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Human metapneumovirus: a new pathogen in children and adults

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ABSTRACT

In 2001, human metapneumovirus (hMPV) was discovered in young children with respiratory tract infection of unknown origin. In the two years since its discovery the clinical characteristics of this new virus have been clarified. In children, especially those younger than one year of age, hMPV is responsible for 5 to 10% of respiratory tract infections requiring hospitalisation; its clinical course is somewhat milder, but otherwise indistinguishable from respiratory syncytial virus (RSV) infection. Human MPV can also be found in adults, in influenza-like illnesses, but also as a cause of pneumonia. Especially in the latter cases immunosuppressive conditions may be present.

INTRODUCTION

Respiratory tract infections (RTI) are among the most common infections in humans. Many infectious agents can cause RTI. However, in a substantial proportion of RTI the aetiology is not established. For instance, in adult community-acquired pneumonia an aetiological agent is commonly identified in only 50% of cases. One explanation for this large proportion of unknown aetiologies is insufficient sensitivity of current diagnostic tests, but another explanation might be the presence of unknown pathogens. In 2001, human metapneumovirus (hMPV) was discovered in young children with respiratory tract infection of unknown origin.[1] Cytopathic effects of this virus in tertiary monkey kidney cells were comparable with those caused by human respiratory syncytial virus (hRSV), and electronmicroscopy of supernatant revealed the presence of paramyxovirus-like particles. Sequence analysis and genomic organisation characterised the virus as a member of the genus *Metapneumovirus* of the family *Paramyxoviridae*, of which the only member until then was the avian pneumovirus (APV), the causative agent of an upper respiratory tract infection in turkeys. The most closely related human virus was RSV, also a paramyxovirus but belonging to the *Pneumovirus* genus. The isolated hMPV strains showed sequence variation, and two main clusters of isolates could be distinguished.[1,2] In the two years since its discovery, the epidemiology and clinical features of this virus have been the subject of further investigations.

EPIDEMIOLOGY

Human MPV is a common respiratory virus; 25% of Dutch children aged between six months and one year have antibodies to the virus, and at the age of five years almost all children have antibodies.[1] Investigation of samples stored at the National Influenza Centre showed that as early as in 1958, 100% of investigated persons had antibodies to hMPV, so the virus has been circulating for at least 50 years in the Netherlands. Comparable serological results were obtained in Japan.[3] Soon after its discovery the virus was also isolated in other countries in Europe, North America, Australia, and Asia.[4-13] In all these countries, the same two hMPV clusters as originally described were found. There is a clear seasonal distribution of disease, with almost all cases occurring between December and April.[4,7,9,10,12,14,15] In the Far East, the peak of hMPV activity is in spring and early summer.[8,11]

DETECTION OF THE VIRUS

The virus can be isolated by cell culture. Originally, the virus was isolated from cultures of tertiary monkey kidney (tMK) cells, displaying cytopathological effects (CPE) within 10 to 14 days post-inoculation similar to those seen with RSV.[1] Boivin *et al.* only showed CPE in LLC-MK2 cells after a mean incubation time of 17.3 days, without large syncytia formation.[14] The virus could not, or only poorly, be propagated in other cell lines commonly used for isolation of respiratory viruses (such as Vero cells, MDCK or A-549). In human laryngeal carcinoma (HEp-2) cells hMPV could be detected from respiratory samples; however, since no CPE was found, RT-PCR examination of cell culture material was necessary.[16]

In clinical samples viral RNA can be detected by reverse-transcription polymerase chain reaction (RT-PCR). Several targets for amplification have been chosen in the design of the RT-PCR, and it has been suggested that amplifying the N and/or the L gene is particularly suitable for hMPV diagnosis.[17,18] Since several laboratories have started to implement PCR as routine diagnostic assay for respiratory virus infections, it can be expected that detection of hMPV RNA will be more widely used as part of a respiratory virus diagnosis package.
Antibodies against hMVP can be measured and serology studies have been performed, but since everyone over the age of five years has anti-hMPV antibodies, antibody detection is not currently implemented as a standard assay in most routine laboratories.

CLINICAL FEATURES IN CHILDREN

In most series, hMPV could be demonstrated in 5 to 10% (range: 1-25%) of children admitted with acute respiratory tract infections.[4,8-13,15] The incidence can vary substantially in consecutive years,[7,10] which partially explains the wide range of incidences found. In up to 30% of cases more than one respiratory virus was isolated.[4,8,10,12,15,18] In all series RSV was isolated more frequently than hMPV.
The clinical picture is comparable with what is seen with RSV infections, with bronchiolitis being the most frequent manifestation, followed by (broncho)pneumonia, pneumonitis, wheezing, and otitis media. Most infections are seen in children younger than one year of age who are otherwise healthy. Compared with RSV infections, the affected children are somewhat older, and the severity of disease is usually somewhat less.[11,12,15,18] The detection of antibodies against hMPV in 100% of older children suggests that most infections in older children are not associated with serious disease.[10] Antibodies against strains from one cluster do not automatically confer immunity against strains from the other cluster. This explains that in the same person more than one episode of hMPV infection can occur.[6,19]

CLINICAL FEATURES IN ADULTS

In a cohort of mainly adult persons with an influenza-like illness of less than five days' duration hMPV was detected in 1.3% of cases.[5] In most of these patients there was evidence of lower respiratory tract involvement. In the Dutch ARIEL study (Acute Respiratoire Infecties in de Eerste Lijn) 448 patients were investigated who had gone to their general physician with an influenza-like illness or another acute respiratory infection. In 3% of cases (and in 0% of controls) hMPV was found.[20]
In several large cohorts respiratory material was collected during (unspecified) respiratory conditions.[7,14,21] Human MPV could be recovered from 2.3 to 14.8% of respiratory samples, and in 4.5 to 24% of patients with hMPV more than one respiratory pathogen was detected.[7,14,21] HumanMPV was detected in all age groups,[21] and during subsequent years substantial differences in hMPV incidence were noted.[7] The clinical characteristics of hMPV infections are not distinctive. Differentiating it from other respiratory viruses on clinical grounds is not possible,[5,21] although as compared with RSV infections hoarseness has been observed more frequently.[7] In around 18 to 50% of cases a pneumonitis was diagnosed, while in the other patients rhinitis, bronchitis or a flu-like syndrome were found.[7,14,21] Of the described patients with pneumonitis a substantial percentage had an immunosuppressive condition.[14] In a recently described Dutch cohort most adult patients also had another disease, or had recently received a bone marrow or kidney transplant.[18] As only hospitalised patients were investigated in this cohort, a population bias is likely to be present. In the described cohort of patients with influenza-like illnesses this association with underlying immunosuppression was not found.[5] Noteworthy is the fact that during the recent SARS epidemic in Hong Kong in patients with proven disease, hMPV could also be demonstrated in 52% of cases.[16] In the Canadian SARS cohort the same observation was done. It is not clear whether hMPV influenced the severity of disease, or whether the two viruses were merely co-circulating in the population during the epidemic.[16]

HUMAN MPV IN IMMUNOCOMPROMISED PERSONS

Although the fatality rate of hMPV appears to be very low, hMPV can be responsible for fatal respiratory insufficiency in severely immunocompromised persons. One young

child with acute leukaemia was described who suffered from hMPV infection in two subsequent winters. The two strains that were recovered during these two episodes were genetically distinct, and the last episode was fatal.[19] Likewise, a fatal case of hMPV infection was described in an adult haematopoietic stem cell transplant recipient.[22]

CONCLUSION

In the two years since its discovery, the clinical characteristics of this new virus have been clarified. In children, especially those younger than one year of age, hMPV is responsible for a substantial number of respiratory tract infections requiring hospitalisation; its clinical course is somewhat milder, but otherwise indistinguishable from RSV infection. Human MPV can also be found in adults, in influenza-like illnesses, but also as a cause of pneumonia. Especially in the latter cases immunosuppressive conditions may be present, and, like RSV, hMPV can be responsible for respiratory insufficiency under these conditions. The incidence of hMPV as a cause of respiratory failure in these patients needs further investigation. Identifying hMPV in such patients is relevant, because *in vitro* reports suggest that ribavirin and intravenous immunoglobulin have antiviral activity against hMPV.[23] Whether these agents have therapeutic value *in vivo* needs to be demonstrated in further studies.

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REVIEW

Thrombophilia screening: a matter of debate

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ABSTRACT

In the last ten years, several risk factors that increase the risk of venous thrombosis have been discovered. Venous thrombosis is a multicausal disease in which several risk factors, both genetic and acquired, have to occur simultaneously to cause thrombosis. This means that most individuals with single thrombophilia are asymptomatic. Although testing thrombosis patients and their relatives for thrombophilia factors seems important for tailoring the duration of (prophylactic) anticoagulant therapy or estimating the risk of recurrence of thrombosis, current data do not support screening for thrombophilia. The risk of recurrences or the duration of anticoagulant therapy are generally not altered by thrombophilia. Future research should focus on identifying clusters of thrombosis risk factors to better estimate the individual risk of thromboembolic events.

INTRODUCTION

Before 1993, an inherited risk factor was detectable in only 10% of symptomatic patients with venous thrombosis. In the last ten years, the knowledge of risk factors for venous thrombosis has increased significantly. With the discovery of several inherited coagulation abnormalities associated with an increased tendency for venous thrombosis, such as factor V Leiden and the prothrombin 20210A mutation, many patients with a first episode of venous thrombosis have a detectable disorder.

Rudolph Virchow stated that the development of thrombosis was the result of changes in blood composition (hypercoagulability), reduced blood flow, or changes in the vessel wall.[1] Disturbance of this balance favours fibrin formation and may ultimately lead to the formation of occlusive thrombi. Examples of this pathophysiological phenomenon are trauma, immobilisation, pregnancy, surgery, malignancy and infection. These are acquired risk factors for venous thrombosis that may cause tissue damage, stasis of the blood or changes in blood composition. Both family studies and case-control studies led to important discoveries of heritable causes of thrombosis. The Leiden Thrombophilia Study (LETS), a population-based large case-control study, assessed the importance of various risk factors for thrombosis, which in most cases had been identified by family studies.[2] *Table 1* summarises the main results of the LETS. The thrombophilia factors can roughly be divided in two groups: deficiencies in the anticoagulant proteins antithrombin, protein C, and protein S are loss of function mutations and are rare in the general population. The prothrombotic abnormalities have a gain of function through subtle changes in the regulation of the gene activity. Factor V Leiden is relatively resistant to inactivation by activated protein C (APC) and the prothrombin mutation leads to increased prothrombin levels. High levels of procoagulant factors, such as factor VIII, IX and XI, lead to prolonged formation of fibrin as a result of excessive generation of thrombin. Finally, high thrombin-activatable fibrinolysis inhibitor (TAFI) levels result in prolonged down-regulation of fibrinolysis. Since no mutations have been found that elevate these coagulation factors, we do not know whether a gain or loss of function is responsible.

Table 1
Results from the Leiden Thrombophilia Study

RISK FACTOR	PREVALENCE IN PATIENTS (%)	PREVALENCE IN CONTROLS (%)	OR	95% CI
ANTICOAGULANT PROTEINS				
Protein C <0.67 U/ml	4.6	0.8	3.8	1.7-7.0
Protein S <0.67 U/ml	1.1	1.3	0.8	0.2-3.0
Antithrombin <0.80 U/ml	1.1	0.2	5.0	0.7-34
PROTHROMBOTIC MUTATIONS				
Factor V Leiden mutation	19	3	7.9	4.4-14
Prothrombin 20210A mutation	6.2	2.3	2.8	1.4-5.6
ELEVATED LEVELS OF PROCOAGULANT FACTORS				
Factor VIII >150 IU/dl	25	11	6.2	3.4-11
Factor IX >129 U/dl	20	10	2.5	1.6-3.9
Factor XI >120.8%	19	10	2.2	1.5-3.2
FIBRINOLYTIC FACTORS				
TAFI >122 U/dl	17	10	1.7	1.1-2.5
Protein C inhibitor >125.5%	13	10	1.4	0.9-2.0
OTHER LABORATORY ABNORMALITIES				
Homocysteine >18.5 μmol/l	10	5	2.5	1.2-5.2
APC resistance for wild-type factor V <0.92	36	16	4.4	2.9-6.6

INTERACTION, REGULATION AND CLUSTERING OF RISK FACTORS

Interaction

Venous thrombosis like many other diseases is multicausal. The discovery of common risk factors was a prerequisite for the study of interaction and made it clear that risk factors for thrombosis result from genetic differences or differences brought about by the environment or even behaviour. Plasma levels of proteins can, for instance, be determined by polymorphisms in the functional allele and by age or hormones. A good example of this complicated regulation is factor VIII. ABO blood group is an important genetic determinant of plasma factor VIII levels.[3] Von Willebrand factor is the carrier protein of factor VIII in plasma and also determines the factor VIII level.[4] If both blood group and von Willebrand factor are taken into account, a clear familial clustering remains, suggesting a third set of genes that regulate factor VIII levels.[5] Apart from the genetic causes, factor VIII is also influenced by environmental factors such as acute phase reactions and age. It is clear that not only is thrombosis a multicausal disease, but that the level of coagulation factors also reflects a mixture of genetic and environmental determinants.[6,7] The mean age at first thrombosis for patients from thrombophilic families is much younger than for consecutive patients with thrombosis.[8] This phenomenon is probably due to interaction of several genetic defects. In thrombophilic families, the risk of thrombosis in combination with protein C deficiency and factor V Leiden was much higher than for relatives with only protein C deficiency.[8] This gene-gene interaction results in variation within and between families. Homozygous disease is another example of this interaction. More commonly, a gene-environment interaction is present in patients with thrombosis. The synergistic effect of factor V Leiden and oral contraceptive use was described in 1994.[9] The annual absolute risk of women who were taking oral contraceptives and were carriers of factor V Leiden was 28.5 per 10,000 people, whereas this risk was 5.7 per 10,000 women per year for those with factor V Leiden without contraceptives and 3.0 per 10,000 per year for women with contraceptives without factor V Leiden.[9] An example of environment-environment interaction is oral contraceptive use and age.[9] This all shows that the nature of thrombosis is complex. The model of multicausal disease is not always sufficient to explain why the clustering of these different risk factors is sufficient to cause thrombosis in one patient but not in the other. Refinement of this model by including the dynamic influence of age is more useful for an individual risk estimate.[6] In this way we can better incorporate interaction of different risk factors. *Figure 1* shows the hypothetical situation of a patient who is followed through life.[6] This person has a certain basic thrombosis potential, which is formed by genetic factors (in this case factor V Leiden). Through life, several events lead to an increased thrombosis potential.

