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EDITORIAL

The rise and fall of postprandial lipids

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ABSTRACT

The remnants of absorbed fat particles which circulate after a meal might cause atherosclerosis, but such a causal role is still unproven. High levels of such lipoprotein remnants are often found in patients with the metabolic syndrome. Increased activity and weight loss will diminish the cardiovascular risk factors caused by this syndrome including elevations of postprandial remnants.

It is now 25 years since Donald Zilversmit published his hypothesis that atherogenesis is a postprandial phenomenon.[1] At that time the proposal met with a warm reception because it appeared to solve two dilemmas. The first dilemma was the vexing question why low-density lipoproteins (LDL) cause atherosclerosis. Although the evidence for a causal role of high LDL levels was less complete than it is now, it was already impressive: the epidemiology was highly consistent, mutations that caused high LDL levels caused premature coronary heart disease, and diets that lowered (LDL) cholesterol lowered the incidence of coronary heart disease in randomised clinical trials.[2] The one piece of the puzzle that refused to fit was the mechanism. Cholesterol was probably deposited in atherosclerotic plaques by macrophages that had accumulated cholesterol-rich lipoproteins, but LDL does not accumulate in macrophages:[3] when the concentration of LDL outside the cell becomes too high, macrophages simply shut down their LDL receptors and so avoid being overloaded with cholesterol. The lipoproteins that do accumulate in macrophages are remnants of triglyceride-rich lipoproteins.[3] These arise during digestion of such lipoproteins in the capillaries of fat tissue and of muscles that can use fatty acids as fuel. Digestion of the triglyceride core leaves remnants that are relatively rich in cholesterol, and such remnants are avidly taken up by macrophages. What Zilversmit now proposed was that people who eat a lot of triglycerides (i.e. fat) and who do not efficiently clear triglyceride-rich particles will have remnants in their circulation which cause atherosclerosis. Although this hypothesis did not explain how LDL causes atherosclerosis, it seemed a promising explanation for the occurrence of atherosclerosis in coronary patients with normal LDL values.

The Zilversmit hypothesis had a second attraction. At the time that it was proposed, the most effective way to lower cholesterol was through diets high in polyunsaturated fatty acids, i.e. high in vegetable oils such as soybean or sunflower oil which are rich in linoleic acid. However, there was increasing concern that such high-fat or high-oil diets caused obesity or cancer. The evidence supporting such adverse effects of high-oil diets was soft, and has subsequently eroded,[4,5] but at the time there was a groundswell in favour of diets low in fat and high in carbohydrates. The Zilversmit hypothesis nicely fitted with that mood, because low-fat high-carbohydrate diets were thought to produce fewer chylomicrons and therefore fewer remnants of triglyceride-rich lipoproteins circulating after a meal.

The paper by Van Oostrom and co-workers[6] in this issue illustrates that things have turned out to be less simple. Van Oostrom *et al.* tried to explain why at a given (LDL) cholesterol level, Northern Europeans have higher rates of coronary heart disease than Southern Europeans. The

authors hypothesised that the Mediterranean diet high in unsaturated fatty acids – mainly monounsaturates from olive oil – might produce lower levels of chylomicron remnants throughout the day than the Dutch diet, and that this might explain the lower rates of coronary heart disease seen in Mediterranean countries. The results of the study were negative: young men and women from Barcelona in Spain and from Utrecht in the Netherlands had similar levels of triglycerides in their blood throughout the day when eating their habitual diets. The only difference was that the Spanish had their highest blood triglyceride levels after lunch and the Dutch after dinner, which fits with the Spanish habit of eating the main meal of the day at noon.

The present study of postprandial lipoproteins was not the only one to yield a negative result in spite of much effort. Over the past 25 years a vast research effort has gone into studies of postprandial lipoproteins but many questions have remained open, including the seemingly simple question of which types of fat cause the greatest increase in postprandial remnant levels. Why has it been so hard to verify the hypothesis put up 25 years ago? One reason is technical. Measuring post-meal lipoprotein levels is cumbersome, and it is hard to tell which component of which particle should be quantified at which point in time. Van Oostrom *et al.*[6] took an innovative approach here in that they had subjects measure their own blood triglyceride levels six times throughout the day with a hand-held self-monitoring device. This allowed measurement over three days in each group, which reduced variability, and it obviated the need to admit subjects to the clinic for frequent blood letting. However, it is uncertain how well blood triglyceride levels after a meal reflect the presence of the hypothetical harmful particles that cause atherosclerosis. Another issue is how baseline triglyceride levels, i.e. the fasting or pre-meal levels, should be factored in. Low-fat high-carbohydrate diets cause less of a postprandial rise in triglycerides than high-fat diets, but high-carbohydrate diets cause higher baseline levels so that the absolute level reached after a meal may be the same. Which is worse?

A more fundamental question is that of causality. Postprandial lipaemia is associated with coronary heart disease,[7] but does the one cause the other? Poor lipid clearance after a fat meal is typically seen in patients with central obesity and low physical activity, and such patients usually have a host of abnormalities which are all associated with coronary heart disease, such as low HDL, high fasting triglycerides, insulin resistance, high blood pressure, high levels of C-reactive protein and the other paraphernalia of the metabolic syndrome. The only way to prove that post-meal remnants are causal would be to apply a treatment that changes only the level of remnants while leaving all other lipoproteins undisturbed. Such a treatment does not exist, for clearance of one type of lipoprotein affects that of other types.

Thus proof of a causal role of postprandial lipoproteins may be a long way off. Does that matter to the clinician? From a scientific point of view it would be highly satisfactory if the role of remnants in atherogenesis could be cleared up, but from a clinical point of view the advice to patients with postprandial hyperlipaemia would probably remain the same: eat less, exercise more, and lose weight.

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Pathophysiology of antiphospholipid antibodies

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ABSTRACT

The presence of antiphospholipid antibodies in plasma is a risk factor for thromboembolic complications. *In vitro*, however, the same antibodies can prolong clotting times in coagulation assays, a classic marker for a bleeding tendency. For years this contradiction has puzzled many scientists. Recently new insights into the interaction between antiphospholipid antibodies and their main target, the protein beta-2-glycoprotein I, have opened new avenues for the understanding of the pathology of this syndrome.

INTRODUCTION

The antiphospholipid syndrome (APS) is a noninflammatory autoimmune disease characterised by the presence of antiphospholipid antibodies (aPL) in the plasma of patients with venous and/or arterial thrombosis and/or recurrent complications of pregnancy.[1,2] The presence of aPL in plasma of patients can be detected by either a prolongation of the phospholipid dependent coagulation test (lupus anticoagulant, LAC), or by solid phase immune assays (anticardiolipin ELISA).[3] Antiphospholipid antibodies that cause LAC activity and anticardiolipin antibodies (aCL) are closely related but not identical autoantibodies. Originally, it was thought that aPL were directed against anionic phospholipids. We now know that the antibodies are directed against plasma proteins with affinity for anionic phospholipids. After the discovery of β_2-glycoprotein I (β_2-GPI) as an important antigen in the anticardiolipin ELISA,[4-6] over time a large number of possible other target proteins have been described (*table 1*).

The only two antibodies that are frequently present are anti-β_2-GPI antibodies and antiprothrombin antibodies; of these the most important and relevant protein involved in APS seems to be β_2-GPI. Beta-2-GPI is a plasma protein with no obvious function and persons or mice lacking this protein seem to be completely healthy.[7]

The antiphospholipid syndrome is a very unusual syndrome because the clinical symptoms such as thrombosis occur relatively often but in most cases are not due to the

Table 1

List of published targets for antiphospholipid antibodies. The assays used in diagnostic laboratories to detect the presence of these antibodies recognise β_2-glycoprotein I (LAC assay and anticardiolipin ELISA) or prothrombin (LAC assay). The presence of antibodies to the other targets is not detected in a normal diagnostic setting. The relevance of all other antibodies is questionable.

LIPID / POLYSACCHARIDE SUBSTRATES	PROTEIN SUBSTRATES
Cardiolipin	β_2-glycoprotein I
Phosphatidylserine	Prothrombiin
Phosphatidyl ethanolamine	Protein C
Phosphatidyl inositol	Protein S
Phosphatidic acid	High-molecular weight kininogen
Lysobisphosphatidic acid	Factor XII
Heparan sulphate	Annexin A5
Oxidised low-density lipoprotein	Tissue factor pathway inhibitor
7-ketocholestryl-9-carboxy-nonanoate	Complement factor H
	Phospholipases
	Plasminogen
	Tissue-type plasminogen activator

presence of antiphospholipid antibodies. The detection of the antibodies in the blood of a patient with thrombosis or complications of pregnancy is an essential step to define the syndrome. However, a major assay to detect the antibodies is a prolongation of a coagulation assay and normally prolongation of a clotting test is used to detect a bleeding disorder and not a risk for thrombosis. Given this contrast between the *in vivo* clinical manifestations and the laboratory observations, a large number of hypotheses to explain the pathology of the syndrome have been proposed.[8,9] The first suggested mechanisms were based on the interference of antiphospholipid antibodies with protein-protein or protein-phospholipid interactions that are essential for optimal haemostasis. However, none of the proposed mechanisms survived studies in which the hypothesis was tested with plasma samples of larger cohorts of patients. Nowadays, possible stimulation of blood and endothelial cells by antiphospholipid antibodies has been emphasised. Two important assumptions must be considered to understand a possible cellular action of antiphospholipid antibodies. Firstly, the pathological autoantibodies are not directed against phospholipids *per se*, but against a protein, β_2-GPI. Secondly, it is now generally accepted that aPL do not inhibit the functional activity of β_2-GPI but they do induce a new function for β_2-GPI, namely a significantly increased affinity for cellular surfaces containing anionic phospholipids. Thus, the affinity of β_2-GPI for negatively charged phospholipids only becomes high enough to interact with cells after interaction with the antibodies. In this overview, we will discuss current insights into the protein β_2-GPI, how it interacts with antibodies and (cellular) surfaces and the consequences of the binding of protein-antibody complexes to the cell on cellular functions.

BETA-2-GLYCOPROTEIN I

Beta-2-glycoprotein I is a glycoprotein present in plasma at concentrations ranging from 10 to 300 μg/ml (0.25-5.0 μM).[10-12] Messenger RNA is found in endothelial cells,[13] placenta,[14] central nervous system cells[15] and hepatocytes,[16] but its major source of synthesis is the liver.[17] Beta-2-GPI is synthesised as a 326 amino acid long single chain polypeptide with a calculated molecular mass of 36.3 kDa.[17] It contains four potential glycation sites and the glycans account for approximately 20% (w/w) of the total molecular mass of about 45kD as determined by SDS-PAGE gelelectrophotesis.[17] As early as in 1968, a deficiency of β_2-GPI was described without any clinical consequences,[18] an observation that has since been confirmed several times.[10,11,19]

The mature sequence of β_2-GPI consists of five repeating units of the same type, termed short consensus repeat (SCR) domains.[17] SCR domains are present in many proteins functioning in the complement system.[20] They consist of about 60 residues and they have two fully conserved disulphide bonds. Sequence homology among SCR domains ranges between 20 and 40%. Beta-2-glycoprotein I is built up out of four regular SCR domains and one aberrant domain. This fifth domain contains a six-residue insertion and a 19-residue C-terminal extension, which is C-terminally cross-linked by an additional C-terminal disulphide bond.

