months earlier.

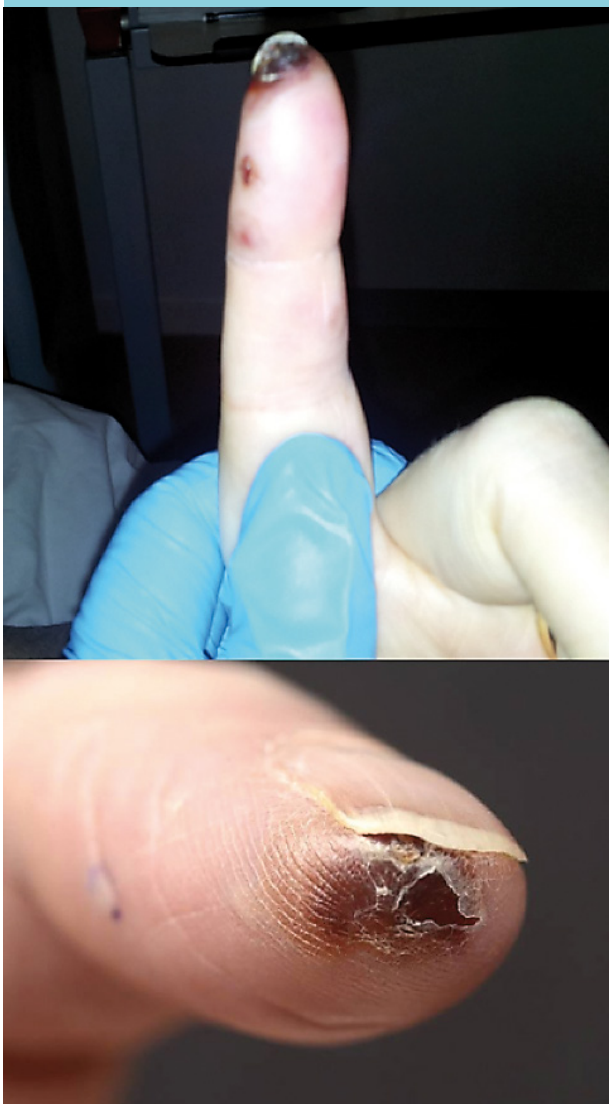
The skin lesion started as a small blister after mild trauma, developing into a blue spot on the tip of the left index finger. This resolved spontaneously, but after six weeks multiple small, painful vesicles and a purple papule arose again.

Her immunosuppressive treatment consisted of tacrolimus, prednisolone and mycophenolate mofetil. Her diabetes was treated with insulin therapy. She did not smoke, drink, or abuse any kind of drugs.

On physical examination there were a few clear-pale lenticular vesicles on the distal phalanx of the finger and on top a blue-purple papule with a haemorrhagic crust (*figure 1*). The finger was warm, arterial pulsations on the left arm were normal and she did not have any fever.

Laboratory results showed a stable serum creatinine, and normal levels of calcium, phosphate and thrombocytes.

Figure 1. *Purple papule with haemorrhagic crust on the left index finger.*



WHAT IS YOUR DIAGNOSIS?

See page 95 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 90)

TAKE A DEEP BREATH…

DIAGNOSIS

Lung hernia is an uncommon diagnosis, defined as the protrusion of lung tissue beyond the confines of the thoracic cavity through an abnormal opening in the chest wall, diaphragm or mediastinum lined by pleura.[1] Pulmonary hernias were first described by Morel-Lavallée and classified based upon both the aetiology and anatomical location.[1]

Anatomically, pulmonary hernias can be divided into cervical, thoracic, mediastinal and diaphragmatic.[1] Pulmonary hernias can also be categorised into the congenital and acquired type, the latter being further classified into traumatic (e.g. costal fractures and/or intercostal muscle rupture), spontaneous (an acute increase in thoracic pressure resulting in a thoracic disruption), pathological (destruction of thoracic wall by cancer) and postsurgical (both video-assisted thoracoscopic surgery (VATS) and thoracotomy are known to cause postsurgical lung hernia's[1,2]).

The most typical presentation of a lung hernia is a painful subcutaneous mass varying in size during inspiration and expiration, increasing when performing the Valsalva manoeuvre, frequently with a history of chest trauma, surgery or chest tube placement.[3] The differential diagnosis of a painful palpable mass of the chest wall should include subcutaneous emphysema, chest wall lipoma, abscess or (sub)cutaneous metastasis, together with the differential diagnosis of localised chest pain such as pulmonary embolus, pleuritis and costal pathology.