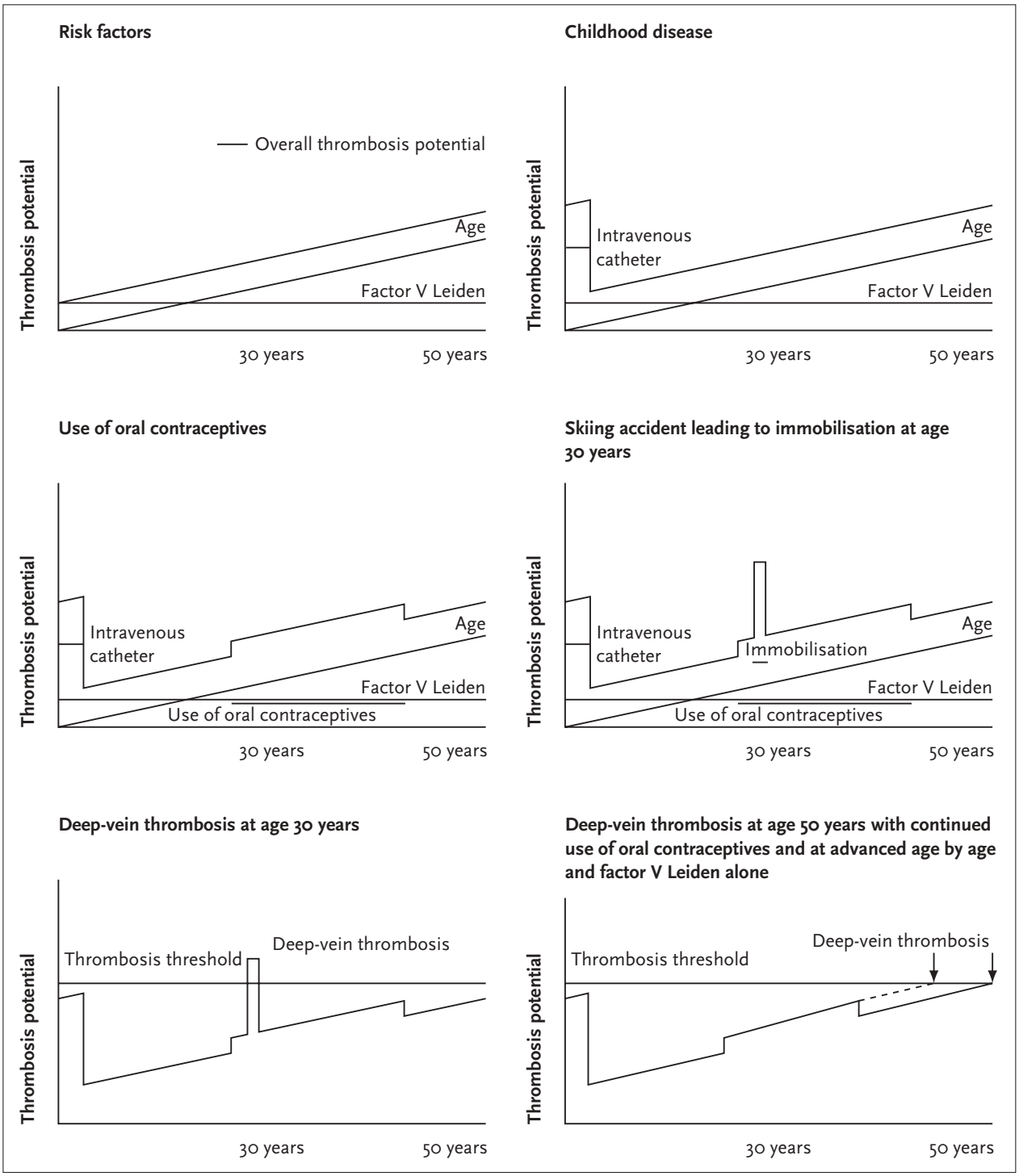

Figure 1

Models of thrombosis risk.[6] In each panel, the figure shows the thrombosis potential of each risk factor during an individual's life and the resultant thrombosis potential

At the age of 30 years, the combination of several risk factors and the thrombosis potential exceeds the thrombosis threshold and leads to clinical disease. Since increasing age itself is a risk factor for thrombosis, the threshold will be reached easier at later age and less risk factors will be needed to cause thrombosis.

Clustering and regulation

Since several procoagulant risk factors for thrombosis are closely related in the haemostatic system, a common genetic determinant of these coagulation factor levels could regulate these levels additionally to environmental determinants. A significant genetic component of coagula-

tion factors has been found in the Spanish population,[10] the United Kingdom[11] and the USA.[12] Interestingly, six families with a thrombotic tendency were reported in which high levels of coagulation factors XI, IX and VIII aggregated.[13] The inheritance pattern seemed to be dominant autosomal.[13] To date, the genetic basis of high levels is unknown. It is, however, possible that regulatory genes outside the genes of the coagulation factors regulate the protein levels. These levels would then cluster in an individual due to pleiotropic effects.

The evaluation between a potential risk factor and the occurrence of thrombosis is becoming more difficult, since adjustment is needed for more and more already known thrombotic risk factors. To better estimate the role of possible confounders and clustering of these factors, *a priori* knowledge of the interrelations of procoagulant and anticoagulant factors is important. With the data from the LETS, factor analysis was conducted using principal-component analysis with varimax rotation.[14] The number of variables is reduced by constructing relatively independent summary factors (the so-called principal components), which explain most of the variation in the data. In large studies where several risk factors seem to cluster, it is important to find the smallest number of principal components that still reflects the original data and variance. The newly formed principal loadings can be compared with the original variables by factor loadings, comparable with Pearson's correlation coefficients. When all the measured coagulation factors of the LETS were analysed, three relatively separate cluster patterns were found (*figure 2*). There was a clustering of the vitamin K dependent factors II, VII, IX and X, together with coagulation factors XI and XII. The second cluster consisted of factors V, VIII, IX, and fibrinogen. The third 'cluster' was made up of only one clotting factor, namely factor XIII subunit levels. These results show that interrelations exist between different coagulation factors in the haemostatic system. Therefore, common shared genetic mechanisms may be responsible for the clustering of these coagulation factors. Transcription factors, such as hepatocyte nuclear factor-4, may contribute to the first clustering pattern.[15-17] Factors V and VIII share a great part of homology and post-translational modifications and could explain the second clustering.[18] By using factor analysis, a better overall estimation of the overall risk associated with coagulant factors may become possible. The described method facilitates the interpretation of epidemiological studies and hopefully the determination of the thrombosis risk for individual patients. Family studies might be helpful in unravelling the genetic basis of these findings.

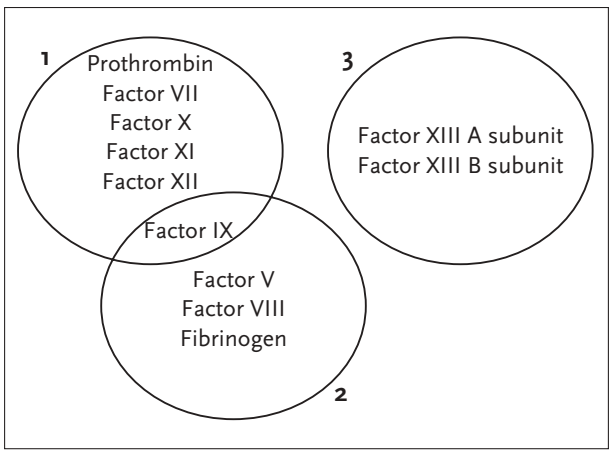

Figure 2
Factor loading pattern of procoagulant factors and fibrinogen in 466 healthy individuals[14]

CONSEQUENCES OF THROMBOPHILIA

Nowadays, a dozen different thrombophilia factors for thrombosis have been elucidated. However, venous thrombosis is a multicausal disease in which several risk factors, both genetic and acquired, have to occur simultaneously to cause thrombosis.[6,7] The interaction between these risk factors is dynamic rather than static, with age as an important contributor. In this complex situation, what is the contribution of inherited thrombophilia? And, now that we know so many thrombophilia factors, what is the consequence of thrombophilia? We will address this question by reviewing the influence of thrombophilia on the intensity and duration of anticoagulant therapy after a thromboembolic event, the risk of recurrence of venous thrombosis and the type of thrombosis. Thrombophilia could further be of importance for asymptomatic individuals.

Treatment of patients with thrombophilia

The intensity of anticoagulant treatment of patients with thrombosis who have a thrombophilia factor usually seems identical to patients without inherited defects, although this subject has never been thoroughly investigated.[19] Even in patients with deficiencies of antithrombin, protein C or protein S the therapeutic approach of thrombosis is generally the same. The optimal intensity of the international normalised ratio (INR) is 2.0 to 3.5, and this regimen is sufficient for preventing recurrences during therapy.[20] Recently it was shown that also in subjects with the antiphospholipid syndrome, moderate intensity anticoagulant therapy is adequate.[21] The optimal duration of anticoagulant therapy is uncertain, but does not seem to be influenced by the common thrombophilia factors. The goal of therapy is mainly to prevent recurrences. Since factor V Leiden and the prothrombin mutation are common in patients with thrombosis, several studies have analysed the risk of recurrent thrombosis in association with these prothrombotic defects. Neither of these mutations seem

to increase the risk of recurrences, although the data are not in complete agreement.[22-27] High levels of factor VIII and homocysteine seem to be associated with recurrences,[28,29] but these results have to be confirmed in other studies. Recurrent venous thrombosis might be more common in patients with a deficiency of antithrombin, protein C or protein S, but these results are based on retrospective data.[30] Given the low prevalence of these defects, it will be difficult to accurately determine the risk of recurrent thrombosis. From the other known prothrombotic defects, the effect on recurrent thrombosis is unknown. The combination of defects or homozygous factor V Leiden is probably associated with an increased risk of recurrence, although the information on patients studied so far is low.[31-36] So, apart from the antiphospholipid syndrome,[37] combined or homozygous defects, and possibly antithrombin deficiency, the impact of thrombophilia on the optimal duration of therapy to prevent recurrent thrombosis is probably small.[38]

Clinical manifestations of thrombophilia

Thrombosis in patients with thrombophilia usually manifests as deep vein thrombosis or pulmonary embolism. In patients with thrombophilia, thrombosis can also occur at unusual sites, such as the cerebral, visceral and axillary veins (*table 2*). Superficial thrombophlebitis is more common in protein C or protein S deficiency. In rare cases coumarin skin necrosis can occur.[39] Recurrence of thrombosis, a family history of thrombosis and first episode of thrombosis at young age are more common in patients with thrombophilia. In unselected thrombosis patients with a prothrombotic defect, such as factor V Leiden or prothrombin mutation, the difference with thrombosis patients without a defect is less clear.[8]

Table 2
Clinical manifestations of thrombophilia

Venous thrombosis at unusual site: mesenteric, pelvic, cerebral sinuses, portal, axillary
Family history of venous thromboembolism
Onset of thrombosis at young age
Recurrent episodes of venous thromboembolism
Warfarin induced skin necrosis
Recurrent foetal loss
Thrombophlebitis
Neonata purpura fulminans

Thrombophilia in asymptomatic patients

In women with the factor V Leiden or prothrombin mutation, oral contraceptive use, hormone replacement therapy and pregnancy further increase the risk of thrombosis, but the absolute risk seems to be low. Middeldorp *et al.* prospectively followed asymptomatic carriers of the factor V Leiden mutation.[40] In 470 individuals, the annual incidence of venous thrombosis was 0.58%, which does not justify routine screening of family members. Also in risk situations, such as pregnancy or oral contraceptive use, the rate of thrombosis was low.[40] In pregnant asymptomatic women heterozygous for factor V Leiden or the prothrombin mutation, absolute risk of thrombosis is less than 3%,[41,42] whereas a deficiency of antithrombin, protein C or protein S leads to a risk of 4.1%.[43] Taken together, the risk of thrombosis in asymptomatic carriers of thrombophilia defects seems low and does not justify screening. The optimal strategy of thrombosis prophylaxis of asymptomatic carriers is probably not different from patients without heritable thrombophilia, but this subject remains controversial as long as there are no trials comparing prolonged prophylaxis with standard prophylaxis in high-risk situations or prophylaxis *vs* placebo during pregnancy.[44]

IMPLICATIONS OF THROMBOPHILIA SCREENING

Testing for thrombophilia is subject to an intense pro-con debate.[45,46] Clinicians who perform thrombophilia screening usually argue that a better understanding of the pathogenesis of thrombosis is important for both the treating physician and for the patient. Family members of the proband with a prothrombotic defect can also be screened, in order to tailor prophylactic treatment during high-risk situations.[47] Others argue against screening since screening is not cost-effective and leads to anxiety among asymptomatic carriers or false reassurance in those without the defect.[46] Apart from the discussion whether screening should be performed, it is important how to interpret the results of studies for thrombophilia. What are the implications for an individual patient, for the family members, the treating physician, researcher or even the society?