The crystal structure of β_2-GPI reveals an extended chain of SCR domains in a fishhook-like form (*figure 1*).[21,22] The first four domains have common SCR forms. The fifth domain deviates strongly from the common SCR folding. Similar to the other domains it has the central antiparallel β-sheets and the common two disulphide bonds. However, half of the domain, in particular the parts that contain the 6 amino acid insertion and the 19 amino acid

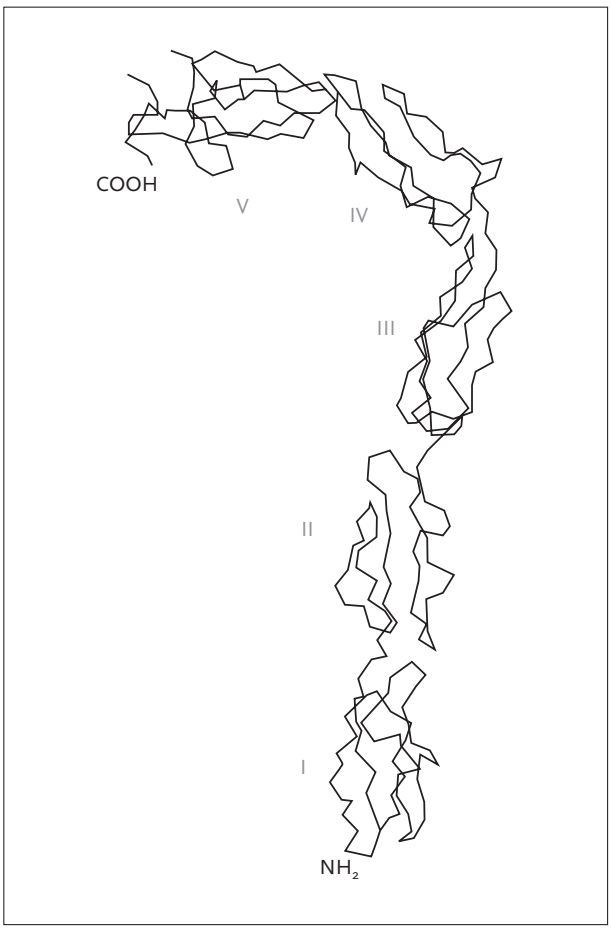

Figure 1

The three-dimensional structure of β_2-glycoprotein I. β_2-glycoprotein I consists of five comparable SRC repeats. The phospholipid binding site is located in domain V. This domain contains a flexible loop that is not visible in the crystal structure. This loop penetrates into the membrane when β_2-glycoprotein I binds to cellular membranes.

extension, form a unique structural element which is the phospholipid binding site. A 2000Å large patch of 14 positively charged amino acids provides the electrostatic interactions with the anionic head groups of the phospholipids. In the middle of this patch a hydrophobic group is present that can insert into the membrane, thereby anchoring the protein to the membrane. Amino acid replacement studies have shown that the presence of hydrophobic amino acids in this loop is crucial for phospholipid binding.[23]
The binding site for phospholipids is located in the fifth domain at the outer curve of the fishhook, the top of the molecule. When β_2-GPI binds to a membrane surface, the domains I and II are exposed far away from the cellular surface. As domains III and IV are heavily glycosylated and therefore shielded from protein-protein interactions, domains I and II are ideally exposed for interactions with other proteins and can provide binding sites for anti-β_2-GPI antibodies, for example.

SPECIFICITY OF ANTIBODIES

Antiphospholipid antibodies is a generic term that describes a collection of closely related but not identical antibodies: LAC activity, anticardiolipin antibodies and anti-β_2-GPI antibodies. This immediately raises two fundamental questions: what are the differences between the different types of antibodies and which one is the most relevant? A meta-analysis on the predictive value of the different types of aPL antibodies showed that the antibodies that induce LAC activity correlate best with a history of thromboembolic complications.[24,25] Apparently, an assay that measures a functional activity, inhibition of a clotting reaction, better predicts a thrombotic risk than assays that measure the presence of autoantibodies that comprise both those that influence a functional activity and those that do not. But besides the fact that one assay is based on functional activity and the other not, there are more reasons why the assays do not measure an identical population of antibodies. In the first place LAC can also be caused by antiprothrombin antibodies.
Antiprothrombin antibodies are not detected in an anticardiolipin ELISA.[26] Antiprothrombin antibodies are probably of little clinical significance.[27] Secondly, the ELISAs developed to detect the presence of anticardiolipin or anti-β_2-GPI antibodies are poorly standardised. A major reason for the poor comparison between the different types of antibodies might be that a plasma sample that is positive in one laboratory can be negative in another.[28,29] Even between laboratories with extensive experience in the detection of aPL antibodies, discordant findings with low titre antibodies samples are more the rule than an exception.

Since 1990, it is known that the pathological anticardiolipin antibodies are in fact anti-β_2-GPI antibodies. To better understand the pathophysiology of anti-β_2-GPI antibodies it is important to characterise the epitopes on β_2-GPI involved in the recognition by the autoantibodies. The first published experiments suggested that anti-β_2-GPI antibodies were a heterogeneous group of antibodies because antibodies were found directed against every possible epitope on the protein.[30-34] However, the assays to detect the presence of autoantibodies are rather aspecific and proper standardisation of the anti-β_2-GPI-antibody ELISA is lacking.[28,29] Improvements in the detection of the antibodies by preventing the binding of low affinity, aspecific antibodies and the use of deletion mutants of β_2-GPI have resulted in strong evidence that the major, if not the only, epitope on β_2-GPI responsible for the binding of pathological autoantibodies is situated in domain I, probably near Lysine 43.[35,36]

In summary, there is a heterogeneous population of antiphospholipid antibodies but only a subpopulation of these antibodies is pathological. One of the major challenges is to improve our serology in such a way that specifically the pathological antibodies are detected.

CONSEQUENCES OF ANTIBODY BINDING

Originally, it was thought that aPL antibodies prolonged clotting times by means of competition with clotting factors for binding to negatively charged phospholipids that are essential for optimal coagulation. The discovery that not negatively charged phospholipids but β_2-GPI was the antigen pointed to another explanation. Beta-2-GPI on its own has a relatively low affinity for negatively charged phospholipids. However, the presence of anti-β_2-GPI antibodies causes two phospholipid-bound β_2-GPI molecules to cross-link, thereby increasing its affinity a hundredfold.[37-39] Only the antibody-β_2-GPI complexes are able to interfere with the binding of clotting factors with their catalytic phospholipid surface, not β_2-GPI alone (*figure 1*). Studies in a hamster model with monoclonal anti-β_2-GPI antibodies showed that the antibodies and not their Fab fragments induce increased thrombus formation, indicating that the dimerisation of β_2-GPI by antibodies is essential not only for the induction of LAC but also for the induction of thrombotic complications.[40] Recently, we showed that dimerisation is also essential for the activation of platelets by β_2-GPI.[41] All these studies indicate that the anti-β_2-GPI antibodies are gain-of-function antibodies. They induce a new function in β_2-GPI, namely an increased affinity for negatively charged phospholipids. Beta-2-GPI on its own is unable to interfere with membrane-bound reactions, it

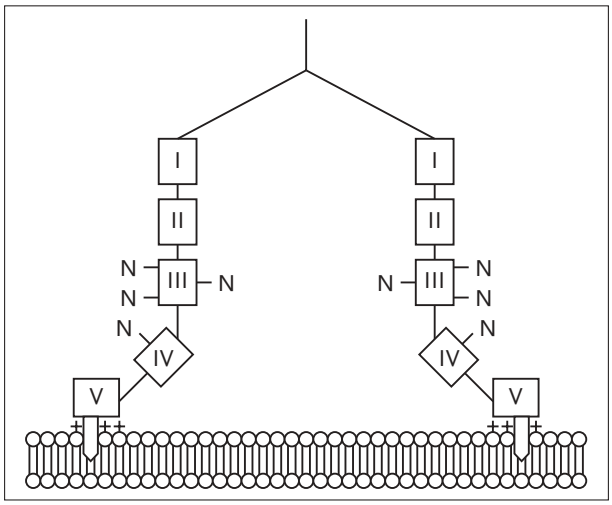

Figure 2
Mechanism by which anti-β_2-glycoprotein I antibodies increase the affinity of β_2-glycoprotein I for phospholipids. The antibodies dimerise β_2-glycoprotein I thereby doubling the number of binding sites. Without antibody, the affinity for a PS/PC surface is 170 nM, with antibody the affinity increases to 5nM. The affinity of the complex is now high enough to compete with clotting factors for the available catalytic phospholipids in clotting assays. (N = carbohydrate side chain).

can only interfere with physiological functions after an interaction with antibodies. These observations are now the lead in the hypotheses that explain the pathophysiology of the antibodies.

PATHOPHYSIOLOGY

Antiphospholipid antibodies are notable because they increase the risk for both venous and arterial thrombosis. Almost all other known risk factors increase only venous or only arterial thrombosis.[42] In general, markers related to humoral coagulation factors result in venous thrombosis while markers related to platelets correlate with arterial thrombosis. We cannot exclude that the risk for arterial thrombosis and the risk of venous thrombosis are the result of two separate actions of β_2-GPI -antibody complexes. The most important mechanisms that have been put forward by which anti-β_2-GPI could increase a thrombotic risk is that they interfere with phospholipid dependent antithrombotic pathways or that they bind to blood and/or endothelial cells, thereby activating these cells. The major antithrombotic pathway is the protein C pathway. Protein C, activated by thrombin bound to thrombomodulin, cleaves factors Va and VIIIa thereby preventing further thrombin formation.[43] The whole protein C reaction cascade takes place on a phospholipid surface. Indeed, 'protein C resistance' has been found in patients with antiphospholipid antibodies *in vitro*.[44] Whether this is also very relevant *in vivo* is unknown. Apparently the antibodies inhibit both the prothrombotic pathway (coagulation) and the anticoagulant pathway (protein C axis) and the overall result may be neutral. Some authors have reported that the presence of phosphatidyl ethanolamine in lipid vesicles might shift the balance towards a more pronounced inhibition of the antithrombotic pathway.[45] More information (animal experiments) is necessary to judge a possible role of acquired protein C resistance as an important pathological mechanism in the antiphospholipid syndrome. Recently, evidence has revealed that β_2-GPI-antibody complexes are able to activate platelets, monocytes and endothelial cells.[46-48] Activation of these cells results in increased platelet activation and induction of tissue factor activity, the major inducer of the coagulation cascade. The activation of the cells is not due to binding to phospholipids on the surface of the cells but to binding to specific receptors on the cells. A number of receptors have been suggested, apoER2' on platelets, annexin A2 on monocytes and a Toll-like receptor on endothelial cells. It is remarkable that β_2-GPI only has affinity for these receptors when bound to an antibody. This can be explained in two ways. The binding of β_2-GPI-antibody to the phospholipids of the membrane is an essential condition before β_2-GPI can bind to a receptor. Thermodynamically, this can be understood because binding to a membrane reduces the enthropy of the reaction, also allowing low-affinity interactions. A second explanation is that binding of the antibodies to β_2-GPI induces a conformational change into β_2-GPI, exposing a neo-epitope that is involved in the interaction with the receptor.[49]
The activation of cells by antiphospholipid antibodies is normally weak and not enough to fully activate the cell. It is now generally believed that antiphospholipid antibodies make the cells more sensitive for other activators or that other activators, in the presence of the antibodies, can activate cells at a lower concentration. A second hit is necessary. This explains why although the antibodies are permanently present in the plasma of patients, the patients do not suffer continuously from thrombotic complications. Only the risk to develop thrombosis or pregnancy complications is increased.

CONCLUSIONS

We have now reached a fascinating area in the research into the pathology of the antiphospholipid syndrome. New findings on the specificity of the antibodies open the possibility to develop better and more specific assays for the detection of patients at risk for thromboembolic

complications. With a better definition of the patients that really suffer from the syndrome, future patient studies will not be disturbed by the inclusion of incorrectly classified patients within the patient cohorts. The notion that antiphospholipid antibodies do not inhibit a certain metabolic function but that due to binding of the antibodies to β_2-GPI a new function for β_2-GPI is induced was a major step forward. The research into the pathology of the syndrome was also blinded too much by the idea that negatively charged phospholipids were the central theme in the explanation of the syndrome. Nowadays we envision the concept of cells and cell activation as the consequence of 'classic' receptor-substrate interactions as the major cause of the pathophysiology. With the new tools and ideas that have been developed in the last few years we are now able to test the current hypotheses in animal models and possibly large patient cohort studies. It is, of course, still unknown whether the pathology of venous thrombosis is the same as the pathology of arterial thrombosis.