When intercostal lung herniation results in lung tissue strangulation and pneumothorax, surgical intervention is indicated. The goal of surgery should be recovering the integrity of the thoracic wall by reduction of the hernia followed by closure of the hernia with or without mesh repair, depending on the localisation and size of the defect.[4]

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ANSWER TO PHOTO QUIZ (PAGE 91)

A MAN WITH ERYTHEMA AND BLISTERS ON HIS FOREARMS

DIAGNOSIS

Phytophotodermatitis caused by contact with a fig tree (*Ficus carica*)

Delving into the anamnesis, the patient acknowledged that he had been pruning a fig tree. The diagnosis of phytophotodermatitis was finally made. Fusidic acid ointment was prescribed to treat overinfection given the impetiginised crusts. After one week of follow-up, the skin lesions had disappeared.

Phytophotodermatitis is a phototoxic inflammatory skin reaction consisting of erythema, accompanied or not by blistering and late hyperpigmentation due to the combination of a topical or oral photosensitising agent followed by exposure to sunlight or the appropriate wavelength of UV radiation.[1-3]

The key point about phytophotodermatitis is that these photosensitising agents (furocoumarins, psoralens) are usually produced in the vegetable kingdom although there are phytophotodermatitis-like lesions caused by applying psoralens for psoralen plus ultraviolet A (PUVA) therapy.[1]

There are several plant families that can cause phytophotodermatitis. *Moraceae* (mulberry) family, to which the *Ficus carica* belongs, is a frequent cause of phytophotodermatitis due to its psoralens and 5-MOP (5-metoxipsoralen, bergapten), especially abundant in the sap of the leaves and shoots.[4]

This entity is equally prevalent in both sexes, and it can occur at any age. It is significantly more frequent in some employments such as bartenders, grocery staff and agricultural farm workers,[2] although it can occur at non-occupational situations too. Phytophotodermatitis tends to have seasonal behaviour since it more often occurs in the spring and summer. While any ethnicity can be affected, dark-skinned people usually develop the disease with only the post-inflammatory pigmentation and without previous erythema and blisters.[1] Diagnosis can be easily made without any complementary tests if the pattern is well recognised and a detailed history is taken. Other entities to be considered in the differential diagnosis are pemphigoid, porphyria cutanea tarda, herpes simplex or thermal burns, and particularly acute irritant contact dermatitis, but this disease is eczematous and phytophotodermatitis is not.

Active treatment for phytophotodermatitis is not usually effective. Phototoxic reaction could only be obviated by washing exposed areas. To avoid new reactions, preventive strategies such as covering the extremities with gloves and boots when working with psoralen-containing plants and not pruning these plants during hours of maximum sun exposure may be useful.[1]

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ANSWER TO PHOTO QUIZ (PAGE 92)

A PURPLE FINGER TWO MONTHS AFTER KIDNEY TRANSPLANTATION

DIAGNOSIS

The differential diagnosis of a black or purple coloured finger is: vascular obstruction (microangiopathy, vasculitis, septic or cholesterol embolism), calciphylaxis, malignancy (melanoma, paraneoplastic tumour thrombi) or local infection (bacterial or viral).

A skin biopsy showed subepidermal clefting with several multinucleated keratinocytes and a ground glass appearance of the nuclei (*figure 2*). There was a perivascular mononuclear cell infiltrate in the dermis without signs of micro-angiopathy or thrombi. The histology was characteristic for a herpes infection, which was confirmed by a positive polymerase chain reaction for herpes simplex virus (HSV) type 1.

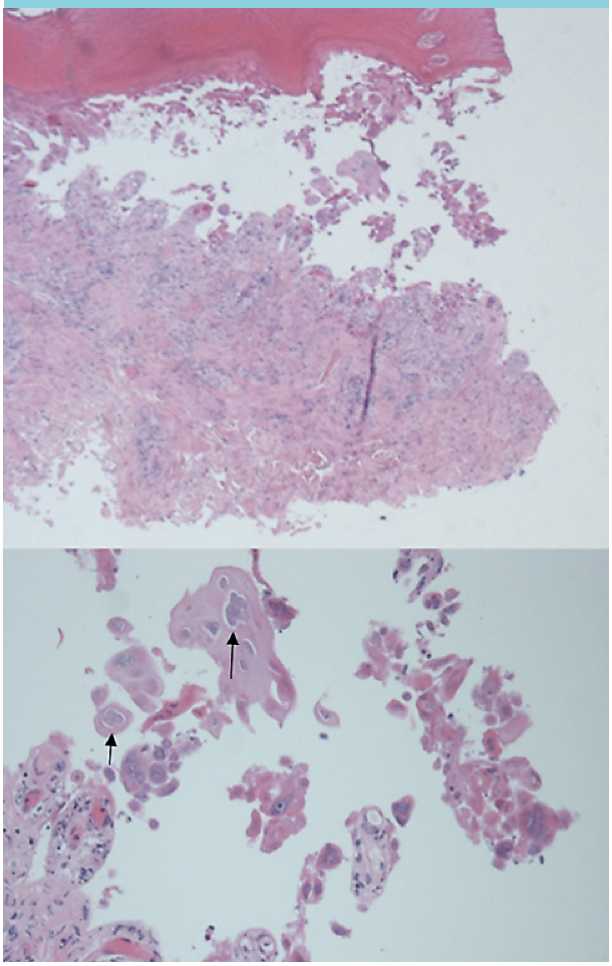
The patient told us that she had had a cold sore a few weeks before, which was probably the source of the herpes infection of the finger.