Influence of patient selection on the association of thrombophilia and thrombosis

The strength of an association between an inherited coagulation defect and venous thrombosis can be influenced by the type of study and the selection of thrombosis patients and controls.[7] In cohort (follow-up) studies, quantitative estimates (i.e. absolute risks) can be obtained. In case-control studies one can estimate relative risks (as an odds ratio) by comparing thrombosis patients with healthy individuals. This figure indicates how much higher the thrombosis risk is in the presence of a certain risk factor than in the absence of that factor. In unselected cases from population-based studies, relative risks can be applied to all individuals with that particular risk factor, provided cases are well selected. Population-based case-

control studies can be used to calculate the attributable risk, i.e., the proportion of all thrombotic events that would have been prevented by removing the risk factor. Family studies often consist of subjects that were selected because of a conspicuously high frequency of thrombosis. In these studies, the occurrence of thrombosis is compared between family members with and without the risk factor. These studies are ideal for studying the type of inheritance of a certain risk factor and to qualitatively estimate the thrombosis risks. These thrombophilia families usually have more than one thrombophilic defect and results cannot be extrapolated to the general population. The influence of selection is well reflected in the age of onset of thrombosis that clearly differs between individuals from thrombophilia families and unselected thrombosis patients.[48] Finally, other aspects such as an objective diagnosis of thrombosis and prospective *vs* retrospective studies also influence the estimates of risk.

Importance of a risk factor for thrombosis

With so many new risk factors emerging, the question is what impact they have in daily clinical practice. In other words, how can the results from research be translated into practical clinical guidelines? First of all, we must make sure that the new risk factor is independent and clinically relevant. This requires full adjustment for potential confounders, such as age, sex, body mass index, and other coagulation factors. This does not apply for genetic risk factors, since these are by definition unconfounded.

It is important to appreciate and interpret the differences between absolute and relative risks. The relative risks that have been calculated from case-control studies are mainly important to the researcher, whereas absolute risk estimates (the probability to develop thrombosis and the possibility to lower this probability) is relevant to the individual patient and his physician.[49] Population-attributable risk estimates are also important for the population and can influence decision-making. Asymptomatic females with deficiencies of antithrombin, protein C, or protein S have an eightfold increased relative risk of thrombosis during pregnancy.[30] The absolute risk is 4.1% (7 in 169 pregnancies). So, these deficiencies have a high magnitude of risk, but because of the low prevalence, account for only a small percentage of the overall thrombosis risk. Likewise, oral contraceptives and hormone replacement therapy both increase the risk of the thrombosis approximately fourfold. Since the baseline risk of thrombotic disease is nearly tenfold higher in the older HRT users, their basic risk of thrombosis is greater than in women who are on oral contraceptives.[49] Hypertension is a moderate risk factor for congestive heart failure, but accounts for nearly half of the cases of heart failure in a population.[50] So, in general, common risk factors usually have moderate relative risks, but are important at population level when its prevalence is high. Strong risk factors are generally less important for healthy individuals.

If we consider thrombophilia screening for risk factors, the measurement must be reliable (low coefficient of variation) and reflect a true representation of the risk factor. For prothrombotic mutations, such as factor V Leiden and prothrombin mutation, this is generally not a problem. Most coagulation factors, such as factor VIII, however, have a large intra-individual and inter-assay variation, as reflected in the decision to use the factor VIII measurements of three different plasma samples to calculate the probability of carriership of haemophilia A. Further, there is the important question how we should interpret the result of a measurement in terms of risk of a first thrombotic event and risk of recurrences. Most risk factors have wide ranges of values with large overlap between individuals with and without thrombosis. These risk factors typically increase with increasing levels, without a clear threshold. So, artificial cut-off values which were used in clinical research are being used now for decision-making in individual patients. It is unknown whether these cut-off values are practical and reliable. We do not know the sensitivity or specificity of most risk factors for predicting future occurrences of thrombosis. Factor VIII levels can easily rise above the cut-off value of 150 IU/dl due to acute phase reactions, such as a thrombotic event. This transient rise may cause a mislabelling of a person with venous thrombosis who normally has a low factor VIII level.[51] Since factor VIII levels may be associated with the risk of recurrences,[28] a treating physician might decide to prolong anticoagulant therapy on the basis of a single measurement. This shows that the results from research cannot simply be extrapolated to patient care and can even lead to wrong decisions (*primum non nocere*).

IMPLICATIONS OF THROMBOPHILIA SCREENING FOR THE INDIVIDUAL PATIENT

As already stated, relative risk has no value in the clinic, and only knowledge of the absolute risk of developing thrombosis may have relevance for the individual patient, and then still only if this leads to the possibility of prevention. This would imply that for each patient at risk of a first episode of thrombosis or for a recurrent event, an individualised risk profile should be available with age, sex, current risk factors and the possibility of future risk factors, such as trauma, surgery and pregnancy, while for each factor its strength should be known, as well as its interaction with the other factors. This scenario is still far away. It is not even feasible to readily identify patients with thrombophilia unless all thrombosis patients are screened, since half of the first thrombotic events in patients with

thrombophilia are not idiopathic and occur in high-risk situations. Practical recommendations have been suggested to guide screening strategies in patients with thrombosis, in which patients are divided in 'strongly' and 'weakly' thrombophilic.[19] The 'strongly' thrombophilic patients include patients with age at onset <50 years, patients with recurrent thrombosis, or first-degree family members with a thrombotic event before 50 years of age. All other patients are 'weakly' thrombophilic and should be screened for the common defects such as factor V Leiden and prothrombin mutation, while the former group should also be tested for the more rare defects such as deficiencies of protein C, protein S and antithrombin. This strategy optimises the likelihood of finding a prothrombotic abnormality, but does not necessarily benefit the patient. With the current knowledge it is questionable whether the presence of a risk factor leads to any difference in clinical management, and therefore screening does not seem helpful. The most compelling question is whether, based on laboratory tests, we can predict the risk of recurrence and, while the various studies are not in complete agreement, it may well be that the risk of recurrence is not increased in the presence of prothrombotic defects. In that case it makes more sense to base clinical strategy on clinical history, i.e., the severity of the event or the age of the patient, than on laboratory tests. The next question concerns asymptomatic relatives: is it useful to screen asymptomatic individuals from a family with hereditary thrombophilia, for instance women who intend to become pregnant or want to start oral contraceptives? Again, the literature offers little assistance, except that in most cases the risk of thrombosis appears to be low. Women from families with a strong history of thrombosis may consider not using oral contraceptives.

CONCLUSION

The last decade revealed several new risk factors that contribute to a better understanding of the pathogenesis of venous thrombosis. Well-designed large population-based case-control studies were a prerequisite for establishing new risk factors, such as factor V Leiden, prothrombin 20210A mutation, procoagulant factors, as factor VIII, IX, and XI, and antifibrinolytic factors, such as TAFI. Since many individuals with a thrombophilic factor are asymptomatic, a single defect is seldom sufficient to cause thrombosis. Thrombosis is thus a multicausal disease, in which genetic and environmental factors interact dynamically. The common risk factors with a high prevalence in the general population make a major contribution to the overall risk of thrombosis. These risk factors are likely to occur simultaneously in an individual with thrombosis. When these clusters of risk factors can be identified, preventive measures can be installed, mainly for those individuals with a genetic predisposition. This can only be assessed adequately through sufficient knowledge of important risk factors for thrombosis, their effect and interaction with other genetic and environmental factors, and the beneficial effect of intervention. Until that time, screening for thrombophilia will remain a matter of debate.

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Helicobacter pylori and gastro-oesophageal reflux disease: a cross-sectional epidemiological study

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ABSTRACT

Background: *H. pylori* infection is accompanied by a lower prevalence of reflux disease. There is still an ongoing debate as to whether *H. pylori* actually protects against the development of reflux oesophagitis or is merely an epiphenomenon. A cross-sectional study was performed to study the relation of *H. pylori* with reflux oesophagitis, hiatus hernia and Barrett's oesophagus.

Material and methods: Consecutive patients undergoing upper gastrointestinal endoscopy in a period of ten years were studied. Included were patients with active reflux oesophagitis and/or hiatus hernia and/or Barrett's oesophagus. As a reference group, patients without macroscopic abnormalities were included. *H. pylori* was detected applying routine diagnostic modalities.

Results: In the ten years 11,691 consecutive patients were studied. Reflux oesophagitis was seen in 1535 patients, 307 patients had Barrett's oesophagus and a hiatus hernia was present in 2116 patients. The reference group consisted of 5341 patients. *H. pylori* was significantly less often detected in patients with reflux oesophagitis or Barrett's oesophagus compared with the reference group, 20 *vs* 29% (p<0.001). Also presence of *H. pylori* was significantly lower in patients with hiatus hernia 20 *vs* 29% (p<0.0001).

Conclusion: The present study confirms, in a very large group of patients studied in one single centre, the findings of earlier papers. Patients without *H. pylori* gastritis suffer more often from reflux disease. There is a relation between *H. pylori* and reflux disease. However, the consequence of this relation will not be the same in every patient.

INTRODUCTION

The discovery of *H. pylori* has been a major breakthrough in understanding and treatment of gastritis and ulcer disease. Despite the effects of *H. pylori* infection on the gastric acid production,[1,2] the bacterium does not play a role in the pathogenesis of reflux disease. On the contrary, presence of *H. pylori* is accompanied by a lower prevalence of reflux disease.[3-7] There is still an ongoing debate as to whether *H. pylori* actually protects against the development of reflux oesophagitis or is merely an epiphenomenon.[8-10] In earlier studies, it was shown that patients with reflux disease exhibit *H. pylori* infection less often than a reference group of patients without signs of reflux oesophagitis or Barrett's oesophagus.[6,11] These findings have been confirmed in many other papers. In most studies relatively small populations of patients were studied. Significant differences in study design were present (prospective, retrospective case control or trial). In the present cross-sectional study the number of patients was extended considerably and the relation of *H. pylori* with reflux oesophagitis, hiatal hernia and Barrett's oesophagus in a large population of patients undergoing upper gastrointestinal endoscopy for various reasons was studied.

MATERIAL AND METHODS

All consecutive patients undergoing upper gastrointestinal endoscopy in a period of ten years were included. Endoscopies carried out as follow-up because of newly developed or recurrent symptoms were excluded. Included in the study were patients with active reflux oesophagitis and/or hiatus hernia and/or Barrett's oesophagus. As a

reference group, patients without macroscopic abnormalities in oesophagus, stomach or duodenum, with the exception of endoscopic signs of gastritis, were included. Biopsy specimens were taken from the gastric antrum if judged necessary by the endoscopist or if a clinical reason for detection of *H. pylori* was present. *H. pylori* was detected using Gram's stain with culture, standard haematoxylin and eosin stain, and immunoperoxidase stain. Culture has been used since 1994 as a standard diagnostic method. A patient was judged *H. pylori*-positive if one or more of the applied methods were positive. A patient was considered *H. pylori*-negative if all methods failed to detect the bacterium.

All endoscopy results were noted in a standardised endoscopy report.

A hiatus hernia was defined as a distance of more than 2 cm between the oesophageal gastric junction and the diaphragm. Barrett's epithelium was judged to be present if the typical coloured metaplastic mucosa was seen in the tubular oesophagus.

Statistical analysis was done with chi-square test for contingency tables. A result was judged statistically significant if the value was below 0.05.

RESULTS

In the ten-year period 14,909 consecutive diagnostic upper gastrointestinal endoscopies were performed in 11,691 patients. A total of 3218 endoscopies were excluded because these procedures were carried out as follow-up after previously diagnosed abnormalities (peptic ulcer, cancer) or because of recurrent or newly developed upper gastrointestinal symptoms in the same patient.

Four groups of patients were seen. Group 1 consisted of 1535 patients with active reflux oesophagitis; Barrett's oesophagus was seen in 307 patients (group 2). A hiatus hernia was diagnosed in 2116 patients (group 3) and, finally, group 4 consisted of 5341 patients without any macroscopic abnormalities in oesophagus, stomach or duodenum or with signs of endoscopic gastritis (reference group). *Table 1* shows details of the different groups. Patients in group 4 (reference group) were significantly younger than all other groups ($p<0.001$); however, overlap in age cohorts is present.