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Clinical consequences of antiphospholipid antibodies

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ABSTRACT

Antiphospholipid antibodies (aPL), notably the lupus anticoagulant and anticardiolipin antibodies, are the serological hallmarks of the antiphospholipid syndrome. Thrombosis and pregnancy complications are the most prominent clinical manifestations of this syndrome. This paper provides the clinician with guidelines for ordering and interpreting tests for aPL and discusses consequences for treatment if persistently positive tests are found.

INTRODUCTION

Twenty years ago it was recognised that in patients with systemic lupus erythematosus (SLE) the presence of circulating antiphospholipid antibodies (aPL), notably lupus anticoagulant (LAC) and anticardiolipin antibodies (aCL), was associated with thrombosis, pregnancy complications and thrombocytopenia. This association was termed antiphospholipid syndrome (APS).[1] It was soon recognised that APS can also occur in patients without an underlying systemic autoimmune disease (primary APS, PAPS). With the current wide availability of aPL tests, clinicians need to know when such tests should be ordered, how results should be interpreted and what the consequences are of a positive test.

HISTORY OF APL

It was in 1906 that aPL were described for the first time as complement-fixing antibodies that react with alcoholic extracts of beef heart in patients with syphilis.[2] Later on, the essential component within the complex antigen was identified as cardiolipin, a negatively charged mitochondrial phospholipid.[3] This observation led to the development of an agglutination test known as the Venereal Disease Research Laboratory (VDRL) test, which is currently still used as a screening test for syphilis. Mass screening of blood during and after the second world war led to the recognition that the VDRL test can be transient or persistently positive without clinical or serological evidence of syphilis. Transient biological false-positive reactions mainly occurred with (nonsyphilitic) infections and persistent positive reactions were found in patients with systemic autoimmune disorders, mainly systemic lupus erythematosus (SLE).[4] Associations between a positive VDRL test and clinical manifestations in SLE patients were never reported. In 1952, Conley and Hartman described in patients with SLE a peculiar inhibitor of *in vitro* coagulation,[5] which has been known as lupus anticoagulant (LAC) since 1972.[6] The phenomenon refers to antibodies that interfere with the assemblage of proteins of the coagulation cascade on a phospholipid template. *In vitro* plasma clotting times normalise when extra phospholipids are added to the test system. For many years the only importance of identification of LAC was that, in contrast to most other inhibitors of coagulation, it was not associated with bleeding.
As many patients with LAC had a biologically false-positive VDRL test and coagulation tests are relatively complicated, requiring among other things adequately processed plasma samples and a relatively long hand-on time, sensitive solid phase immunoassays for the detection of antibodies to cardiolipin were developed in the 1980s.[7] In contrast to

what was originally presumed, tests for aCL and LAC detect overlapping but not identical antibodies.

In 1990, it was reported that autoimmune aCL as detected in an ELISA system are not directed to phospholipids *per se*, but to a phospholipid binding plasma protein termed β_2-glycoprotein 1.[8,9] It was soon recognised that LAC is more heterogeneous than aCL as antibodies causing LAC use β_2-glycoprotein 1, prothrombin or other plasma proteins as cofactors for phospholipid binding.[9-11] Strictly speaking, the widely used term aPL is incorrect as most APS-related aPL are directed against plasma proteins and not phospholipids *per se*.

THE ANTIPHOSPHOLIPID SYNDROME

Currently used criteria to classify a patient as having APS are given in *table 1*.[12]

By definition, a diagnosis of APS requires persistent presence of medium to high levels of aCL (IgG or IgM isotype), presence of LAC or both. In general, antibodies causing LAC are more specific for APS, whereas aCL are more sensitive. The specificity of aCL for APS increases with titre and is higher for the IgG than for the IgM isotype.[13] However, multiple tests for aPL should be applied since patients may be negative according to one aPL test and positive in another.

Table 1

Preliminary classification criteria for antiphospholipid syndrome[12]

Vascular thrombosis
a) One or more clinical episodes of arterial, venous or small-vessel thrombosis in any tissue or organ AND
b) Thrombosis confirmed by imaging or Doppler studies or histopathology, with the exception of superficial venous thrombosis AND
c) For histopathological confirmation, thrombosis present without significant evidence of inflammation in the vessel wall.
Pregnancy morbidity
a) One or more unexplained deaths of a morphologically normal foetus at or beyond the 10th week of gestation, with normal foetal morphology documented by ultrasound or by direct examination of the foetus OR
b) One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or severe placental insufficiency OR
c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal, anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded.
Laboratory criteria
a) Anticardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titre on at least two occasions at least six weeks apart, measured by standard ELISA for β_2-glycoprotein-1 dependent anticardiolipin antibodies OR
b) Lupus anticoagulant present in plasma on two or more occasions at least six weeks apart, detected according to the guidelines of the international Society on Thrombosis and Haemostasis.[23]

Definite APS is considered to be present if at least one of the clinical and one of the laboratory criteria are met.

Clinical criteria include objectively verified vascular thrombosis and well-described pregnancy complications. APS-related thrombotic events occur in both arterial and venous vessels and may comprise both large and small vessels. APS-related thrombosis has been described for almost any vascular bed of the human body and reported clinical manifestations are consequently very diverse. Deep vein thrombosis in the legs, pulmonary emboli and ischaemic stroke are the most frequent APS-related thrombotic manifestations.[14] APS-related thrombosis tends to recur. The vascular pattern of thrombotic recurrences seems fairly consistent in APS. Retrospective studies found that venous thrombosis is followed by another venous thrombosis in more than 70% of cases, and an arterial thrombosis by another arterial event in more than 90% of cases.[15,16] Additional risk factors are often present in patients with aPL-related thrombosis. This holds in particular for pregnancy, surgical procedures, hypertension and smoking.[17]

The term catastrophic APS refers to a life-threatening condition in which aPL-positive patients develop progressive thrombosis in at least three different organ systems in a period of days to weeks. In this accelerated form of APS, vascular occlusion afflicts predominantly small vessels, although in a minority of patients thrombosis also occurs in large vessels.[18] The condition resembles thrombotic thrombocytopenic purpura, haemolytic uraemic syndrome and diffuse intravascular coagulation.

The APS criteria differentiate between pregnancy complications that occur before and after ten weeks gestation (viz. 70 days from conception), which implies a segregation between the (pre-)embryonic and foetal periods of pregnancy. This is based on observations in the general population where (pre-)embryonic loss is frequent (occurring in 10 to 15% of recognised pregnancies) and foetal loss after 14 weeks gestation is rare (2%). More than half of sporadic (pre-)embryonic losses are related to chromosomal abnormalities of the conceptus and in many cases a visible embryo never forms. Therefore, epidemiological evidence dictates that the definition of recurrent miscarriage should include three or more consecutive (pre-)embryonic losses.[19] Furthermore, the APS criteria recognise that a preterm live birth accompanied by severe pre-eclampsia or severe placental insufficiency is comparable with a loss late in pregnancy.

Apart from thrombosis and pregnancy complications, the presence of aPL also relates to thrombocytopenia (often mild), livedo reticularis, heart valve abnormalities, movement disorders (chorea), myelitis transversa and

microangiopathic nephropathy.[14] With this last complication, histological examination of a kidney biopsy characteristically shows vascular occlusions, cellular intima fibrosis, fibroelastic intima hyperplasia, ischaemic glomeruli and signs of cortical ischaemic atrophy.[20] The prevalence of APS nephropathy in patients with primary APS is not exactly known. In the original paper by Nochy *et al.*[20] the 16 described patients came from a database from three university hospitals in Paris comprising seven years. However, it is likely that with increasing awareness of this complication the real prevalence will be higher than these data suggest. The frequency of APS nephropathy in patients with SLE is about 30%.[21] In SLE patients the characteristic histopathological abnormalities of APS nephropathy may be isolated or occur together with classical findings of lupus nephritis. The most frequent renal manifestations in primary APS are hypertension (93%), renal insufficiency (87%) and proteinuria (75%).[20] In the series from Nochy *et al.*[20] hypertension was malignant in two patients (12.5%). Patients with SLE and histological proof of APS nephropathy have significantly more often hypertension (60 *vs* 28%) and significantly higher initial serum creatinine levels compared with SLE patients with renal involvement in absence of microangiopathic nephropathy. For the prevalence and extent of proteinuria no significant differences were found.[21]

EPIDEMIOLOGY

Because assays for aPL are poorly standardised and there are no generally accepted cut-off levels that discriminate negative from low-positive results and low-positive from clinically relevant aCL levels as determined in ELISA, the range in reported frequencies of aPL in different studies is wide. Among young, apparently healthy control subjects the prevalence lies between 1 and 5%.[22] In the elderly, the frequency of aPL increases.[22] Similar to what is found in conditions as infection, cancer, haemodialysis and the use of certain drugs, these aPL are usually of IgM isotype, present at low levels and not associated with thrombotic events.[13] Among patients with SLE, reported prevalences for aPL range from 12 to 34% and in women with recurrent (pre-)embryonic pregnancy loss from 10 to 20%.[13] Although prospective studies have shown an association between aPL and the first episode of venous thrombosis, the first myocardial infarction and recurrent stroke,[13] there are insufficient data to determine what percentage of healthy subjects with aPL will eventually have a thrombotic event or a complication of pregnancy consistent with APS. In patients with SLE, APS may develop in 50 to 70% of patients with aPL over 20 years of follow-up.[13] Traditional risk factors for venous and arterial thrombosis are associated with aPL-related thrombosis[17] supporting the importance of a second-hit theory. However, in daily practice we still do not know what the characteristics are of asymptomatic aPL-positive persons with high risks for APS.

WHO SHOULD BE TESTED FOR APL?

Accepted conditions for aPL testing in patients are the presence of SLE, an obstetric history that meets the criteria for obstetric APS (*table 1*), arterial or venous thrombosis before the age of 45 years, recurrent thrombosis, thrombosis in an unusual site and an association of both venous and arterial events.

INTERPRETATION OF AN APL TEST

At first glance the laboratory criteria for APS (*table 1*) are simple: a positive test for LAC and/or a medium to high IgM and/or IgG titre. However, many laboratories still use insensitive coagulation tests to diagnose LAC and do not adhere to the international guidelines for testing LAC.[23] As no single assay is 100% sensitive for LAC at least two different tests should be used for screening. False-negative results occur when platelets are not sufficiently removed from the test sample and presence of heparin in the test sample causes false-positive results. With respect to the aCL ELISA, it is widely recognised that the assay is difficult to standardise. With the same samples tested, different (commercial) tests often give discordant results.[24-26] Despite many efforts at standardisation, cut-off levels for negative, low, medium and high titres remain a matter of dispute, especially at the lower ranges. A good dialogue between the clinic and the laboratory is essential. Furthermore, in the interpretation of test results, clinicians should take into account the age of the patient, use of aPL-inducing drugs, presence of infection, use of immunosuppressive drugs and if the patient has SLE the degree of disease activity at the time of blood sampling.[27] A positive test should always be repeated after six to eight weeks with a second sample to establish persistent positivity.