A HSV infection involving the hand is called herpetic whitlow.[1] The infection can be caused by HSV type 1 (usually presenting as gingivostomatitis) or type 2 (mostly presenting as genital herpes).[2] Direct contact of the finger with secretions or lesions of infected areas is the likely mechanism of transmission. It is especially seen in young children (finger sucking), dentists, young adolescents, and immunocompromised patients.[3] Classically, a single vesicle or a cluster of vesicles arises near the nail, 3-4 days after skin irritation or minor trauma. Vesicles are first clear-pale yellow, and later become turbid or haemorrhagic. Most patients have intense localised pain. The lesion might resemble a felon (also called whitlow).[1,4] The natural course is usually benign with complete resolution within 2-3 weeks. Systemic viraemia or ocular infection are rare, but severe, complications. After resolution of the infection, the herpes virus passes into a latency phase, inhabiting a host neural ganglion. One or more recurrences are experienced in 20-50% of patients and usually arise in the same dermatome as the original infection. Immunocompromised patients are at greater risk of more frequent and more severe HSV recurrences.

The diagnosis is usually made on clinical grounds, but may be confirmed with polymerase chain reaction or immunofluorescence microscopy in which scrapings of the lesions are examined for herpes antigens.[1,4]

Treatment is symptomatic, but antiviral drug therapy can be beneficial. Acyclovir seems to prevent or decrease recurrence rates. Intravenous acyclovir is occasionally required in cases of immunosuppression and disseminated HSV infection.[1] We started valacyclovir orally, after which the patient recovered fully.

Figure 2. *Subepidermal clefting with multinucleated keratinocytes.*



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LETTER TO THE EDITOR

An unusual craving!

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To the Editor,

Pica refers to an abnormal appetite for non-edible substances usually occurring in children with developmental disability or brain injury. However, it can also occur during adulthood, as it is associated with iron deficiency. Pica requires medical attention in case of severe eating behaviour that can be potentially harmful. Here we present a case of craving for Johnson's® baby talcum powder in a patient with iron deficiency anaemia.

CASE REPORT

A 41-year-old woman was referred to our outpatient clinic by her general practitioner because of severe iron deficiency anaemia. She was supposed to take iron tablets because of anaemia caused by frequent epistaxis due to hereditary haemorrhagic telangiectasia. Unfortunately, she had discontinued taking her iron supplementation without a specific reason eight months previously. Laboratory results revealed severe iron deficiency anaemia (haemoglobin 5.9 g/dl, mean corpuscular volume 62 fl). During the consultation, the patient told us that she consumed one bottle of Johnson's® baby powder daily. She also told us that her behaviour had subsequently been picked up by her daughter. Fortunately, after four weeks of iron supplementation, our patient felt no more craving and completely stopped the consumption of Johnson's baby powder. Her general practitioner was informed and immediately intervened pertaining to her daughter, in whom iron deficiency was excluded.

DISCUSSION

To our best knowledge this is the first described pica syndrome of talcum powder craving associated with severe iron deficiency. Pica occurs in up to 45% of non-pregnant iron deficient adults and most often presents with a craving for ice, which is called pagophagia, but substances such as rubber and toothpicks have been reported as well. This craving generally rapidly reverses within a few weeks with iron repletion. The mechanism leading to this syndrome is unknown.[1] Pica for Johnson's® baby powder might be a potentially harmful for a patient's health, because it contains hydroxylated magnesium silicate and zinc oxide minerals that inhibit iron absorption, which might worsen the iron deficiency. Furthermore, when consuming the powder straight from its bottle it is likely that, besides aspiration, amounts of the powder will be inhaled, which might cause acute respiratory problems (e.g. bronchoconstriction, chemical pneumonia). Chronic lung damage could be due to granulomatous or interstitial lung disease or pulmonary talcosis, which might over time lead to pulmonary fibrosis.[2,3]

In a case of severe iron deficiency it is important to ask about unusual cravings in order to detect pica, especially since potentially harmful substances may be consumed. Furthermore, clinicians should be aware of the possibility that such behaviour may be copied by relatives as well.

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