H. pylori was significantly less often detected in patients with reflux oesophagitis or Barrett's oesophagus compared with the reference group, 20 *vs* 29% ($p<0.001$). Also presence of *H. pylori* was significantly lower in patients with hiatus hernia (group 3), 20 *vs* 29% ($p<0.0001$) (*table 2*). Unfortunately the *H. pylori* status was not known in all patients. There was no difference in the number of missing biopsy specimens in the different groups of patients. Assuming that *H. pylori* was present in 30 or 40% of the missing specimens (this is a normal prevalence of the bacterium in the Western world), than the numbers in each group would have been higher but the final significant differences would not change.

Table 3 shows the differences in *H. pylori* presence in three major age cohorts.

Table 1
Numbers of men and women and H. pylori*-positives and negatives in the four groups of patients*

	MEN	WOMEN	HP+		HP-		NO BIOPSY SPECIMEN		MEAN AGE
			n	%	n	%	n	%	
Group 1	937	598	312	20	770	50	453	30	56
Group 2	193	114	55	18	124	40	128	42	65
Group 3	938	1178	416	20	994	47	706	33	58
Group 4	2159	3182	1550	30	2425	45	1366	25	50

Hp means no biopsy specimens available, the numbers in brackets indicate percentages. Age of the different groups is compared. Group 1 vs group 2: p = ns, group 1 vs group 3: p = ns, group 1 vs group 4: p<0.001, group 2 vs group 3: p = ns, group 2 vs group 4: p = 0.03, group 3 vs group 4: p<0.0001.

Table 2
Numbers of H. pylori*-positive and negative patients*

	HP+		HP-		HP NOT KNOWN	
	n	%	n	%	n	%
Group 1 + 2	367	20	894	49	581	31
Group 3	416	20	994	47	706	33
Group 4	1550	29	2425	45	1366	26

Table 3
Presence of H. pylori *in three different age cohorts of patients in the four different groups*

PATIENTS >50 YEARS	HP+		HP-		NO BIOPSY SPECIMEN	
	n	%	n	%	n	%
Group 1 + 2	247	20	489	41	465	39
Group 4	747	29	988	38	863	33
	p<0.001					

PATIENTS 30 TO 50 YEARS	HP+		HP-		NO BIOPSY SPECIMEN	
	n	%	n	%	n	%
Group 1 + 2	94	18	326	63	99	19
Group 4	534	29	940	52	340	19
	p<0.001					

PATIENTS <30 YEARS	HP+		HP-		NO BIOPSY SPECIMEN	
	n	%	n	%	n	%
Group 1	26	21	75	65	17	14
Group 4	269	29	497	53	163	18
	p = 0.04					

DISCUSSION

The present study confirms, in a very large group of patients studied in one single centre, the findings of earlier papers. Patients without *H. pylori* gastritis suffer more often from reflux disease. This observation has led to the hypothesis that *H. pylori* protects against reflux oesophagitis. There is an ongoing debate in the literature as to whether reflux disease actually develops after successful anti-*H. pylori* therapy.[12-15] Is *H. pylori* protective or is this merely coincidence? A point of criticism can be that in the reference group patients are included with endoscopy negative reflux disease. The so-called typical reflux symptoms are not very specific for reflux disease. They are also present in ulcer disease and patients with functional dyspepsia. In addition, pH monitoring in the oesophagus, often considered as gold standard, can produce false-negative results. The only true gold standard for reflux disease is the presence of reflux oesophagitis or Barrett's metaplasia.
The major problem in all studies on *H. pylori* and reflux disease is that many different types of patients have been studied: patients treated with maintenance acid suppressive therapy because of peptic ulcer disease or reflux disease, patients with peptic ulcer disease with coexisting reflux disease and patients with newly developed disease without ever having been treated before. This makes comparisons difficult. Also many patients with functional dyspepsia or genuine reflux oesophagitis have been included.[16-18]
Patients with a chronic *H. pylori* associated corpus gastritis induced by the use of acid suppressive therapy started for whatever reason will have higher acid production once *H. pylori* has been eradicated compared with patients without corpus gastritis.[19,20] Obviously the presence of corpus gastritis, induced by acid suppressive therapy, in a studied patient population is a confounding factor.
The most likely mechanism by which *H. pylori* may protect against reflux is by decreasing the potency of the gastric refluxate in patients with corpus predominant gastritis.[21] It has been shown that colonisation with CagA-positive *H. pylori* provides significant protection against the development of reflux disease and its long-term complications.[4] While *H. pylori* infection itself does not cause or really protect against developing reflux disease, it may protect certain susceptible individuals from developing the condition and its possible complications.[23]
The prevalence of reflux disease and oesophageal adenocarcinoma is rising, while the prevalence of *H. pylori* infection has been decreasing[24] in the Western world. Since it is known that the acquisition of *H. pylori* at young age is decreasing this could be an explanation. The rising prevalence of reflux disease can also be explained by changes in dietary habits and body mass index. *H. pylori* in the stomach is possibly responsible for other feeding habits. Recent studies indicate that *H. pylori* has effects on production of leptin and plasma ghrelin levels. Leptin is produced in the mucosa of the gastric fundus. Gastric distension due to eating will lead to a decrease of fundic leptin.[24] Eradication of *H. pylori* does not change plasma leptin levels. However, leptin immunoreactivity in the gastric fundus significantly decreases after successful

eradication of the bacterium. In the studied patients, this was accompanied by a significant correlation with changes in body mass index. Since the serum leptin levels did not change this must be due to a local effect.[24]

Ghrelin, a newly discovered gastric hormone, is an important factor in appetite. After *H. pylori* cure plasma levels increase significantly.[25] This could lead to increased appetite and hence weight gain. Whether these levels are also higher in people who were always *H. pylori* negative is yet to be determined. The concept, however, is appealing.

It is conceivable to assume that individuals without *H. pylori* have more appetite resulting in increase in body weight, more transient lower oesophageal sphincter relaxation and hence induction of reflux disease. It is also possible that dietary habits change after eradication of *H. pylori* to such an extent that the BMI rises. Together with healing of corpus gastritis, this may be a risk factor in developing reflux disease.

A rising body mass index takes time. It is well known that the majority of people gain weight with rising age. Since patients with reflux disease are older then patients in the reference group it is tentative to assume that reflux patients could have a higher body mass index. This observation could be an extra argument in favour of a relation of dietary habit and body mass index.

Long-term prospective studies on the prevalence of reflux disease with information of dietary habits, body mass index and presence of *H. pylori* are mandatory.

It can be concluded that there is a relation between *H. pylori* and reflux disease. However, the consequence of this relation will not be the same in every patient.

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ORIGINAL ARTICLE

The influence of pretreatment on cure rates of *Helicobacter pylori* eradication

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ABSTRACT

Background: Many patients treated for *H. pylori* infection have been taking a proton pump inhibitor beforehand. There is conflicting evidence whether pretreatment influences the efficacy of *H. pylori* eradication. The aim of this study was to investigate the influence of pretreatment on cure rates of *H. pylori* eradication.

Methods: Patients with *H. pylori* positive peptic ulcer disease or functional dyspepsia were treated with two-day quadruple therapy (lansoprazole 30 mg twice daily, and colloidal bismuth subcitrate 120 mg, tetracycline 250 mg and metronidazole 250 mg, all eight times a day). Patients were randomised to receive either three-day pretreatment with lansoprazole 30 mg twice daily or no pretreatment. *H. pylori* was diagnosed using CLO, histology and culture.

Results: Twenty-five (66%) of 38 patients with pretreatment and 32 (84%) of 38 patients without pretreatment were cured (p=0.06). After adjustment for diagnosis, smoking status and metronidazole resistance the influence of pretreatment became slightly less pronounced (OR 0.44, 95% CI 0.1-1.7). Nonsmokers and patients with peptic ulcer disease were more likely to achieve *H. pylori* eradication than smokers and patients with functional dyspepsia, respectively (adjusted odds ratios: 4.79 (1.2-19) and 4.32 (1.0-18)).

Conclusions: This two-day quadruple therapy reached an overall cure rate of 75%. Nonsmokers and patients with peptic ulcer disease were more likely to achieve *H. pylori* eradication. Three-day pretreatment with a proton pump inhibitor may decrease cure rates of this two-day quadruple therapy.

INTRODUCTION

During the past decade it has been established that not only patients with peptic ulcer disease but also a subgroup of patients with functional dyspepsia benefit from *Helicobacter pylori* eradication.[1,2] Therefore *H. pylori* test-and-eradication has been incorporated in most guidelines for treatment of patients with dyspeptic symptoms.[3] As a result, many patients now receive therapy for *H. pylori* infection. Triple and quadruple therapies are usually used and achieve high cure rates[4] but none of the current therapies have reached a 100% cure in clinical trials[5] and several studies reported that cure rates in routine clinical practice are even lower.[6] Cure rates are influenced by antibiotic resistance,[7] duration of therapy[8] and compliance.[9]
Another factor that has been implicated in therapy failure is pretreatment with a proton pump inhibitor. This may be an important factor as many patients treated for *H. pylori* infection are already on proton pump inhibitors.[10]
Although pretreatment was advocated in the assumption that elevating gastric pH before starting the antibiotics would increase cure rates, several studies showed that pretreatment was related to therapy failure for dual therapy with omeprazole and amoxicillin. Eradication rates were 30 to 70% lower in patients with pretreatment.[11-14]
The few studies investigating the influence of pretreatment on triple and quadruple therapies did not find differences in eradication rates for patients with and without pretreatment.[15-17] However, the high eradication rates of seven-day triple and quadruple therapies make it difficult to study factors associated with therapy failure.
In this paper we used a very short quadruple therapy to study the influence of pretreatment. In our area, fairly high cure rates were reached with this quadruple regimen, and

because of its short duration we assumed it to be more vulnerable to the effect of pretreatment. That renders this regimen suitable for studying the effect of pretreatment in a fairly small population. The aim of this study was to evaluate the influence of three-day pretreatment with lansoprazole on cure rates of a two-day, intensified quadruple therapy, combining lansoprazole, bismuth, metronidazole and tetracycline.

MATERIALS AND METHODS

Study population

The study was conducted at Bernhoven Hospital, the Netherlands, in 1997, with approval of the local ethics committee. Patients over 18 years with *H. pylori* positive peptic ulcer disease or functional dyspepsia were eligible. Exclusion criteria were use of bismuth compounds/antibiotics/proton pump inhibitors during the past four weeks, prior *H. pylori* eradication, pregnancy or lactation and known allergic reaction to the study medication. All participating patients gave written informed consent.

Investigations

All patients underwent upper gastrointestinal endoscopy both before and four to six weeks after treatment. At endoscopy seven biopsies were taken: four from the antrum (two for histology, one for CLO® (Delta West, Australia), one for culture) and three from the corpus (two for histology and one for CLO®). Biopsies for histological examination were fixed in neutral buffered 4% formaldehyde and *H. pylori* identification was performed on Giemsa-stained sections of paraffin embedded tissue. For culture Belo-Horizonte medium was used and plates were incubated microaerobically for seven days. Resistance to metronidazole and clarithromycin was determined by E-test® (AB Biodisk, Sweden) with cut-off values of 2 and 8 μg/ml, respectively. Patients were considered *H. pylori* positive when two out of three tests (CLO®, histology, culture) were positive. Patients were regarded to be cured when all three tests were negative.
Patient compliance was assessed both by interview and pill count. Side effects were registered using the questionnaire developed by De Boer *et al.*[18]

Intervention

Patients received open-label therapy with two-day quadruple therapy consisting of lansoprazole 30 mg twice daily, together with colloidal bismuth subcitrate (De-Nol®) 120 mg, tetracycline 250 mg and metronidazole 250 mg (all taken eight times a day, at 9, 11, 13, 15, 17, 19, 21, and 23 hours). Patients were randomly allocated to three-day pretreatment with lansoprazole 30 mg twice daily or no pretreatment at all.

Randomisation procedure

After inclusion each patient received a (sequentially) numbered, sealed, opaque, envelope containing the prescription (with or without pretreatment according to randomisation) and instructions on how to take the drugs. The envelopes were filled before the start of the study using a computer-generated randomisation list.

Data analysis

Primary outcome of the study was *H. pylori* eradication. The study was designed as a pilot study with 80% power to detect a 20% decrease in cure rate due to pretreatment, for an estimated 85% cure rate of this quadruple therapy without pretreatment (a=0.05).
Baseline characteristics and eradication rates for both groups were compared using the χ^2 test. Pretreatment and baseline characteristics were related to *H. pylori* eradication by means of unadjusted and adjusted logistic regression analyses, using the SAS® statistical software package (SAS Institute Inc., USA). Statistical significance was defined as a $p<0.05$. Missing values were excluded from analyses.

RESULTS

Study population

Altogether, 76 patients were randomised. *Table 1* shows the baseline characteristics of these patients. Unfortunately, despite adequate randomisation, the pretreatment group contained more patients with functional dyspepsia.