CONSEQUENCES OF A POSITIVE TEST

The incidental presence of a positive aPL test or a low titre aCL has no clinical consequences. At present, most authorities agree that there is no indication for chronic primary prophylactic treatment in asymptomatic persons with persistently positive aPL tests.[13] However, it seems justified to offer thromboprophylaxis to these persons during high-risk situations such as immobilisation, surgery and the postpartum period, and to consider the

aPL status when a method of contraception is chosen.
In the general population, standard treatment for patients with venous thrombosis and embolic cerebrovascular events is oral anticoagulation targeting an international normalised ratio (INR) of 2.0 to 3.0. After the first venous thrombotic episode treatment is continued for three to six months. Longer duration of anticoagulation implies less recurrences, but the risk for bleeding apparently outweighs the benefits. For patients with nonembolic ischaemic stroke, antiaggregants, notably aspirin, are the standard treatment. The clinician has to decide whether these strategies also hold if the thrombotic patient has aPL. The retrospective study by Khamashta *et al.*[16] including 147 patients with a median follow-up of six years suggested that all patients with thrombosis who fulfil the laboratory criteria for APS should receive life-long high-intensity oral anticoagulants (target INR ≥3). Lower intensities of anticoagulation and aspirin were found to be significantly less effective and the period of six months following cessation of oral anticoagulation had an extraordinarily high risk for recurrent thrombosis. The conclusions of this paper were adopted by many centres worldwide, despite the notion that the study had many methodological shortcomings, such as its retrospective design, treatment according to physicians' and patients' choices, thrombosis taken as the endpoint without discrimination between arterial and venous events and that single patients contributed to different strategies evaluated.[28]
Recent data indicate that the conclusions from the study by Khamashta *et al.* can not be generalised[16] and that prophylaxis with intermediate-intensity anticoagulation and even aspirin may be effective in selected patients. The best evidence comes from a randomised, double-blind trial on anticoagulant treatment of patients with persistently positive aPL tests and thrombosis (over 75% venous).[29] This study, which excluded among others patients with a recurrent event while anticoagulated at an INR of 2.0 or greater, concluded that high-intensity anticoagulation (target INR 3.1 to 4.0) was not superior to anticoagulation at moderate intensity (INR 2.0 to 3.0). This prospective study found a recurrence rate of 2.6 per 100 patient years with anticoagulation. The study supported similar conclusions from some previous small studies.[28]
For current clinical practice this implies that prophylaxis with intermediate-intensity anticoagulation can be provided to most aPL patients with venous thrombosis. The optimal duration of treatment is an open question. In particular questions on whether treatment can be stopped earlier when thrombosis is triggered by surgery, use of oral contraceptives, or by other nonrecurring triggers, or in case traditional aPL tests become negative are important but await further studies. Most authorities currently advise continuation for years if not lifelong. There may also be a role for aspirin for secondary prophylaxis in patients with aPL-related nonembolic stroke.[30] The prospective randomised AntiPhospholipid Antibody in Stroke Study (APASS) found similar rates of recurrence when aPL-positive patients received 325 mg aspirin or low-dose oral anticoagulation (target INR 1.4 to 2.8).[31] Of note, patients in the APASS did not by definition have APS as patients with low titre aCL were included and the aPL status was based on the test result with a single sample.
Because of the rarity of the condition, there are no prospective studies on treatment of catastrophic APS. From an analysis of case histories and small series, guidelines for treatment have been published.[18] These include for all cases treatment of known precipitating factors (in 35% infections), treatment with effective anticoagulation and high-dose corticosteroids. With a life-threatening condition administration of intravenous γ-globulins and/or plasma exchange with fresh frozen plasma is indicated. Treatment should be extended with cyclophosphamide if the condition is associated with a lupus flare. The survival rate of catastrophic APS is about 50%. Poor prognostic factors are older age and a higher number of involved organs. About 60% of patients who survive initial catastrophic APS remain symptom-free with anticoagulation during a follow-up of more than 5.5 years. About a quarter of patients will have further APS-related events during follow-up.[32]
Based on results from retrospective studies, pregnancy outcome in aPL-positive patients who meet the obstetric APS criteria is poor without pharmacological treatment, as there is about a 60% chance of recurrent loss. In considering the literature on pharmacological treatment of obstetric APS, it should be realised that obstetricians will consider a pregnancy a high risk for complications if aPL are found and consequently optimise obstetric care. This in itself will increase the chances of a live birth.[33]
The first pharmacological treatment widely applied in APS pregnancies was the combination of prednisone and low-dose aspirin. When a small randomised study showed similar outcome (over 70% live births) with aspirin and heparin and less side effects, the enthusiasm for prednisone waned. In women with primary obstetric APS randomised studies compared heparin plus aspirin with aspirin alone, aspirin with placebo and heparin plus aspirin with heparin plus aspirin and intravenous γ-globulin. In general, outcome was relatively good with in most studies 70% or more live births in treated pregnancies. Superiority of heparin plus aspirin over aspirin alone in terms of live birth rates was found in some,[34,35] but not in other[36] controlled trials. It is supposed that differences in patient selection, notably on laboratory criteria for obstetric APS, are important denominators for these discrepant results.[13] At present most authorities believe that a combination of low-dose aspirin and a prophylactic dose of low-molecular-weight heparin is the preferred treatment for pregnant women with obstetric APS. This conclusion

was also reached in a recent meta-analysis.[37]

It should be noted that patients with SLE or previous thrombosis were excluded from all previous randomised trials. Whether a history of thrombosis characterises a subset of patients with worse prognosis for pregnancy is unknown. Most physicians will advise use of (low-molecular-weight) heparin in APS patients with a thrombotic history. The dose should be individualised based on the circumstances at which thrombosis occurred, its location and its severity. We advise starting (low-molecular-weight) heparin before conception or, at the latest, within two weeks of the missed period, because oral anticoagulants cross the placenta, are teratogenic when given between 6 and 12 weeks' gestation and may cause intracranial bleeding in the foetus. As pregnancy progresses the volume of distribution for heparin increases and dose-adjustments in proportion to weight gain or based on APTT or anti-factor Xa levels can be considered. In selected cases a switch from heparin to oral anticoagulants may be practical between 15 and 34 weeks' gestation[33]

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Daylong triglyceridaemia in healthy Mediterranean and Northern European subjects

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ABSTRACT

Background: A Mediterranean eating pattern and diet enriched in monounsaturated fatty acids may result in a favourable daylong lipid profile.
Methods: 19 Spanish males (aged 32 ± 8 years) and 28 females (34 ± 8 years) were matched to Dutch subjects on the basis of fasting capillary triglycerides (TGc), gender and age. TGc were self-measured at six fixed time points over three days. Daylong TGc profiles were calculated as areas under the curve (TGc-AUC).
Results: Anthropometric parameters and fasting plasma lipids were comparable between Spanish participants and Dutch subjects. Insulin sensitivity (expressed as HOMA) was highest in the Dutch females (1.41 ± 1.09 *vs* 2.09 ± 1.23 in the Spanish females, p<0.05). Daylong TGc values were not different between Spanish and Dutch participants. Male Spanish subjects showed the largest daylong TGc increase after lunch, while in the Dutch males, the largest TGc increase was seen after dinner. Total daytime dietary energy and total fat intake were comparable when analysed by gender. However, the Spanish participants had a higher intake of monounsaturated and polyunsaturated fatty acids as percentage of energy.
Conclusion: There are no major differences in daylong triglyceridaemia between Dutch and Spanish subjects, despite different eating habits and a diet enriched in monounsaturated and polyunsaturated fat in the latter.

INTRODUCTION

Coronary heart disease (CHD) is the major cause of death in Western populations.[1] Due to different lifestyles and genetic background, there are large geographical differences in CHD mortality.[2] Dyslipaemia plays an important role in the development of atherosclerosis. However, approximately 40 to 50% of all premature atherosclerosis develops in fasting normolipidaemic individuals.[3-6] Since triglycerides (TG) are highly variable during the day due to food intake, and humans are in a postprandial state for the most part of the day, postprandial triglyceridaemia could be a concealed risk factor for CHD. Indeed several studies have demonstrated delayed clearance of TG-rich particles and their direct relation with atherosclerotic disease in different patient groups.[5,7-10]
Recently it was shown that CHD patients on a Mediterranean diet had a 50 to 70% reduction of cardiac endpoints when compared with people who did not receive dietary recommendations, and that this effect was independent of fasting plasma lipids.[11] A Mediterranean diet, which is enriched in unsaturated fatty acids, could have beneficial effects on postprandial TG when compared with a Northern European diet.[12-14] This may be either indirectly via reduction of fasting TG and therefore less remnants of TG-rich lipoproteins, or directly by improved metabolism of postprandial lipoproteins containing unsaturated fatty acids.[14] On the other hand, there are also studies showing undesirable effects of the Mediterranean diet on postprandial TG.[15,16] In addition, it has been shown that, after a similar test meal, people from Mediterranean countries have accelerated postprandial TG clearance when compared with Northern Europeans.[17] This may suggest increased lipolytic activity or decreased intestinal absorption of lipoproteins.[17] Furthermore, the different eating patterns of people from Mediterranean countries, e.g. the main meal in the afternoon instead of the evening,

could be beneficial with regard to postprandial TG. It is known that TG from an oral fat load given in the evening are cleared at a slower rate compared with the same fat load given in the morning.[18]

All the above-mentioned studies have assessed postprandial triglyceridaemia after a standardised oral fat load. In real life there are generally three eating occasions throughout the day with lower food intakes than the single oral fat-loading test. Recently, ambulant self-measurement of capillary TG (TGc) was described to study postprandial lipaemia in a free-living situation.[19-23] Using this technique we have confirmed reports in metabolic ward conditions showing postprandial hyperlipidaemia in males compared with females,[19] in obesity,[24] in diabetes mellitus type 2[25] and in patients with premature CHD.[26] We have previously shown that diurnal TGc profiles correlate well with standardised oral fat-loading tests that are regarded as the golden standard for testing of TG metabolism.[19] Major determinants of diurnal TGc profiles are gender, insulin sensitivity and age, besides fasting TGc. Furthermore, we have described positive associations between increments in diurnal TGc and the carbohydrate, protein and total energy content of the diet, whereas fat intake determined the total but not incremental TGc response.[19,22,23]

Geographical differences, including a different genetic background, lifestyle and diet, may affect daylong triglyceridaemia. We studied daylong TGc in healthy normolipidaemic subjects from Spain and the Netherlands in an uncontrolled out-of-hospital setting. Since fasting TGc have been shown to be the best predictor of daylong triglyceridaemia,[19,22] Spanish and Dutch subjects were matched for fasting TGc. In addition, determinants of the daylong TGc profiles were evaluated.

METHODS

Subjects

Healthy normolipaemic volunteers from the Departments of Internal Medicine in Utrecht (the Netherlands) and Valencia (Spain), aged 20 to 55 years, were recruited by advertisement. Exclusion criteria were fasting plasma cholesterol concentration >6.5 mM, fasting plasma TG concentration >2.3 mM, body mass index (BMI) >30 kg/m^2, smoking, renal or liver disease, diabetes mellitus, use of lipid-lowering medication, menopause or a postmenopausal state, and a family history of premature myocardial infarction (males <55 years, females <65 years) or type 2 diabetes mellitus. On the morning of inclusion, anthropometric measurements were performed using standard techniques. The Spanish subjects were matched to Dutch subjects on the basis of gender, age and fasting TGc. All subjects gave written informed consent before participating. The study was approved by the Independent Ethics Committee of Institutional Review Board of Utrecht University Medical Centre (the Netherlands) and Valencia University Hospital (Spain).

Self-measurements of TGc

TGc was self-measured with a TG-specific point-of-care testing device (Accutrend GCT; Roche Diagnostics, Mannheim, Germany)[19,21-23] after the subjects had received instructions from the same investigator. Subjects were instructed to wash and dry their hands thoroughly before each measurement. A drop of blood (30 µl) obtained from the finger using a lancing device was applied to the test strip in the device. Subsequently, TGc was measured by a process of dry chemistry and colorimetry. If there was not enough blood on the test strip, subjects were asked to repeat the measurement. The reference range for TGc is 0.80 to 6.86 mM. In a previous study, the coefficients of variation for different TGc concentrations ranged from 3.3 to 5.3%.[21] The correlation coefficient between TGc using the device and plasma TG according to enzymatic methods is 0.94.[21] Similar results were obtained in our laboratory.[19,22]

Subjects were instructed to measure their TGc concentrations on three different days (preferably Monday, Wednesday, and Friday; not in weekends) at the following six time points: fasting, before and three hours after lunch and dinner, and at bedtime. The three-hour postprandial measurements were performed exactly three hours after the meals, regardless of the intake of snacks, and the results were recorded in a diary. Subjects were requested to refrain from heavy physical activity, although normal daily activities such as riding a bike to work, were allowed. When one or more measurements were missing for a day, the data for that particular day were not used to create an average daylong TGc profile. The mean daytime TGc profile was used for statistical analysis.

Dietary intake

Dietary intake was recorded in the same diary in which the TGc concentrations were written. Subjects received no recommendations concerning the frequency and composition of the meals and were requested to consume their usual diet during the study. Quantities of intake were estimated according to instructions given by a dietician and by using a table with standardised portion sizes.[27] Other details, such as illness, were also recorded in the diary. The diaries were evaluated by a trained physician together with each subject. Foods consumed were converted into nutrients by using the Dutch Nutrient Database[28] and nutrition tables for Spain.[29] Dietary intakes were compared with the average diet in the Netherlands[27,28] and in Spain.[30] Dietary intakes were calculated per day and as an average of two or three days.

Analytic determinations

On the morning of inclusion, after an overnight fast of at least ten hours, blood was collected for measurement of plasma lipid, insulin and glucose concentrations. Total cholesterol, HDL cholesterol obtained after precipitation with Phosphotungstate/$MgCl_2$ and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits, respectively (Roche diagnostics, Germany).[7,31] LDL cholesterol was calculated using the Friedewald formula. Glucose was measured by glucose oxidase dry chemistry and colorimetry (Vitros GLU slides; Johnson & Johnson, Clinical Diagnostics, Rochester, NY), insulin was measured using a competitive radioimmunoassay with polyclonal antibodies. The HOMA index (homeostasis model assessment = glucose*insulin/22.5) was calculated to estimate insulin sensitivity.[32] All clinical chemistry determinations were performed at the laboratory of clinical chemistry of Utrecht University Hospital.

Statistics

Data are given as mean ± SD in the text and tables and as mean ± SEM in the figure. Daytime TGc profiles were calculated as total and incremental (after correction for fasting TGc) areas under the curve (TGc-AUC and dTGc-AUC, respectively). Dietary intakes and AUCs were calculated by using averages over two or three days. Differences in dietary intakes or TGc-AUC between three separate days were tested by paired *t* test. Differences between the study groups were tested with an unpaired Student's *t* test. Individual time points of daylong TGc were compared back to baseline by a 1-factor RM ANOVA, using time as within-subject factor, with Bonferroni adjustment for multiple comparisons. All comparisons were performed by gender because, after fasting TGc, this is the major determinant of daylong TGc.[19] To study variables associated with TGc-AUC and dTGc-AUC, univariate correlations were calculated using Pearson's correlation coefficients. Stepwise multiple regression analysis was performed with TGc-AUC and dTGc-AUC as dependent variables and with the significantly associated variables identified by univariate regression analysis as independent variables. Plasma TG, insulin and the HOMA index were analysed after logarithmic transformation because of the nonparametric distribution. SPSS version 10.0 (SPSS Inc, Chicago) was used for the statistical analysis. Areas under the TGc curve were calculated with PRISM version 3.0 (Graph Pad Software, San Diego) by using non-logarithmically transformed TGc concentrations. Statistical significance was set at $p<0.05$ (two-sided).

Table 1

Baseline characteristics (mean (SD)) and daylong triglycerides of the study group (20 to 55 years, n=94)

	SPANISH MALES (N=19)	DUTCH MALES (N=19)	SPANISH FEMALES (N=28)	DUTCH FEMALES (N=28)
Age (years)	32 (8)	33 (11)	34 (8)	34 (9)
Length (m)	1.76 (0.05)	1.83 (0.08) †	1.63 (0.05)	1.69 (0.06) ‡‡
Weight (kg)	78 (11)	79 (10)	60 (8)	65 (8) ‡
BMI (kg/m²)	24.9 (3.0)	23.4 (2.8)	22.7 (2.5)	22.7 (2.5)
Waist (m)	0.87 (0.10)	0.83 (0.08)	0.75 (0.08)	0.74 (0.08)
WH	0.90 (0.05)	0.86 (0.07)	0.79 (0.06)	0.77 (0.05)
Plasma TG (mM)	1.17 (0.65)	1.33 (0.48)	0.76 (0.35)	0.75 (0.37)
Cholesterol (mM)	4.73 (1.01)	5.01 (0.76)	4.90 (0.92)	4.32 (0.89) ‡
LDL cholesterol (mM)	3.00 (0.91)	3.17 (0.89)	3.16 (0.80)	2.46 (0.64) ‡
HDL cholesterol (mM)	1.19 (0.18)	1.24 (0.29)	1.42 (0.24)	1.52 (0.38)
Glucose (mM)	5.4 (0.5)	4.9 (0.8) †	4.9 (0.5)	5.3 (0.6) ‡
Insulin (iU/l)	7.1 (3.1)	8.4 (3.8)	6.3 (4.7)	8.9 (4.9) ‡
HOMA	1.72 (0.78)	1.85 (0.90)	1.41 (1.09)	2.09 (1.23) ‡
TGc-fasting (mM)	1.44 (0.52)	1.42 (0.49)	1.15 (0.29)	1.12 (0.29)
TGc-AUC (mM*h/l)	27.2 (8.9)	26.7 (9.3)	19.9 (5.8)	17.3 (6.0)
dTGc-AUC (mM*h/l)	7.0 (7.5)	7.7 (5.2)	3.9 (4.0)	3.2 (3.9)
dTGc pre-3h postlunch (mM)	0.92 (0.82)	0.48 (0.81)	0.13 (0.59)	0.15 (0.65)
dTGc predinner-bedtime (mM)	0.12 (0.85)	0.44 (0.83)	0.08 (0.86)	-0.03 (0.31)

WH = waist-to-hip ratio, HOMA = homeostasis model assessment, TGc = capillary triglycerides, TGc-AUC and dTGc-AUC = total and incremental area under the TGc curve, dTGc pre-3h postlunch = TGc change from lunch to three hours after lunch, dTGc predinner-bedtime = TGc change from dinner to bedtime, Student's t test = † p<0.05, †† p<0.005 Spanish vs Dutch males, ‡ p<0.05, ‡‡ p<0.005 Spanish vs Dutch females.

RESULTS

Subject characteristics

In total 100 subjects (50 in each country) were screened for inclusion. Six subjects were excluded due to elevated BMI (n=2), smoking (n=2) and a positive family history for premature atherosclerosis (n=2). Data are shown per gender and ethnicity (*table 1*). The Dutch participants were taller than the Spanish; however, body mass indexes were not different. Fasting plasma lipid values were within normal limits and comparable between the groups, except for a higher fasting total plasma cholesterol due to higher LDL cholesterol in the Spanish females when compared with Dutch females. As a result of differences in fasting glucose and insulin, insulin sensitivity was higher in the Dutch females when compared with the Spanish females. Four of the Spanish females and 12 of the Dutch females were on oral contraceptives.

Self-measurements of TGc and dietary intake

Mean fasting TGc values were not different between Dutch and Spanish participants (*table 1*). In the males all daylong TGc values, except TGc before lunch, were higher than at baseline (*figure 1*, upper panel). In the Spanish females, all postprandial TGc measurements were higher than at baseline, while in the Dutch females only TGc's after dinner were higher than at baseline (*figure 1*, lower panel). In the Spanish males, the largest TGc increment was observed after lunch (*table 1*); however, this increase was not higher than that in the Dutch males (p=0.1), while in the Dutch males the largest TGc increment was seen after dinner (*table 1*). In both groups of males, there was no TGc decline at bedtime. The differences in daylong TGc increments in the males did not result in different total and incremental AUCs (*table 1* and *figure 1*). In both groups of females, small gradual daylong TGc increments were seen that did not result in different total and incremental AUCs (*table 1* and *figure 1*). Subanalysis of TGc-AUC and dTGc-AUC according to the use of contraceptives did not show significant differences in the Spanish or Dutch women (data not shown). Both total and incremental TGc-AUC were higher in males compared with females in the Spanish as well as in the Dutch participants (p<0.05 for all comparisons, *table 1*). When fasting TGc, TGc-AUC and dTGc-AUC were compared between the two ethnic groups; this did not result in statistically significant differences (data not shown). In the males the total energy intake was comparable; however, the Spanish males had a higher monounsaturated and polyunsaturated fat intake and ingested more cholesterol, when compared with Dutch males (*table 2*). The females showed a comparable total energy intake. Similarly to the Spanish males, the Spanish females had a higher intake of monounsaturated fat and cholesterol when compared with the Dutch females (*table 2*).

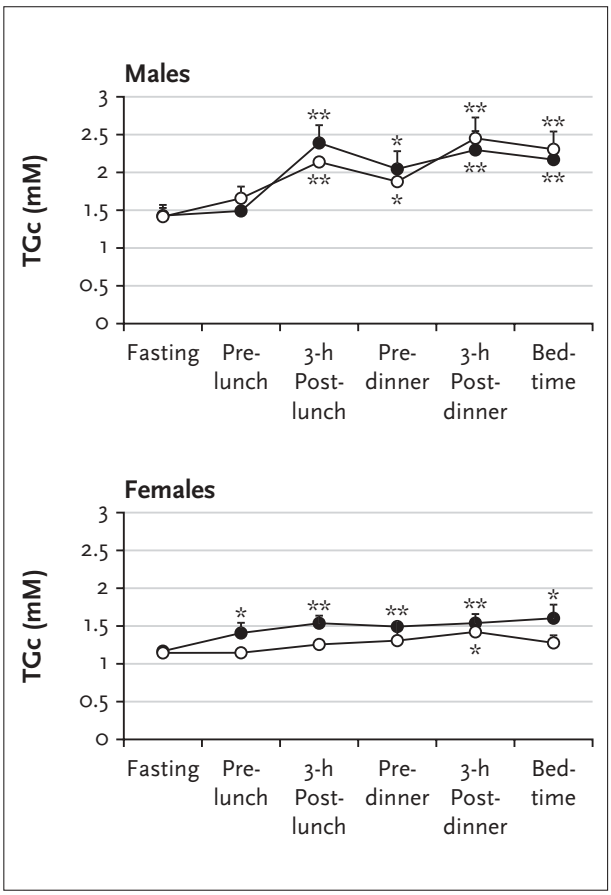

Figure 1

*Mean (± SEM) daylong capillary triglycerides (TGc) in Spanish (n=19, closed circle) and Dutch males (n=19, open circle), [upper panel] and in Spanish (n=28, closed square) and Dutch females (n=28, open square), [lower panel]. Between group differences (unpaired Student's t test for total and incremental AUCs): p=ns for both figures. Differences back to fasting (repeated measures ANOVA): *p<0.05, ** p<0.005.*

Determinants of daylong TGc

When all subjects were analysed together, TGc-AUC was significantly related to fasting TGc and plasma TG (r=0.73 and 0.65 respectively, p<0.001 for each), waist-to-hip ratio (r=0.43, p<0.001), cholesterol (r=0.31, p<0.005), HDL cholesterol (r=-0.30, p<0.005) and HOMA (r=0.22, p<0.05). From all dietary parameters, total energy intake (r=0.29, p<0.01), total intake of carbohydrates (r=0.32, p<0.005), total MUFA intake (r=0.26, p<0.01), total alcohol intake (r=0.28, p<0.01) and protein intake as percentage of energy (r=-0.28, p<0.01) were significantly related to TGc-AUC. Stepwise multiple regression revealed fasting TGc as best predictor (standardised β=0.72) explaining 51% of the TGc-AUC (p<0.001), the model improved significantly when carbohydrate intake, ethnicity and gender were entered (adjusted r^2=0.62, p<0.001).