Table 1
Baseline characteristics (intention-to-treat population)

	WITH PRETREATMENT (N=38)	WITHOUT PRETREATMENT (N=38)
GENDER		
Male	21 (55%)	29 (76%)
Female	17 (45%)	9 (24%)
AGE		
≤50 years	17 (45%)	14 (37%)
>50 years	21 (55%)	24 (63%)
DIAGNOSIS (p<0.05)		
Peptic ulcer disease	14 (37%)	23 (61%)
Functional dyspepsia	24 (63%)	15 (39%)
CURRENT SMOKING	15 (39%)	19 (50%)
ANTIBIOTIC SUSCEPTIBILITY		
Metronidazole resistant	7 (23%)	5 (19%)
Metronidazole susceptible	24 (77%)	22 (81%)
Clarithromycin resistant	0 (0%)	0 (0%)
Clarithromycin susceptible	31 (100%)	27 (100%)

Eradication rates, compliance and adverse events

Of 38 patients with pretreatment, 25 (66%) were cured, whereas 32 (84%) of 38 patients without pretreatment were cured (p=0.06). All patients reported to have taken more than 90% of their pills.

The questionnaire on side effects was returned by 67 patients. Eighty-five percent of patients reported 'no side effects', or 'slight discomfort, not interfering with daily activities', 10% reported 'moderate side effects, sometimes interfering with daily activities' and 4% reported 'severe side effects'. None of the patients discontinued therapy because of side effects. Most frequently reported side effects were metallic taste, nausea and diarrhoea. There were no differences in incidence or severity of side effects between the treatment arms.

Factors associated with treatment outcome

Table 2 shows that there is a tendency towards treatment failure for patients with pretreatment. For these patients the risk of treatment failure almost triples, although this effect becomes somewhat less pronounced after adjustment for diagnosis, smoking and metronidazole resistance. Furthermore, *table 2* shows that diagnosis and smoking status are important predictors of treatment outcome. After adjustment, patients with peptic ulcer disease have an over four times greater chance of treatment success compared with patients with functional dyspepsia, whereas smokers have an almost five times greater chance of treatment failure compared with nonsmokers.

DISCUSSION

The aim of this study was to investigate the influence of pretreatment with a proton pump inhibitor on *H. pylori* eradication. Many patients treated for *H. pylori* infection receive pretreatment, either intentionally, in an attempt to enhance cure rates of *H. pylori* eradication as used to be advocated, or unintentionally, by using a proton pump inhibitor for treatment of gastrointestinal symptoms, peptic ulcer disease or reflux oesophagitis before starting *H. pylori* eradication. This warrants the need to further investigate the influence of pretreatment.

Theoretically, pretreatment with a proton pump inhibitor may influence eradication rates in several ways. Firstly, proton pump inhibitor therapy prevents degradation of acid labile antibiotics and decreases the minimum inhibitory concentration of the antibiotics.[19] Consequently, pretreatment may increase the effectiveness of the first doses of antibiotics by elevating gastric pH before starting eradication therapy. Secondly, proton pump inhibitor therapy decreases bacterial load, especially in the antrum.[20] This may seem an advantage because less bacteria have to be killed. However, the remaining bacteria are in a less active, dormant, state[21] and are therefore less vulnerable to the actions of antibiotics.

In the present pilot study we evaluated the effect of three-day pretreatment with lansoprazole on eradication rates of a two-day intensified quadruple therapy. The results show a trend for patients with pretreatment towards lower eradication rates. But, although patients with pretreatment have an 18% lower cure rate, this difference does not reach statistical significance (p=0.06). This may be due to type II error, as the power of this pilot study was only sufficient for detection of a difference of over 20%. Furthermore, adjustment for diagnosis, smoking status and metronidazole resistance slightly decreased the influence of pretreatment. This may be explained by the higher number of patients with functional dyspepsia, who have lower cure rates than patients with peptic ulcer disease, in the pretreatment group.

However, a 10 to 20% decrease may well be possible with

Table 2

Factors associated with treatment outcome

	UNADJUSTED ANALYSIS			ADJUSTED ANALYSIS*		
FACTOR	ODDS RATIO	95% CI	P VALUE	ODDS RATIO	95% CI	P VALUE
Pretreatment (*vs* no pretreatment)	0.36	0.1-1.1	0.06	0.44	0.1-1.7	0.23
Diagnosis (peptic ulcer disease *vs* functional dyspepsia)	2.58	0.9-7.8	0.09	4.32	1.0-18	0.05
Smoking (*vs* no smoking)	0.37	0.1-1.1	0.06	0.21	0.1-0.8	0.03
Metronidazole resistance (resistant *vs* susceptible)	0.44	0.1-1.7	0.22	0.51	0.1-2.3	0.39
Gender (male *vs* female)	1.58	0.5-4.6	0.40			
Age class (>50 years *vs* ≤50 years)	1.08	0.4-3.1	0.89			

* *Adjusted for pretreatment, diagnosis, smoking and metronidazole resistance.*

this two-day quadruple therapy. An effect of that magnitude would be clinically relevant and might have consequences for clinical practice. Possibly, patients on a proton pump inhibitor should be advised to either interrupt the proton pump inhibitor therapy before starting *H. pylori* eradication or take an eradication regimen of longer duration.
We used a two-day quadruple therapy in order to be able to demonstrate the influence of pretreatment without the necessity to study a large number of patients. Seven-day quadruple regimens have higher cure rates and may possibly overcome any deleterious effect of pretreatment. However, there are no published data on this. For seven-day proton pump inhibitor triple therapy, two studies investigating 89 and 101 patients found no difference in cure rates between patients with and without pretreatment.[16,17] However, the high cure rates of these therapies require large study populations to detect a 10 to 15% difference in eradication rates. Therefore more research is necessary to definitely settle the issue of pretreatment.

The overall eradication rate of this two-day quadruple therapy was 75%, which is comparable with other research with two-day quadruple therapy.[22,23] Although this is inadequate for use in routine clinical practice, these results after just two days of therapy emphasise the efficacy of quadruple therapy.
Being a nonsmoker and having peptic ulcer disease were associated with a greater chance of achieving *H. pylori* eradication. Smoking has been identified by several studies to be an important factor associated with treatment failure.[24-26] The underlying mechanism is still unknown, although decreased gastric blood flow,[27] damage to the gastric mucosa,[28] and increased acid secretion[29] have been implicated.
The higher cure rates for patients with peptic ulcer disease (*vs* functional dyspepsia) are consistent with other studies, typically reporting 5 to 15% higher eradication rates for patients with peptic ulcer disease.[30-32] This may be caused by the higher prevalence of more virulent *H. pylori* strains[33,34] which cause more inflammation[35] in patients with peptic ulcer disease, as several studies have shown that patients with more virulent strains[36] and with more inflammation[37] can be cured more easily.

In conclusion, this two-day quadruple therapy reached an overall cure rate of 75%. Although this is not sufficient for use in routine clinical practice, these results after just two days of therapy emphasise the potency of quadruple therapy in general. Nonsmokers and patients with peptic ulcer disease were more likely to achieve *H. pylori* eradication. Three-day pretreatment with a proton pump inhibitor may decrease cure rates of two-day quadruple therapy, but more research is necessary to definitely establish the influence of pretreatment with a proton pump inhibitor on routine therapy for *H. pylori* eradication.

NOTE

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An immunocompromised host with bilateral pulmonary infiltrates

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CASE REPORT

A 36-year-old man was admitted at our haematology ward in the winter season because of fever and a nonproductive cough for two days. He had a myeloablative allogeneic blood stem cell transplantation ten months earlier for chronic myelogeneous leukaemia in the first chronic phase. The transplantation had been complicated by a treatment-related pneumonitis for which he received immunosuppressive drugs and by Epstein-Barr virus-related lymphoproliferative disease for which he had received a single dose of a humanised mouse anti-CD20 monoclonal antibody (rituximab). On admission he was given ciclosporin and steroids as immunosuppression and claritromycin and valaciclovir as primary prophylaxis. Physical examination revealed crackles over the left lower lung field, a temperature of 39.5°C and a peripheral oxygen saturation of 92% at room air. Laboratory examination showed mild anaemia, thrombocytopenia and leucopenia, with a marked neutropenia of 0.55 x 10^9/l. A radiograph of the chest showed bilateral pulmonary infiltrates in the lower lobes and bilateral shadowing of the frontal sinuses (*figure 1*). He was treated with broad-spectrum antibiotics (imipenem/cilastatin) and a bronchial alveolar lavage was performed the next day.

WHAT IS YOUR DIAGNOSIS?

See page 210 for the answer to this photo quiz.

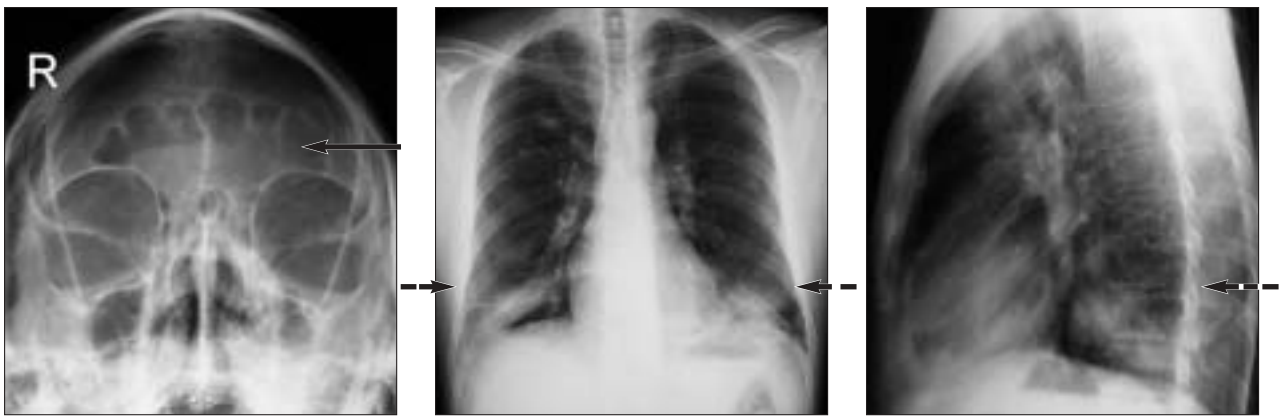

Figure 1
Bilateral shadowing of the frontal sinuses (◄) and bilateral pulmonary infiltrates of the lower lobes (◄ -)

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CASE REPORT

Cryptosporidiosis leading to an unsuspected diagnosis of AIDS

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ABSTRACT

We describe a 68-year-old woman with an episode of diarrhoea, malaise and weight loss, caused by infection with *Cryptosporidium*. The diagnosis was hampered because this patient had a low risk of HIV infection, a two-year history of Crohn's disease, and a simultaneous candidal infection. An infection with *Cryptosporidium* was demonstrated with electron microscopic examination, and subsequent tests revealed positive HIV serology. AIDS was probably contracted through her husband.

INTRODUCTION

The differential diagnosis of diarrhoea is very extensive. In patients with Crohn's disease a new episode of gastrointestinal symptoms can usually be ascribed to an exacerbation. When the symptoms do not respond to adequate therapy, further investigations must be performed. We report here on a patient with previously diagnosed Crohn's disease who developed therapy-resistant diarrhoea.

CASE REPORT

A 68-year-old woman presented with a three-month history of nausea, abdominal cramps, diarrhoea without blood or mucus, malaise and a weight loss of 14 kg. Three years earlier, in 1998, Crohn's disease had been diagnosed after an episode of fever, diarrhoea and weight loss. Biopsies then showed a granulomatous colitis. Since treatment, she had been without symptoms.