Table 2
Mean (SD) dietary intake of the study group (20 to 55 years, n=94)

	SPANISH MALES (N=19)	DUTCH MALES (N=19)	SPANISH FEMALES (N=28)	DUTCH FEMALES (N=28)
Energy (kJ)	10832 (1950)	11288 (2485)	7954 (2414)	8577 (1766)
Total fat (g)	108 (25)	96 (24)	81 (29)	83 (26)
(% of energy)	37.4 (5.1)	32.4 (4.8) ††	38.1 (4.0)	36.2 (6.4)
Saturated fat (g)	41 (14)	36 (11)	30 (12)	32 (10)
(% of energy)	14.2 (3.3)	12.0 (2.8) †	14.1 (2.4)	13.9 (3.0)
MUFA (g)	45 (9)	38 (9) †	35 (13)	31 (9)
(% of energy)	16.0 (2.8)	12.8 (2.1) ††	16.9 (3.1)	13.4 (2.8) ‡‡
PUFA (g)	18 (6)	14 (4) †	11 (5)	14 (8)
(% of energy)	6.2 (1.8)	4.9 (1.2) †	5.5 (1.9)	6.0 (2.3)
Carbohydrates (g)	294 (65)	314 (64)	215 (73)	235 (56)
(% of energy)	45.5 (6.4)	47.8 (5.5)	45.0 (5.9)	47.0 (7.2)
Protein (g)	101 (23)	105 (22)	76 (19)	79 (16)
(% of energy)	15.7 (2.8)	15.9 (1.9)	16.5 (2.8)	15.9 (2.6)
Cholesterol (mg)	456 (213)	217 (92) ††	345 (179)	183 (67) ‡‡
Alcohol (g)	6.7 (8.6)	19.8 (28.5)	2.4 (5.2)	6.8 (7.3) ‡

MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, Student's t-test = † p<0.05, †† p<0.005 Spanish vs Dutch males, ‡ p<0.05, ‡‡ p<0.005 Spanish vs Dutch females.

The dTGc-AUC of the total study group was significantly associated with fasting TGc and plasma TG (r=0.20 and 0.40 respectively, p<0.05 for each), waist (r=0.21, p<0.05), cholesterol (r=0.26, p<0.01), HOMA (r=0.23, p<0.05) and diastolic blood pressure (r=-0.36, p<0.05). From all dietary parameters, total carbohydrate and total alcohol intake (r=0.27 for both, p<0.01 for each) and protein intake as percentage of energy (r=-0.25, p<0.05) were significantly related to dTGc-AUC. The best model to predict dTGc-AUC included gender only (standardised β=-0.66), predicting 42% of variation (p<0.001).

DISCUSSION

A Mediterranean diet consists of a higher amount of monounsaturated fatty acids when compared with a Northern European diet,[11] as was also observed in the present study, in particular in the male subjects. In the Lyon Diet Heart Study, the Mediterranean diet had an impressive beneficial effect on cardiovascular complications, despite unchanged fasting lipids, suggesting an alternative mechanism as for instance postprandial lipaemia.[11] However, there is controversy about the beneficial effects of unsaturated fatty acids on postprandial triglyceridaemia. Some studies have shown a reduction of postprandial TG,[12-14] whereas others have shown the contrary.[15,16] In all these studies, unphysiological oral fat-loading tests were used to study postprandial lipaemia. In the present study, in a nonstandardised setting reflecting the normal daily situation, we were not able to detect differences in daylong triglyceridaemia between Spanish and Dutch participants, despite a higher monounsaturated and polyunsaturated fat intake in the Spanish groups, while anthropometric and baseline laboratory values were similar and the dietary intakes reflected that of the general population.[27,28,30] Therefore, the effects of this diet on postprandial lipaemia in real life may be questioned. It could be quite possible that the beneficial effects of unsaturated fatty acids on the process of atherosclerosis depend on other mechanisms than postprandial lipaemia. In this regard, inhibition of endothelial activation by unsaturated fatty acids has been described[33] and others have shown improvement of postprandial endothelial function by antioxidant-rich components of the Mediterranean diet.[34] On the other hand, certain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are believed to be beneficial with regard to CHD[35] and lipid metabolism,[36] may be unequally distributed among the two ethnic groups that we have studied. Unfortunately, we were not able to calculate these fatty acids separately in the present study due to the software used. It was, however, remarkable that the intake of dietary cholesterol was higher in the Spanish subjects than in the Dutch participants. A high dietary cholesterol may increase plasma cholesterol levels,[12] but effects on triglyceridaemia are unlikely since in another study no TG change was observed in type 2 diabetes patients after cholesterol supplementation.[37] Furthermore, as we already observed, we have never found a correlation between dietary cholesterol and

daylong triglyceridaemia in our previous studies.[19,22,23] Daylong triglyceridaemia was similar in both groups; however, the male Spanish participants showed the largest TGc responses after lunch while in the Dutch males this was seen after dinner. It is known that in Mediterranean countries most people have their main (hot) meal at noon, while this is uncommon in West European countries. The timing of the major meal could be of importance for the total daylong triglyceridaemia.[18] Assuming a difference in eating pattern between the Spanish and Dutch subjects, we did not observe beneficial effects on daylong triglyceridaemia. This suggests that the number of atherogenic chylomicron remnants generated by each meal may have been similar in both groups.
Daylong triglyceridaemia was relatively low and comparable in the Spanish and Dutch premenopausal females. However, in the Spanish females there was a more pronounced postprandial TGc increase. The lower insulin sensitivity in the Spanish females may have caused the small difference in daylong TGc. However, in previous studies in Dutch subjects, we have shown that insulin sensitivity affects daylong TGc more in males than in females.[19] In the present study we did not correct for the phase of the menstrual cycle, since it is unlikely that the oestrogen status of the premenopausal women influenced our results. It has previously been shown that in premenopausal women, despite fluctuations in plasma TG during the menstrual cycle, overall intraindividual TG variability was comparable with that of men.[38] It should be underlined that a subgroup of the female participants were on oral contraceptives. We can not rule out that this may have affected daylong triglyceridaemia. However, we do not believe this to be the case since a subanalysis did not show differences in daylong triglyceridaemia.
Table 1 suggested that fasting plasma TG were lower than capillary TG. We have previously shown that in a direct comparison TGc are slightly higher than plasma TG.[22] Secondly, plasma TG given in *table 1* represented a single measurement, whereas TGc comprised the average of three measurements at different days. Since TG are highly variable within individuals, repetitive measurements may have reduced the variation. Thirdly, plasma TG was determined on the day of inclusion after an overnight fast of at least ten hours, whereas TGc was self-determined without prior overnight restrictions since this measurement was intended to represent real-life fasting TG.
Ethnicity was one of the predictors of daylong TGc in the present study; however, the predictive value was much weaker than that of fasting TGc, and total and incremental triglyceridaemia were not different between Spanish and Dutch subjects. Nevertheless, with the present study we cannot exclude that genetic differences may have influenced the results. It is well known that the apolipoprotein E gene and many other genes can influence postprandial lipoprotein metabolism.[39] In this regard a novel gene, the apolipoprotein AV gene, has very recently been linked to daylong TGc.[40] In addition, differences in the activity of lipolytic pathways such as lipoprotein lipase and hepatic lipase could have influenced the data. Unfortunately we were not able to study differences in genotypes and activity of lipolytic enzymes. Furthermore, it is known that physical activity enhances lipolysis.[41] In the present study only normal daily activities were allowed on the days of TGc self-measurement. We did not quantify separately the daily activity.
In conclusion, there are no major differences in daylong triglyceridaemia between Dutch and Spanish subjects, despite different eating habits and a diet enriched in monounsaturated fat in the latter.

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AvO designed the study, was involved in the data collection and analysis and drafted the manuscript. JTR, RC and JFA contributed to the study design, interpretation and analysis of the data and were involved in the writing of the manuscript. MCC devised the study, supervised the data collection and analysis and contributed to the writing of the manuscript. MCC received educational grants from Merck, Pfizer, Novo Nordisk and AstraZeneca. The other authors had no conflict of interest.

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Faecal elastase-1: helpful in analysing steatorrhoea?

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ABSTRACT

Background: The faecal elastase-1 test (FE-1) is considered easy to perform and sensitive to detect severe and moderate exocrine pancreatic insufficiency. However, little information is available on the specificity of this test in the analysis of steatorrhoea. Our aim was to evaluate the clinical value of FE-1 in the analysis of patients sent in for faecal fat determination.

Methods: Stool samples were collected over 24 hours in 40 healthy controls and 119 patients: 58 patients with chronic pancreatitis and 61 nonpancreatic disease patients with chronic diarrhoea. Faecal fat excretion was determined and FE-1 was measured using a commercially available ELISA kit, which employs two monoclonal antibodies to bind to two distinct epitopes of human pancreatic elastase-1.

Results: Faecal elastase-1 test shows good reproducibility. The test lacks sensitivity in detecting exocrine pancreatic insufficiency and chronic pancreatitis (68 and 59%, respectively). However, it is specific with respect to differentiating pancreatic from nonpancreatic causes in patients with steatorrhoea.

Conclusion: FE-1 lacks sensitivity to detect chronic pancreatitis. It can serve as a simple, noninvasive method to determine the aetiology of steatorrhoea.

INTRODUCTION

For evaluation and follow-up of exocrine pancreatic function, several tests are available.[1] The secretin-cholecystokinin test (SCT) is considered the gold standard. However, this test requires duodenal intubation, is time consuming, expensive and lacks standardisation. Its use, therefore, is limited to research purposes. Indirect tests either determine the amount of unabsorbed nutrients in the stool (i.e. faecal fat excretion) or measure directly or indirectly the enzyme activity in blood, stool, urine or breath.[2] These procedures are relatively easy to perform, while discomfort is limited. The indirect tests lack sensitivity in mild and moderate exocrine pancreatic insufficiency, and their specificity is questionable. Some years ago the faecal elastase-1 test was introduced. This test is considered specific for human pancreatic elastase so that exogenous enzyme supplements do not affect the test result.[3] Elastase-1 concentration in faeces is about five times higher than in pancreatic juice, illustrating its stability during intestinal transport.[4] Several studies have compared faecal elastase-1 with other indirect and direct tests in pancreatic disease but varying and contrasting results have been obtained. With steatorrhoea, it is relevant to distinguish between pancreatic and other gastrointestinal causes. Despite extensive data on faecal elastase output in patients with chronic pancreatitis, little is known on the specificity of the faecal elastase test.[5-11]

The aim of our study was to evaluate the clinical value of faecal elastase-1 in patients with chronic pancreatitis and in patients with chronic diarrhoea with or without fat malabsorption due to nonpancreatic gastrointestinal disorders. In steatorrhoea patients we tested the ability of faecal elastase-1 to distinguish between pancreatic and nonpancreatic aetiologies.