At first, the new episode of gastrointestinal symptoms was attributed to an exacerbation of Crohn's disease. She was treated with mesalazine and budesonide, but the symptoms persisted. A colonoscopy showed no signs of active Crohn's disease. A gastro-duodenoscopy revealed a *Candida* oesophagitis, confirmed by histological examination and colon biopsies showed chronic active inflammation with para-aminosalicylic acid (PAS) positive particles in the crypts that were regarded as yeast forms of *Candida*. Because of further weight loss and therapy-resistant gastrointestinal symptoms, she was admitted to our hospital. She weighed 48 kg, whereas her height was 1.60 meter. Her blood pressure was 90/60 mmHg and pulse rate 80 beats/min. The abdomen was slightly distended with lively peristaltic movements. Her mouth showed *Candida* stomatitis. Physical examination showed no other abnormalities. Routine examination of repeated stool specimens showed no pathogenic bacterial or parasitic organisms. Laboratory examination showed an erythrocyte sedimentation rate of 7 mm/hour, haemoglobin 7.3 mmol/l and a leucocyte count of 3.8 x 10^9/l. Serum potassium (2.4 mmol/l) and albumin (20 g/l) were decreased, as were serum urea nitrogen (1.2 mmol/l) and creatinine (47 μmol/l). Liver tests were unremarkable. X-rays of the small bowel and abdominal ultrasounds revealed no abnormalities. Gastroscopy was repeated and jejunum biopsies demonstrated PAS-positive micro-organisms on the epithelial surface of villi and crypts. These were more suggestive of *Cryptosporidia* than of yeasts, which were initially considered on account of the observed *Candida* oesophagitis. Retrospectively, the micro-organisms in the colon were similar. Subsequent electron microscopy of the biopsies confirmed the infection with *Cryptosporidium* (*figure 1*). At the same time, using specific staining techniques,

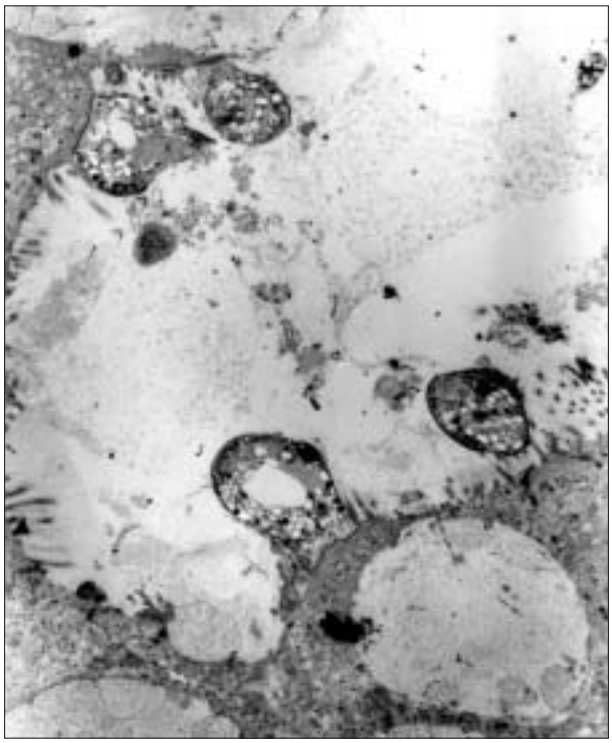

Figure 1
Electron microscopic photograph of intestinal crypt. Below and left, parts of epithelial cells with brush border; in the lumen, four schizonts of Cryptosporidium. *Magnification 3000x*

cryptosporidial oocysts were found in the stools. Because of the presence of opportunistic infections, a diagnosis of AIDS was considered, although this patient had a very low *a priori* risk of HIV infection. She had had no sexual relationships since her husband had died 14 years previously. She had neither a history of blood transfusions nor of intravenous drug use. However, anti-HIV antibodies were demonstrated and HIV-1 infection was confirmed by Western blot. Her husband had received several transfusions during brain tumour surgery in the period 1980 to 1985, when HIV screening on donor blood was not routinely performed in the Netherlands. It is possible that her husband was infected by a transfusion and transmitted the virus to our patient more than 14 years ago. The number of CD4+ T lymphocytes was very low: 0.01 x 10^6 /ml (normal 0.5-1.7 x 10^6 /ml). The viral load was 1.75 x 10^5 eq/ml. A highly active antiretroviral treatment regimen (HAART) was prescribed, which consisted of the combination zidovudine, lamivudine and indinavir. This was combined with cotrimoxazole as prophylaxis for *Pneumocystis carinii*. Symptomatic therapy with loperamide and metoclopramide helped to control the diarrhoea and after six weeks the patient was discharged. As an outpatient further improvement was observed and finally her weight increased by 20 kg. As early as six weeks after starting HAART, HIV-RNA was below the detection limit (<500 eq/ml in that period). Fifteen months later the HAART regimen was successfully changed to the combination of zidovudine, lamivudine and abacavir. The number of CD4+ T lymphocytes had risen to 0.08 x 10^6 /ml.

DISCUSSION

This elderly patient was shown to have cryptosporidiosis, surprisingly as an opportunistic infection secondary to AIDS. The patient probably contracted the HIV infection through the sexual route from her late husband, who had received blood transfusions between 1980 and 1985.
Two years before AIDS was detected the patient had granulomatous colitis, suggestive of Crohn's disease. Cytomegalovirus, histoplasma and atypical mycobacterial infections can mimic Crohn's disease in patients with AIDS.[1-4] Yoshida *et al.* suggest that a Crohn's-like disease secondary to an occult underlying infection can occur.[5] Hing *et al.* reported on six HIV-positive patients with colitis, which was neither typical for ulcerative colitis nor for Crohn's disease but appeared to be a new entity.[6] So, on one hand it is possible that this patient never had Crohn's disease, but had HIV-related colitis two years before. On the other hand, it is also possible that she did have Crohn's disease at the time of the first coloscopy in 1998.
De novo inflammatory bowel diseases after HIV infection have been reported,[7-9] usually diagnosed when patients had near-normal CD4 counts.[7,8,10,11] Our patient had no major opportunistic infections in 1998 and therefore she probably had a (near) normal CD4 count at that time. Many patients have been described in which inflammatory bowel disease went into remission when significant CD4 depression occurred.[8,12,13] Because our patient responded well to therapy specific for Crohn's disease and because granulomas, but no *Cryptosporidia,* were found at the review of the colon biopsies of 1998, the diagnosis Crohn's disease was probably correct.
Cryptosporidium is a small parasite, easily overlooked in bowel biopsies. Because this patient was at low risk for AIDS and had a *Candida* oesophagitis, the particles in the crypts of the jejunum could easily have been misdiagnosed as the yeast form of *Candida*. However, electron microscopic examination clearly proved the micro-organisms to be *Cryptosporidia*, and this finally led to the diagnosis of AIDS.

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CASE REPORT

Duodenal metastasis: an uncommon cause of occult small intestinal bleeding

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ABSTRACT

Duodenal metastases are a very uncommon and peculiar cause of upper gastrointestinal bleeding. However, they should be considered in a patient presenting with upper gastrointestinal bleeding and a previous history of malignancy. The importance of recognising the unusual presentation of duodenal metastasis has to be emphasised. We describe two patients with upper gastrointestinal bleeding due to duodenal metastases. In the first patient a periampullary bleeding due to a metastasis of a renal cell carcinoma was detected five years after nephrectomy of the right kidney. In the second patient an occult bleeding caused by a duodenal metastasis of a melanoma was diagnosed. The first manifestation of this melanoma was eight years earlier.

INTRODUCTION

Upper gastrointestinal bleeding is a case of emergency and is generally diagnosed and treated by upper endoscopy or by surgical intervention. We describe a rare cause of upper gastrointestinal bleeding in two patients who presented with occult upper gastrointestinal bleeding from duodenal metastases. Duodenal metastases are a very uncommon and peculiar cause of upper gastrointestinal bleeding. The purpose of this paper is to present the clinical entity of metastatic malignancy of the duodenum and to discuss the pathogenesis, clinical presentation, diagnosis, management, and prognosis of duodenal metastasis.

CASE REPORT 1

A 76-year-old man was admitted in November 2001 with symptoms of weakness, dizziness and exertional dyspnoea. He had no epigastric discomfort. His medical history revealed removal of the right kidney because of renal cell carcinoma five years previously. The resected lymph nodes were all free of tumour localisation and there was no evidence of renal vein or perirenal capsular invasion. No adjuvant radiation or immunotherapy had been administered. In August 1999 he developed a right radicular syndrome with a peroneus paresis possibly due to an epidural metastasis at the 5th lumbar vertebra seen on magnetic resonance imaging. No biopsy was taken. He received radiotherapeutic treatment (22 cGy on L3 to S1 in five fractions) and recovered without sequelae. He was also known to have hypertension, dyslipidaemia and a mild aortic valve stenosis with insufficiency. He had no history of peptic ulcer disease. He was taking acetylsalicylic acid (80 mg/day), lercanidipine (20 mg/day), chlorthalidone (25 mg/day), metoprolol (50 mg/day) and lisinopril (10 mg/day). Because of anaemia the family doctor subscribed ferrofumarate (600 mg/day).

On physical examination he appeared to be in good general health and nutritional condition. He did not look anaemic. His blood pressure was 180/90 mmHg with a pulse of 72 beats/min, regular and aequal. There was no lymphadenopathy or organomegaly. Apart from a systolic heart murmur there were no other cardiopulmonary abnormalities. The abdomen was soft and not tender. There was no evidence of melaena.
The laboratory investigation showed a normocytic, hypo-

chromic anaemia with iron deficiency, which was diagnosed with a bone marrow examination (no iron pigment seen). The haemoglobin concentration was 4.7 mmol/l (8.7-10.9 10^9 mmol/l) with an MCV of 82 fl (80-100 fl), normal values of thrombocytes and leucocytes with normal differentiation. Urea was 8.6 mmol/l (2.5-7.0 mmol/l), and creatinine 119 µmol/l (65-110 µmol/l). Liver enzymes were normal. Further investigation into the cause of the anaemia with iron deficiency revealed no abnormalities in the colon. Subsequently an oesophagogastroduodenoscopy was performed which demonstrated a lobular mass involving the periampullary area in the pars descendens of the duodenum (3 x 5 cm) (*figure 1a*). Microscopic investigation of a biopsy showed consistency with the diagnosis of metastatic renal carcinoma of the clear cell type (*figure 1b*). Acetylsalicylic acid was discontinued and palliative radiotherapy was initiated. On follow-up the patient remained in a good clinical condition and had a stable haemoglobin concentration (6.3 mmol/l) with iron suppletion and a proton pomp inhibitor. He is still alive.

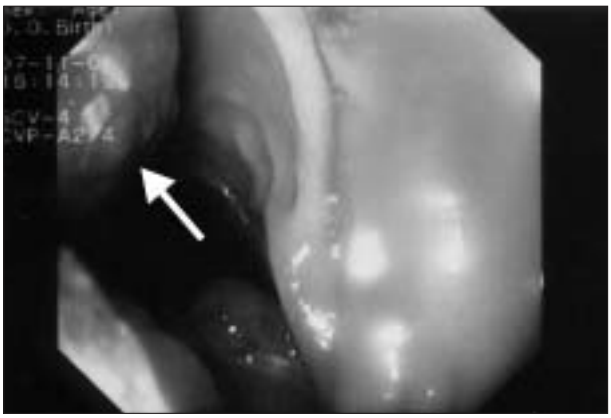

Figure 1a
Lobular mass (arrow) involving the periampullary area in the pars descendens of the duodenum

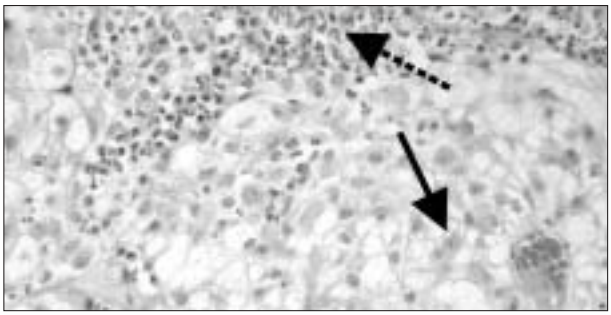

Figure 1b
Renal cell carcinoma in duodenal biopsy. Large tumour (arrow) cells with conspicuous pale and vacuolated cytoplasm and large nuclei with prominent nucleolus. The ulcerated surface is covered with leucocytic debris (dotted arrow) (haematoxylin and eosin, 250x)

CASE REPORT 2

In February 2001, a 65-year-old man was admitted because of a collapse and anaemia. His medical history revealed a malignant melanoma Clark level II and Breslow thickness 0.6 mm (stage I melanoma) of the back in January 1993. Because of tumour localisation within the resection borders, a re-excision was successfully performed. In 1996 he developed lymph node metastases of the right axilla (stage II melanoma). He was not included in an Interferon study because of a possible cerebral metastasis. On follow-up the cerebral process was stable and was compatible with a benign tumour. In 1997, a second malignant melanoma of the back (Clark level III and Breslow thickness 2.63 mm) was diagnosed and radically resected. Endobronchial, intrapulmonary and intrahepatic metastases manifested eight years after the first manifestation of the melanoma (stage III melanoma). At the request of the patient no systemic therapy was given.

On admission his physical examination was unremarkable except for pallor. His blood pressure was 132/66 mmHg with a pulse of 70 beats/min, regular and aequal. There were no lymphadenopathy or cardiopulmonary abnormalities. He had no palpable intra-abdominal masses. The abdomen was soft and not tender. There was no evidence of melaena.

The laboratory investigation showed a microcytic hypochromic anaemia. The haemoglobin concentration was 4.6 mmol/l and the MCV was 79 fl. There were normal values of leucocytes and thrombocytes with normal differentiation, urea 9.5 mmol/l and creatinine 65 µmol/l.

An oesophagogastroduodenoscopy showed a mass involving the pars descendens duodeni (5 x 7 cm) (*figure 2a*). Histological examination of the biopsy confirmed the presence of a melanoma with a similar morphological appearance to the original specimen from 1993 (*figures 2b* and *2c*). This was consistent with the earlier diagnosed melanoma. He received blood transfusions and palliative care and was discharged. He died thirteen months later.

DISCUSSION

Chronic blood loss from the gastrointestinal tract can be a challenging problem for physicians. We describe a rare cause of occult upper gastrointestinal bleeding in two patients who presented with occult upper gastrointestinal haemorrhage from duodenal metastases of a renal cell carcinoma and a melanoma.
The most common causes of upper gastrointestinal bleeding are mentioned in *table 1*. Various other causes,

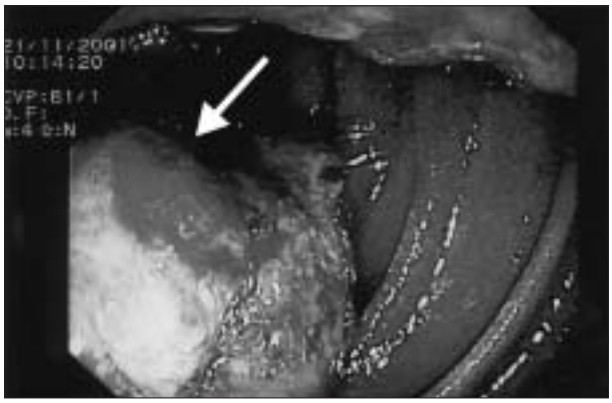

Figure 2a
Mass (arrow) involving the pars descendens duodeni

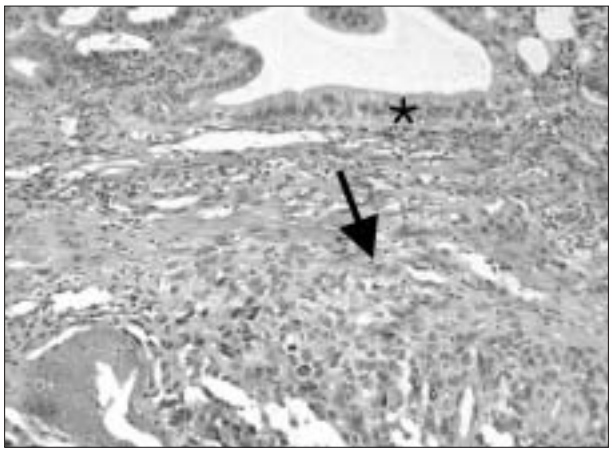

Figure 2b
Duodenal metastasis of melanoma. The cellular tumour (lower half of the picture, arrow) is covered with intact mucosa (asterix) with duodenal epithelium on top (haematoxylin and eosin, 100x)

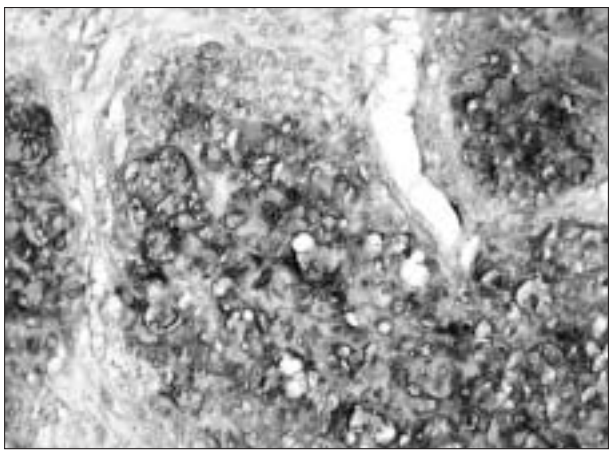

Figure 2c
Immunohistochemical staining with the melanoma specific marker S100-protein (immunoperoxidase, 250x)

Table 1
Causes of upper gastrointestinal bleeding

SOURCES OF BLEEDING	PROPORTION OF PATIENTS (%)
Ulcers	35-62
Varices	4-31
Mallory-Weiss lesions	4-13
Gastroduodenal erosions	3-11
Erosive oesophagitis	2-8
Malignancy	1-4
No source identified	7-25

including neoplasms, account for only 10% of all cases. Identifying an upper gastrointestinal haemorrhage from the small bowel can be difficult. The most common causes of gastrointestinal bleeding of small bowel origin are angiodysplasia and tumours.[1] They account for 5 to 10% of all cases of chronic blood loss of obscure origin.[2]

Neoplasms of the small bowel are uncommonly encountered clinical entities, comprising less than 5% of all gastrointestinal tumours and 0.35% of all malignancies.[3-5] Approximately two-thirds of small bowel tumours are malignant; more than 95% of these are adenocarcinomas, carcinoids, lymphomas or sarcomas (*table 2*). Adenocarcinomas are the most common histological types in Western populations. They are predominantly located in the duodenum. Carcinoids and lymphomas are predominantly located in the jejunum or ileum in contrast to sarcomas which are seen throughout the whole small intestine.

Table 2
Classification of small benign or malignant intestine tumours and their percentual prevalence (between brackets)

BENIGN	MALIGNANT
Adenomas (25)	**Primary malignant**
Leiomyoma (50)	Adenocarcinomas (30-50)
Lipoma (10-20)	Leiomyosarcoma
Hamartomas/ Peutz-Jeghers syndrome	Carcinoid tumors (5-24)
Neural tumours	Lymphoma (15-20)
Islet cell tumours	
Cavernous haemangiomas (<0.05)	
	Metastasis
	Malignant melanoma
	Carcinoma of the lung
	Genitourinary cancers
	Breast cancer
	Kaposi's sarcoma
	Colonic cancer
	Renal cell carcinoma

Metastatic lesions of the small intestine are more frequent than primary tumours. Duodenal metastases are most frequently located in the periampullary region, followed by the duodenal bulb. Common manifestations are gastrointestinal bleeding and anaemia.[6,7] Melanomas are the most common metastatic lesion of the intestine and have the greatest predilection for metastasis to the small bowel,[7,8] followed by lung cancer,[9] cervix carcinoma and hypernephroma, thyroid carcinoma, hepatoma and Merkel cell carcinoma.[10,11] Breast carcinomas metastasise predominantly to the stomach or oesophagus.[7] In immunocompromised patients Kaposi's sarcoma is the most common metastatic neoplasm to the small bowel.[12]

Males have higher incidence rates of small bowel cancer than females (1.5:1) and the incidence increases with age. There is a higher incidence of adenocarcinomas and malignant carcinoid tumours in blacks than in whites.[13] In recent years the overall incidence rates are rising. The main age at diagnosis is about 60 years.

Possible factors for the low incidence of neoplasms of the small bowel are:

- High turn-over of the intestinal mucosal cells which can prevent tumour growth:[14] it has been postulated that every 16 minutes 1 g of intestinal mucosa is replaced.
- Sparseness of bacterial flora in a normal small bowel:[15] the much lower bacterial load may result in minimising the exposure to potential carcinogenic bacterial breakdown products.
- Rapid transit of nutrients through the small bowel, which may also provide shorter exposure of its mucosa to carcinogens.[5]
- Liquefied chyme, which may reduce mechanical trauma and protect the small bowel from damaging effects of carcinogens and may cause less mucosal irritation than the more solid contents of the colon.[5,16]
- Intraluminal alkalinity of the small bowel:[16] this prevents formation of nitrosamines that may be carcinogenic in the acid environment of the stomach.
- Well-developed protective local secretory IgA expression, which may also be protective.[14]

Recent reports suggest that gastrointestinal metastases are more frequent than was previously thought.[17] They often present insidiously with nonspecific abdominal complaints. Signs and symptoms of appendicitis, malabsorption and protein-loosing enteropathy could be present.[18,19] They should also be considered in patients presenting with intermittent, vague abdominal pain of unclear cause, duodenal intussusception, unexplained weight loss and intermittent occult gastrointestinal haemorrhage. Intestinal obstruction and jaundice could be also presenting symptoms.

Secondary tumours involving the duodenum can arise by: (i) peritoneal dissemination, (ii) direct spread from an intra-abdominal malignancy, (iii) haematogenous and (iv) lymphatic spread.[20] Any of these mechanisms could be responsible for the metastases in the cases we reported.

The diagnosis of metastatic lesions of the duodenum may be a vexing experience. Duodenal lesions may be apparent on barium studies. Abdominal computer tomography may demonstrate thickening of the wall and folds in the involved segment of the bowel.[18] Identification of a bleeding metastasis between multiple small bowel lesions can be difficult. Lesions in the duodenum may be diagnosed by using a standard upper endoscopy with tissue sampling. A push upper endoscopy can also diagnose proximal jejunal abnormalities. Sonde enteroscopy and intraoperative or laparoscopically assisted enteroscopy are also good diagnostics.[21-23] Recently, video capsule endoscopy, which allows direct visual access of the entire bowel, has expanded the diagnostic yield.[24] In case of massive gastrointestinal bleeding or ileus, diagnosis is usually made by angiography or at surgery. There is no distinguishing endoscopic feature characteristic of a specific metastasis. The frequency of endoscopic diagnosis of small bowel metastasis is extremely low, approximately 25 per 100,000 upper endoscopies.[7]

Treatment is mainly supportive and palliative. Endoscopic sclerotherapy and radiotherapy of the metastatic lesions could be successful and may improve quality of life. Data on local endoscopic therapy of bleeding from small bowel lesions are limited. Endoscopic haemostasis can be reached by using injection, bipolar or heater probe coagulation. If the patient is still in a good general condition and the primary tumour is known to be chemoresistant, a surgical approach should be attempted. Intractable haemorrhage can also be treated with arterial embolisation of tumour-supplying arteries.[25] The overall long-term prognosis remains extremely poor.

CONCLUSION

The cases presented in this report represent clearly the peculiarity of duodenal metastasis as a cause of occult upper gastrointestinal bleeding. It could be one of the most vexing problems confronting physicians. With the advent of improved diagnostic tests, timely endoscopic diagnosis of this rare entity has become possible, enabling the clinician to make better therapeutic decisions. Physicians should be aware of this clinical entity, especially in patients with a previous history of malignancy. Treatment is mainly supportive and palliative in case of chemoresistant tumours.

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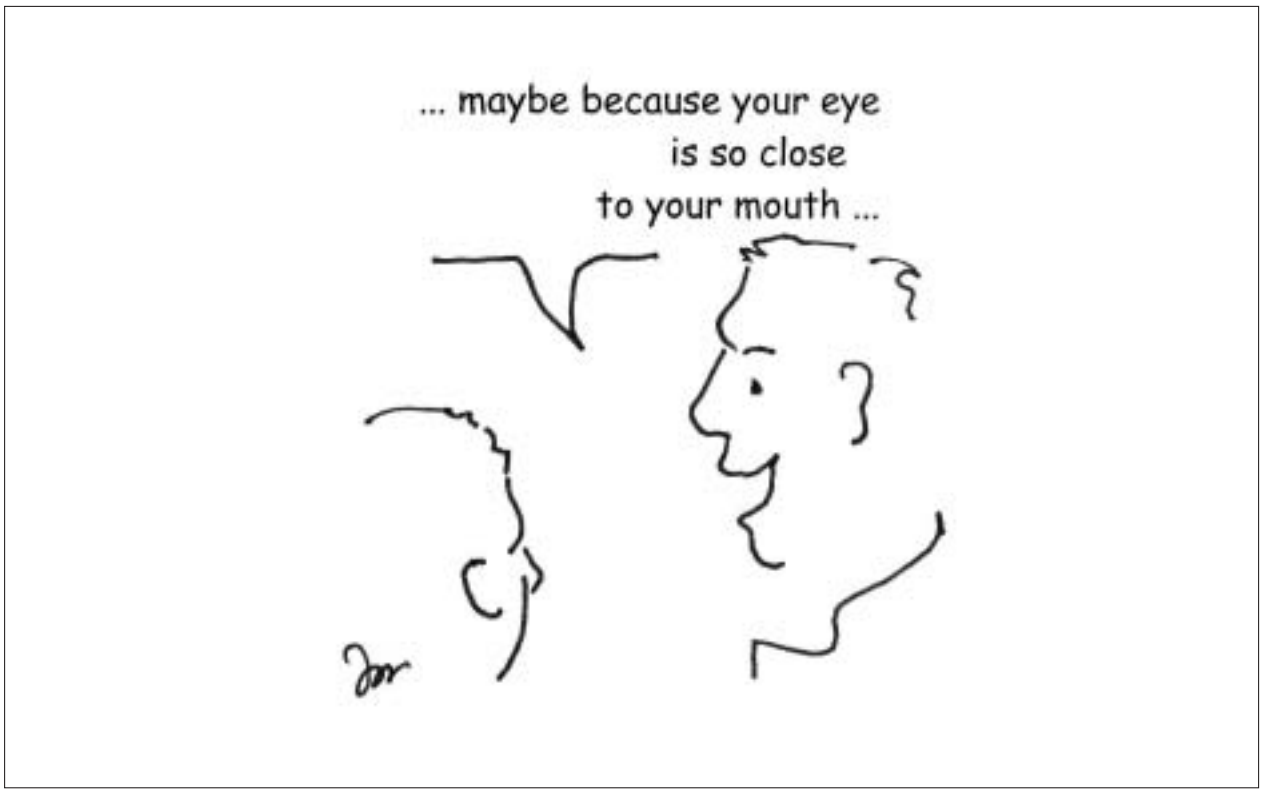

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CASE REPORT

Ocular syphilis acquired through oral sex in two HIV-infected patients

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ABSTRACT

Two cases of ocular syphilis are described in HIV-infected individuals after unprotected oral sex. The primary syphilitic lesion remained unnoticed and lues was therefore only diagnosed after visual symptoms developed.

INTRODUCTION

The risk of acquiring human immunodeficiency virus (HIV) infection through unprotected genito-oral sex is considered low but this may not be the case for other sexually transmitted diseases (STDs), such as syphilis. Furthermore, a primary syphilitic lesion in the oral cavity may be missed and as a consequence, the patient may present at a later stage of the disease with organ manifestations. We describe two cases in which syphilis infection was acquired by HIV-infected individuals after unprotected oral sex and only noticed after visual symptoms developed.