MATERIALS AND METHODS

Patients

Between 1996 and 2000, stools sent in for faecal fat determination were also analysed for faecal elastase-1

concentration. The study group consisted of 119 patients with a mean age of 49 (range 17 to 75 years), 57 male and 62 female. In the patients with chronic pancreatitis, the diagnosis was based on clinical history, morphological changes seen on ultrasonography and/or CT scan, and endoscopic retrograde cholangiopancreatography (ERCP). An elevated faecal fat excretion (a sign of decompensated exocrine pancreatic insufficiency) was present in 38 of the 58 chronic pancreatitis patients. Sixty-one patients with chronic diarrhoea due to nonpancreatic gastrointestinal disorders were included. They consisted of patients with gastrectomy (n=11), systemic sclerosis (n=4), inflammatory bowel disease (n=20) and functional diarrhoea (n=26) patients. All had symptoms of frequent bulky stools and/or diarrhoea, and 30 patients had steatorrhoea. We included 40 healthy controls (mean age 27, range 16-74 years, even gender distribution) who had no history of gastrointestinal or pancreatic disease and had a normal faecal fat excretion (<7 g/24 h). The effect of exogenous enzyme supplements (mean dosage 3 x 25,000 IU lipase) on faecal elastase-1 was assessed in 13 chronic pancreatitis patients. Repeatability was tested in a group of 46 individuals (31 healthy controls, 10 chronic pancreatitis patients and 5 nonpancreatic disease patients). Faecal elastase-1 was determined in stools that were collected over 24 hours on two consecutive days.

Methods

Stools were collected over a 24-hour period while the subject was on a standard diet with a mean daily fat intake of around 100 g. Quantitative fat was determined using the Van de Kamer method. Faecal elastase-1 was measured using an enzyme-linked immunosorbent assay (ELISA kit available from ScheBo-Tech, Giessen, Germany) employing two monoclonal antibodies binding to two distinct epitopes of human pancreatic elastase.[12] In each (24-hour) stool collection, faecal elastase concentration and faecal fat excretion were analysed.

Analysis

The cut-off value for faecal elastase-1 was defined as the mean value in the control group minus twice the standard deviation. Two series of data did not exhibit a normal distribution, namely the chronic pancreatitis group with exocrine insufficiency and a subgroup of the nonpancreatic disease patients (gastrectomy). To be able to apply a parametric statistical model, raw data were transformed by means of the square root method. Statistical differences were analysed using a one-way analysis of variance model (SPSS), contrasts were defined as being significant at p=0.05 or less. Sensitivity and specificity of the faecal elastase-1 test for detecting exocrine pancreatic insufficiency were calculated. The data of the faecal elastase and the faecal fat excretion used to assess the influence of exogenous enzyme did not show a normal distribution, so the nonparametric Wilcoxon signed-rank test was used. The two consecutive faecal elastase values obtained from the healthy volunteers did not exhibit a normal distribution either. Repeatability was therefore analysed using the Wilcoxon signed-rank test. The coefficients of variation and the standard deviation of the measurement error were calculated.

RESULTS

Clinical and biochemical data of the patients with chronic pancreatitis are given in *table 1*. The cut-off value for faecal elastase-1 based on our healthy volunteer population was calculated to be 218 µg/g faeces. *Table 2* shows the results of all the faecal elastase and fat excretion data. Of the chronic pancreatitis patients with steatorrhoea, 26 had a reduced but 12 had a normal faecal elastase-1 concentration. As for the chronic pancreatitis patients with compensated exocrine pancreatic insufficiency (no steatorrhoea), 12 had normal and eight had low faecal elastase-1 concentrations. In the nonpancreatic disease patient group, all but five had normal faecal elastase-1 concentrations. Of these five, four had undergone a gastrectomy and one patient had Crohn's disease. The faecal elastase-1 concentrations in the chronic pancreatitis group with steatorrhoea were significantly (p<0.001) lower than those in the chronic pancreatitis group without steatorrhoea. The entire chronic pancreatitis group had significantly (p<0.001) lower faecal elastase-1 concentrations compared with nonpancreatic

Table 1
Clinical and biochemical data on patients with chronic pancreatitis (CP)

	CP WITH STEATORRHOEA (N=38)		CP WITHOUT STEATORRHOEA (N=20)	
Aetiology of CP				
- Alcohol	19	50%	9	45%
- Idiopathic	14	37%	10	50%
- Hereditary	3	8%		
- Pancreas divisum	1	3%	1	5%
- Hypercalcaemia	1	3%		
Duration of CP (years) mean, range	5.5 (0.3-25)		4.3 (0.3-13)	
Serum amylase (u/l) mean, range	140 (32-237)		182 (61-312)	
Weight loss (kg) mean, range	2.3 (0-10)		1.8 (0-17)	
Endocrine insufficiency:				
- Impaired glucose tolerance	4	(11%)	6	(30%)
- Insulin dependent	13	(34%)	3	(15%)

Table 2

Data on faecal fat excretion determination and faecal elastase-1 estimation in healthy controls, chronic pancreatitis patients and nonpancreatic chronic diarrhoea patients (cut-off value is 218 μg/g faeces)

GROUP	NO. OF PATIENTS	MEAN 24 H FAECAL FAT EXCRETION (± SEM)	MEAN FAECAL ELASTASE-1 CONCENTRATION (± SEM)	NO. (%) WITH FAECAL ELASTASE-1 <218 μG/G
Healthy controls	40	3.4 ± 1.4	636.1 ± 33.1	1 (2.5%)
Chronic pancreatitis	58	16.3 ± 2.6	200.3 ± 28.5	34 (59%)
With steatorrhoea	38	22.5 ± 3.7	145.8 ± 31.2	26 (68%)
Without steatorrhoea	20	4.5 ± 0.4	303.8 ± 52.1	8 (40%)
Nonpancreatic disease patients	61	11.2 ± 1.4	568.6 ± 36.2	5 (8.2%)
With steatorrhoea	31	18.2 ± 2.1	552.9 ± 50.4	3 (9.7%)
Without steatorrhoea	30	3.8 ± 0.3	595.9 ± 49.6	2 (6.7%)

disease patients. There were no statistically significant differences between nonpancreatic disease patients and healthy controls. The sensitivity of the faecal elastase-1 concentrations was 68% for detecting decompensated exocrine pancreatic insufficiency in patients with chronic pancreatitis. The specificity for chronic pancreatitis was calculated to be 93% for the entire chronic pancreatitis group. Our aim was to evaluate the usefulness of faecal elastase-1 in steatorrhoea. Therefore we grouped the patients (n=69) with an elevated faecal fat excretion of all aetiologies. Of these 69 patients, 29 had a positive faecal elastase-1 test (concentration below 218 μg/g): 90% (26) were chronic pancreatitis patients and 10% (3) gastrectomy patients.

Figure 1 shows the effect of exogenous enzyme supplements on faecal elastase concentration and fat excretion. After seven days of enzyme supplement therapy the faecal fat excretion was significantly reduced (p=0.002 compared with basal) but faecal elastase output did not significantly change.

As for repeatability, there was a strong correlation between the faecal elastase value on the first and second day of stool collection (p=0.921). The mean coefficient of variation was 15.7% and the standard deviation of the measurement error was 124.4.

DISCUSSION

Previous studies on the faecal elastase-1 test have shown promising results. Faecal elastase-1 concentration is about five- to six-fold the duodenal concentration and hardly influenced by motility disorders or mucosal defects.[8] As the assay determines concentration, only a single sample of a stool is required. The assay is specific for human elastase suggesting that it is not necessary to discontinue exogenous enzyme supplementation previous to stool sampling, as was confirmed by our data. In our healthy volunteers we determined a cut-off value (218 μg/g) for

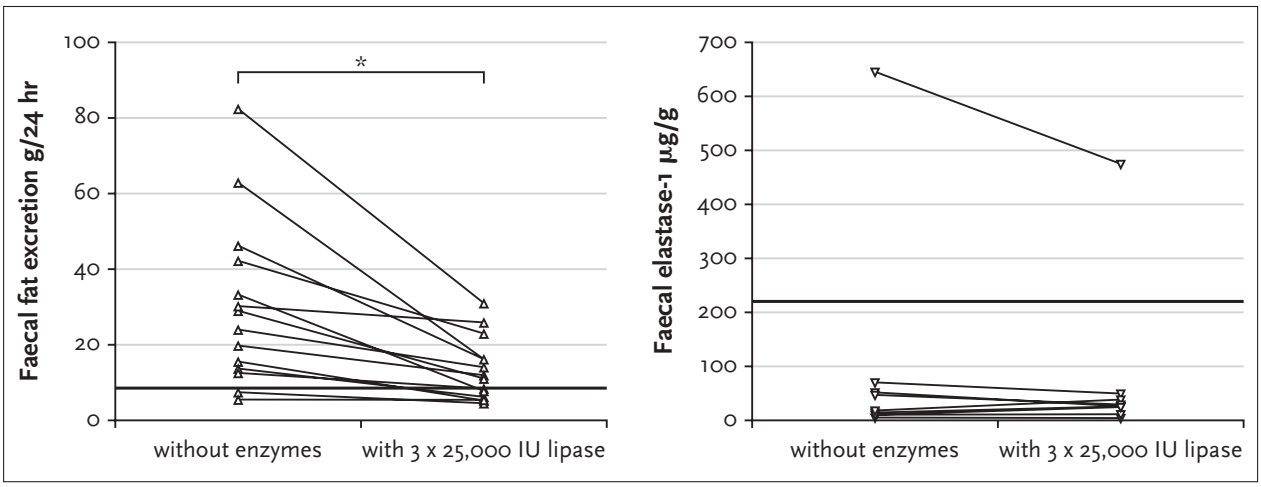

Figure 1

*Effect of exogenous enzyme supplements on faecal fat excretion and faecal elastase concentration in 14 chronic pancreatitis patients of which 12 with steatorrhoea (faecal fat excretion > 7 g/24 h). Faecal fat excretion decreased from 30 g/24 h to 13 g/24 h (p=0.002; indicated by *). Faecal elastase before and during exogenous enzyme use is not affected by exogenous enzyme supplementation.*

faecal elastase, which is comparable with the suggested cut-off by the manufacturer and with results from other studies.[3,6]

Although the faecal elastase concentrations in the chronic pancreatitis group with steatorrhoea were significantly lower than in the chronic pancreatitis patients without steatorrhoea and the nonpancreatic disease controls, the sensitivity compared with faecal fat analysis was poor. Of all chronic pancreatitis patients, 41% had a faecal elastase >218 μg/g. In the chronic pancreatitis group with steatorrhoea, there were still 35% with a normal faecal elastase-1. Our findings certainly do not confirm the high sensitivities reported by others[6-8,12] and are more in accordance with the work by Lankisch *et al.* reporting 82% true-positives of the faecal elastase-1 test in severe exocrine pancreatic insufficiency, but less than 50% in mild and moderate insufficiency. These authors used a functional classification based on the secretin-cholecystokinin test and faecal fat excretion.[13] Amann *et al.* came to the same conclusion (low sensitivity of 40%) but their results where obtained in a group of 14 chronic pancreatitis patients.[14]

Hardt *et al.* reported sensitivities of 45% for faecal elastase-1 in predicting the presence of ductal changes in a large group of patients undergoing ERCP.[11]

Of the patients with steatorrhoea that we analysed, 42% had a faecal elastase concentration below 218 μg/g. Of these patients, 90% had chronic pancreatitis with exocrine pancreatic insufficiency. The three false-positives (patients with steatorrhoea without pancreatic disease) consisted of patients after partial gastrectomy. None of the gastrectomy patients had any evidence of pancreatic disease (normal morphology confirmed by ultrasonography or CT scan) or a history of pancreatic symptoms. In fact four out of five false-positive test results in the nonpancreatic disease patient group were gastric resection patients, three of whom had steatorrhoea. It has been suggested that the presence of dumping symptoms, with rapid intestinal passage and voluminous stools may lead to dilution and a subsequent lower faecal elastase concentration in the stool sample. In an attempt to explain low elastase concentrations in patients with nonpancreatic malabsorption, Amann *et al.* also suggested that liquid stool may perturb accurate determination.[14] This does not explain the results in our gastrectomy patients as the mean faecal mass was 245 g/24 h, which is equal to the 244 g/24 h in the rest of the nonpancreatic disease control patients with a normal faecal elastase-1. Another factor to explain low faecal elastase levels in patients after gastric surgery may be a disturbance in the neurohormonal signals that stimulate exocrine pancreatic secretion.[15]

We prospectively collected data on faecal elastase-1 concentration in stools from patients sent to our laboratory for faecal fat analysis. Based on this cohort of nonselected patients we evaluated the potential clinical value of the faecal elastase-1 test. It is concluded that the faecal elastase-1 test shows good reproducibility. The test lacks sensitivity in detecting exocrine pancreatic insufficiency and chronic pancreatitis. However, the test is specific with respect to differentiating pancreatic from nonpancreatic causes in patients with steatorrhoea.