CASE REPORT 1

A 43-year-old man of Indonesian descent presented to us with visual loss in his left eye. He was a homosexual who regularly visited our infectious diseases outpatients' clinic since he had been diagnosed with HIV three years previously, following an oral gonococcal infection. Highly active antiretroviral therapy (HAART) had been started soon after diagnosis at a CD4 count of 230 x 10^6/ml and a viral load of 100,000 copies/ml. At that time, serological testing for hepatitis B and syphilis were negative. After initiation of HAART, the viral load became undetectable and CD4+ T cells rose to 390 x 10^6/ml. No opportunistic infections had been noticed in the follow-up.

The patient now presented with hazy vision and flashes in his left eye for several days. Ophthalmological examination revealed visual acuity of 4/5 in the right eye and 1/60 in the left. Vasculitis was found in the left eye for which the ophthalmologist initially prescribed oral prednisone 40 mg daily. Opportunistic cytomegalovirus (CMV), herpes simplex virus (HSV), herpes zoster virus (HZV) and Epstein-Barr virus (EBV) infections could not be established but tests for syphilis were strongly reactive: venereal disease research laboratory (VDRL) 1/128, *Treponema pallidum* haemaglutination assay (TPHA) 1/20480, and TPA-Abs positive.

A lumber puncture revealed a leucocyte count of 190 cells/ml, a VDRL titre of 1/4 and a TPHA of 1/2048, confirming the diagnosis of neurosyphilis. The patient was admitted to our infectious diseases ward and treated with intravenous penicillin 18 x 10^6 units/day for two weeks. Following treatment, the ocular manifestations disappeared and the vision in his left eye improved to 2/5.

The patient's sexual history revealed multiple male partners. He claimed always to have used condoms when performing anal sex since being diagnosed with HIV. He had, however, performed oral sex on others without the use of a condom. He had not noticed any oral, genital or anal ulcers.

CASE REPORT 2

A 37-year-old man was admitted to the infectious diseases ward with inflamed eyes. He was a homosexual and had been diagnosed with HIV a year previously after a routine

check. He had also been suffering from idiopathic epilepsy for more than ten years, for which he took valproic acid. HAART had not yet been started, since CD4 counts were still 570 x 10^6/l and viral load stable between 10^4-10^5 copies/ml. The patient had had no major complaints or opportunistic infections since the diagnosis.
He now presented with inflammation, pain and visual loss in both eyes, starting a week before admission; he volunteered no other symptoms. On admission he had vision of 0.4 in the left and 0.05 in the right eye. Ophthalmological examination revealed panuveitis in both eyes and the peripheral retina of the left eye had a necrotic aspect. The central retina of the left eye and the entire retina of the right eye were no longer visible due to vitreal opacities. His visual acuity further decreased to 1/300 in the right eye and 2/60 in the left eye. Further ocular examination revealed an optic neuritis, with the right eye affected more than the left. Further physical examination revealed only a small, eroded, nontender ulcer on the palate and cervical lymphadenopathy.
Initially, an opportunistic herpes virus or toxoplasma infection was suspected, until a PCR on ocular aspirate proved negative for CMV, HSV, EBV, VZV and toxoplasmosis. However, serological examination revealed a VDRL of >1/250, a TPHA of >1/20,000 and a positive fluorescent *Treponema* antibody absorption (FTA-Abs). CSF analysis showed 53 leucocytes/µl and 747 mg/ml protein; liquor VDRL was negative, but the TPHA titre was 1/128.
He was treated with intravenous penicillin 18 x 106 units/day for 14 days, followed by intramuscular benzathine penicillin G 2.4 x 10^6 units/week for a further three weeks. In addition, he was prescribed oral prednisone 60 mg/day for six weeks. His vision has improved considerably.
Our patient had recently started a monogamous relationship with a HIV-negative partner. He claimed to always use condoms for anal sex in order to protect his partner from HIV, but denied oral sex. He said that he had not noticed the oral ulcer.

DISCUSSION

It is a sad fact that in most of the world at the beginning of the 21st century, the incidence of both HIV and STDs is once again on the rise. In the Netherlands, for example, the infection rate for syphilis in men attending STD clinics in Amsterdam rose by 60% in the period 1994 to 1999. Much of this trend is probably due to the increase in risky sexual behaviour since the arrival of HAART.[1]
When safe-sex practices are used, this is often confined to genital and anal contact; it is widely believed that oral sex is 'safe'. For example, current public health advice in the Netherlands regarding oral sex states that the risk of genito-oral transmission of HIV is limited as long as there is no intra-oral ejaculation. The risk of oro-genital transmission is assumed to be negligible.[2] It is often forgotten, however, that genito-oral transmission of other STDs through unprotected oral sex occurs much more easily.[3] It is likely that both our patients acquired their syphilis through genito-oral transmission.

Much has been written on the interaction between HIV and syphilis.[4] The two diseases share a common mode of infection and STDs are known to increase the risk of HIV transmission. Furthermore, syphilis infection appears to follow a more fulminant course in HIV patients, with sometimes rapid progression to second and third stage disease, in particular neurosyphilis.[5] Presentation as secondary disease can occur and signs or history of a chancre may be absent.[6]
Ocular manifestations of syphilis are more common than sometimes assumed and may be the first presenting symptom of the disease[7] or even of underlying HIV.[8] (Pan)uveitis is the most common presentation, although statistics differ on the relative incidence of anterior and posterior uveitic involvement.[9] Syphilis, 'the great imitator', can mimic almost any form of ophthalmological pathology, however, including retinitis, vitreitis, optic neuritis and scleroconjunctivitis,[10] and ocular involvement has been described in all stages of syphilis.[11] In HIV patients, ocular involvement should always be considered as a manifestation of neurosyphilis (see below).
Syphilis serology can be divided into nontreponemal tests (VDRL and rapid plasma reagin (RPR)), which actually measure anticardiolipin antibodies, and treponemal-specific tests (microhaemagglutination assay-*Treponema pallidum* (MHA-Tp), TPHA and FTA-Abs). The reliability of these tests in HIV-infected subjects may be compromised: false-positive results may occur in nontreponemal tests, which are known to be less specific,[12] and false-negative results have been described for both nontreponemal[13] and the FTA-Abs tests.[14] One explanation for this is that the 'prozone' phenomenon,[15] whereby high antibody titres lead to false-negative tests in undiluted specimens, is more common in HIV infection, possibly because of B-cell dysregulation. Confirmation of neurosyphilis can be particularly difficult, with the sensitivity of nontreponemal serology in liquor as low as 20 to 50%.[16,17] In HIV-positive individuals, syphilis serology should therefore be repeated at regular intervals, in order not to miss initially false-negative infections as well as to screen for *de novo* acquisition.
Consensus has existed for several years to treat all ocular manifestations according to neurosyphilis regimens of intravenous penicillin 12-24 x 10^6 units/day for 10 to 14 days, even when overt neurosyphilis cannot be demonstrated.[11] Simple primary or secondary syphilis regimens have been shown insufficient to prevent relapses, particularly in HIV patients.[18] There have been sporadic reports of failure

of neurosyphilis treatment in HIV patients.[19,20] although it is unclear whether such cases represent true recrudescence or simply re-infection. This has led some authors to recommend ocular syphilis in HIV patients be treated with benzathine penicillin G intramuscularly 2.4 x 10^6 units/ week for a further three weeks following the intravenous course.[20-22] Follow-up with quantitative serological tests should be carried out to confirm successful treatment.

CONCLUSION

Syphilis may follow a more fulminant course in HIV-positive individuals, with in particular a more rapid progression to neurosyphilis. We therefore wish to reiterate that unexplained ocular symptoms such as uveitis in HIV-positive patients should always raise the suspicion of syphilis, especially now the incidence of this STD is once again on the rise.
Diagnosis may be delayed if the patient has not previously noticed a primary chancre; this can be the case if this STD is acquired through unprotected oral sex.

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Postpartum amenorrhoea-galactorrhoea

To the editor,

With interest I read the article from my former colleagues Kroese, Grootendorst and Schelfhout.[1] The conclusion that amenorrhoea, galactorrhoea and hyperprolactinaemia associated with enlargement of the pituitary gland are caused by primary hypothyroidism may be correct and is very well supported by the literature. At first glance, the case is clear-cut and other possibilities seem to have been ruled out sufficiently.

In the last paragraph of the discussion the authors state that 'hypothyroidism and hyperprolactinaemia with pituitary enlargement can cause diagnostic difficulties'. Their differential diagnosis consists of 'coexistence of primary hypothyroidism and a pituitary macroadenoma' and 'primary hypothyroidism associated with hyperprolactinaemia and pituitary enlargement'. The former diagnosis was ruled out by the fact that 'replacement therapy with L-thyroxin was associated with the resolution of pituitary enlargement and resumption of the menstrual cycle'. Another possible diagnosis that has not been ruled out in my opinion is the coexistence of primary hypothyroidism and lymphocytic hypophysitis.

Lymphocyte hypophysitis is a rare condition that occurs almost exclusively in young women and has a temporal relationship to pregnancy or (early) postpartum period.[2] It mimics a pituitary tumour and may lead to visual disturbances and to a varying degree of pituitary hormone deficiencies.[3,4] In many patients, transsphenoidal surgical exploration is performed because of a presumed non-secreting pituitary macro-adenoma. Histopathological examination shows extensive mononuclear infiltration of the anterior pituitary gland.[3]

The pathogenesis of lymphocytic hypophysitis is uncertain but autoimmune mechanisms are probably involved. In this context, primary hypothyroidism and lymphocytic hypophysitis may be connected. Of the five patients with lymphocytic hypophysitis reported by Patel et al., one developed thyroiditis.[4] Other authors have also reported lymphocytic hypophysitis, in most cases biopsy proven, in association with autoimmune primary hypothyroidism.[5-8]

On the basis of these reports, coexistence of primary hypothyroidism and lymphocytic hypophysitis may be a plausible explanation. On the basis of 'the resolution of the pituitary enlargement and the resumption of the menstrual cycle after replacement therapy with L-thyroxin' it was concluded that 'primary hypothyroidism was the factor causing the pituitary dysfunction'. However, it should be noted that lymphocytic hypophysitis, when not operated, often runs a benign course with pituitary function spontaneously returning to normal.

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ANSWER TO PHOTO QUIZ (ON PAGE 197)

AN IMMUNOCOMPROMISED HOST WITH BILATERAL PULMONARY INFILTRATES

Because of clinical deterioration the patient was also treated with erythromycin intravenously to cover atypical pulmonary pathogens. In the following days the patient recovered and the fever subsided. Real-time polymerase chain reaction on the bronchial alveolar lavage fluid and of a pharyngeal swab was positive for human metapneumovirus. A sputum culture further revealed *Burkholderia cepacia* and therefore the patient was treated with a fluoroquinolone orally. After further clinical improvement he was discharged from hospital while continuing the fluoroquinolone.

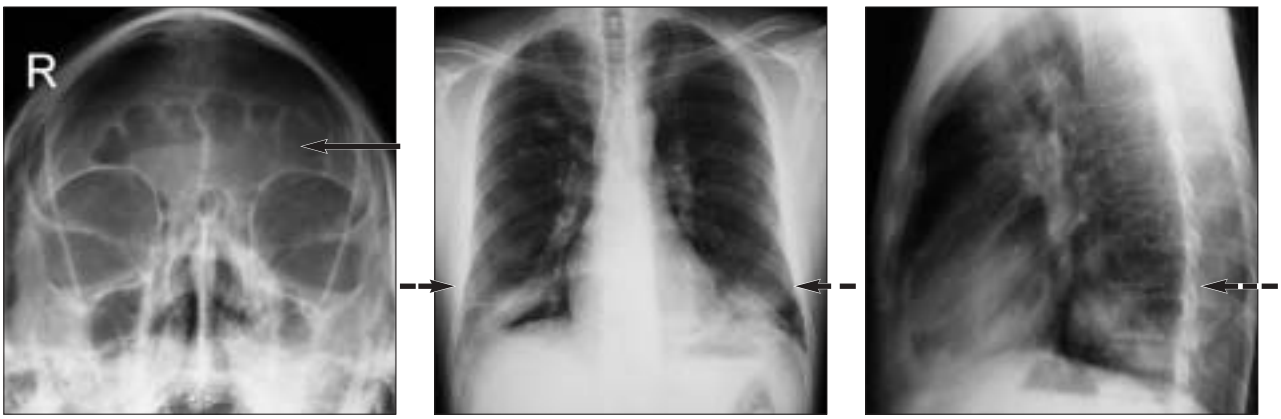

Figure 1
Bilateral shadowing of the frontal sinuses (◄—) and bilateral pulmonary infiltrates of the lower lobes (◄- -)

CONCLUSION

Bilateral pulmonary infiltrates and sinusitis frontalis caused by human metapneumovirus with possible secondary bacterial infection with *Burkholderia cepacia*.

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Shell from Kastrosikia

Caroline Koenders

The technique used for this month's cover is quite new. It involves a photo polymer print which Caroline Koenders learned to work with at a master class at the Amsterdam Graphic Studio two years ago. During her travels, she collects stones and shells and later takes photographs of them. These photos are carried over onto a metal plate and printed as an etching plate. It is possible to keep the collected objects in a way to justify their beauty.

A very limited edition (5) or the original print (size 50 x 65 cm) is available at the price of € 225 at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands, e-mail: galerie-unita@planet.nl or on our website: www.galerie-unita.com.

Bijsluiter

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

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[1.] Smilde TJ, Wissen S van, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.

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