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CASE REPORT

Congestive heart failure in pregnancy: a case of peripartum cardiomyopathy

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ABSTRACT

A healthy 28-year-old woman developed full-blown pulmonary oedema in the 36th week of gestation. Echocardiography revealed a globally enlarged heart with reduced systolic function. A remarkable clinical response with regain of normal ventricular function was noted with early medical intervention. This case report illustrates peripartum cardiomyopathy, a unique form of dilated cardiomyopathy affecting women during/following gestation. Clinician familiarity with this entity increases the probability of prompt appropriate treatment, offering patients the best possible prognosis.

INTRODUCTION

Peripartum cardiomyopathy (PPCM) is a rare and potentially life-threatening complication of pregnancy whose underlying cause remains unknown. An uncommon form of dilated cardiomyopathy, this disorder ultimately results in congestive heart failure late in pregnancy or in the early puerperium. Its natural history is extremely variable, ranging from the spontaneous recovery of ventricular function to refractory disease often necessitating cardiac transplantation. In recent studies, the reported incidence of death or cardiac transplantation were in the range of 12 to 18%, compared with a mortality rate of up to 50% reported in the 1980s. As early intervention is believed to improve prognosis, clinician familiarity with PPCM is essential, thus ensuring timely and optimal treatment to women stricken with PPCM.

CASE REPORT

A 28-year-old primigravid Ghanese woman with an unremarkable previous medical history presented in the 36th week of gestation with respiratory failure. She was mechanically ventilated and an emergency caesarean section was performed. Following surgery the patient was haemodynamically stable and was admitted to the intensive care unit. Physical findings included distension of neck veins, rapid heart sounds, an S3 gallop, a grade 2/6 blowing apical systolic murmur radiating to the axilla, bilateral pulmonary rales, and bilateral pitting oedema of the lower limbs. Laboratory tests including a complete blood count, chemistry profile, coagulation tests and D-Dimers level, erythrocyte sedimentation rate (ESR), thyroid function tests and urinalysis were all in the normal range. The electrocardiogram was interpreted as normal sinus rhythm with no signs of acute ischaemia. The chest X-ray revealed an enlarged cardiac silhouette and pulmonary congestion (*figure 1*). Haemodynamic characteristics are shown in *table 1*.

Once stabilised, the patient was transferred to the department of internal medicine for further evaluation and treatment. Echocardiography revealed an enlarged left ventricular end-diastolic diameter (LVEDD) of 59 mm (normal 46 mm +/- 4), left ventricular end-systolic diameter (LVESD) of 44 mm (normal 30 mm +/- 4), and poor global contraction with a shortening fraction (SF) of 26% (normal 34 to 44%). No valvular abnormalities were seen and regional systolic dysfunction was not detected. A ventilation perfusion scan, duplex imaging of the lower limbs and fundoscopy were interpreted as normal. Conventional treatment for heart failure with sodium restriction, digoxin, diuretics, and vasodilator agents (angiotensin-converting

Table 1
Measured haemodynamic characteristics

	MEASURED VALUE	NORMAL MEAN VALUE REFERENCE (NORMAL RANGE IN BRACKETS)*
Mean pulmonary capillary wedge pressure	30 mmHg	9 mmHg (4-12 mmHg)
Mean pulmonary artery pressure	37 mmHg	9-18 mmHg (systolic 15-30 mmHg, diastolic 4-12 mmHg)
Cardiac index	2.4 L/min/m^2	2.8-4.2 L/min/m^2
Systemic vascular resistance	790 dyne/sec^{-1}/cm^{-5}	1100 dyne/sec^{-1}/cm^{-5} (700-1600 dyne/sec^{-1}/cm^{-5})
Pulmonary vascular resistance	176 dyne/sec^{-1}/cm^{-5}	70 dyne/sec^{-1}/cm^{-5} (20-130 dyne/sec^{-1}/cm^{-5})

** Normal value references are based on: Armstrong WF, Feigenbaum H. Echocardiography. In: Braunwald E, Zipes DP, Libby P, eds. Heart Disease: a Textbook of Cardiovascular Medicine. 6th edn. Philadelphia, Pennsylvania: WB Saunders Company, 2001:160-236.*[13]

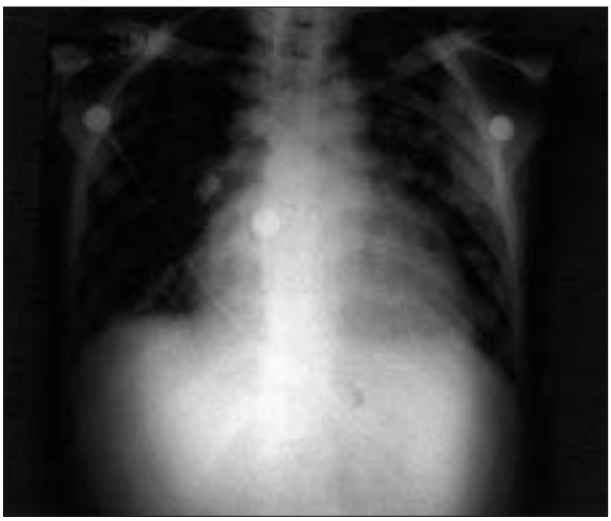

Figure 1
Chest X-ray on admission demonstrating an enlarged cardiac silhouette and pulmonary congestion

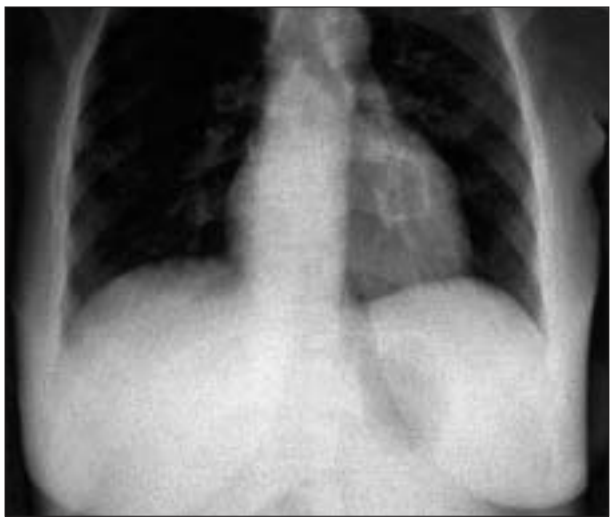

Figure 2
Chest X-ray after three months demonstrating resolution of cardiac enlargement after treatment

enzyme inhibitor) was initiated, with a dramatic improvement clinically, from NYHA functional class 4 to NYHA functional class 2 at the time of discharge. At a follow-up visit three months later, the patient was asymptomatic, with a normal chest X-ray (*figure 2*) and normalisation of the former echocardiography abnormalities (LVEDD 50 mm, SF 42%).

DISCUSSION

A number of disorders may cause heart failure in gravid women with no underlying heart disease. The coincidence of pregnancy and heart failure raises the possibility of high output heart failure, yet echocardiography findings of poor left ventricular contractility minimised the probability of this diagnosis. Myocarditis, often responsible for heart failure in pregnancy, is a possible aetiology, and several studies have demonstrated its occurrence in >50% of women with PPCM who underwent endomyocardial biopsy.[1] However, in the presented case myocarditis was considered unlikely due to the absence of a recent febrile illness history, the normal ESR and creatine phosphokinase values, and the negative serology for autoimmune markers and for infectious agents (viral and bacterial). However, in the current case endomyocardial biopsy was not performed, making definitive exclusion of myocarditis impossible. Given the clinical findings and the exclusion of other potential diagnoses, PPCM seemed the most probable diagnosis.
In 1971, Demakis *et al.*[2] established the diagnostic criteria for peripartum cardiomyopathy which include:
1. Development of heart failure in the last month of pregnancy or within five months of delivery;
2. The absence of another identifiable cause for heart failure;
3. The absence of a recognisable heart disease prior to the final month of pregnancy.
Subsequently, a fourth echocardiography criterion was added: left ventricular dysfunction, as manifested by depressed shortening fraction or ejection fraction.[3]
The true incidence of PPCM is unknown. In the USA it is estimated that PPCM affects 1000 to 1300 women per year.[3] Multiparity, advanced maternal age, multifoetal pregnancy,

toxaemia, and Afro-American descent have been identified as risk factors for PPCM. While the underlying pathophysiological process has yet to be elucidated, theories attempting to explain the pathogenesis of PPCM include abnormalities in the serum level of relaxin, deficiency of selenium, the presence of stress-induced proinflammatory cytokines, an abnormal immune response with high titres of autoantibodies reacting against cardiac tissue proteins,[3] and underlying myocarditis.[1,4] To date, the aetiology of this rare cardiomyopathy is unknown. In contrast to heart failure in gravid women with an underlying heart disease, patients with PPCM present toward the end of gestation or after delivery. Common symptoms include chest pain, dyspnoea, orthopnoea and cough. Echocardiography assessment provides the ultimate diagnosis, and the management of PPCM is based on conventional therapy for heart failure, including oxygen supplementation, sodium restriction, diuretics, digitalis and vasodilator agents. Angiotensin-converting enzyme inhibitors (ACE inhibitors), vasodilator agents commonly used in the treatment of heart failure, are absolutely contraindicated during pregnancy because of the potential of prenatal and postnatal developmental disorders. These disorders include oligohydramnios, intrauterine growth retardation, neonatal renal failure, congenital structural defects (i.e. skull, skeleton, lungs), and early postnatal death.[5] Given the lack of data regarding the use of β-blocking agents in PPCM, these drugs should be considered second-line drugs, preferentially for use after delivery.[3] The risk for thromboembolic events has been reported in PPCM patients. As this complication might occur in as many as 50% of patients,[6] and in particular in those with severely depressed left ventricular ejection fraction,[3] the consideration of anticoagulation treatment in adjunct to standard heart failure management is recommended. Endomyocardial biopsy is recommended only when such therapy fails to yield improvement.[3] For women who fail maximal medical management, the remaining option is cardiac transplantation, with a 60% five-year survival rate.[7] In contrast to earlier data estimating mortality rates ranging from 25 to 50%,[8] Felker *et al.*[9] reported a five year-survival rate of 94%. Despite these encouraging statistics, there remains a small subset of women whose disease follows a rapid and irreversible course with death resulting from an arrhythmia, thromboembolic complications, and ultimately pump failure occurring within three months of diagnosis.

Although there is no consensus regarding the risk of relapse in future pregnancies, cardiac function is ultimately predictive of the patient's prognosis. The persistence of cardiac dysfunction beyond six months, seen in an estimated 50% of cases, usually indicates an irreversible disease process. Elkayam *et al.*[10] found that 21% of the women who regained normal ventricular function suffered heart failure during subsequent pregnancies reinforcing earlier findings of Lampert *et al.*[11] Hence, an event-free future pregnancy, even in women with recovered cardiac function, cannot be guaranteed. Decision-making is perhaps more clear-cut for the group of women suffering persistent heart failure. With a mortality rate of 8 to 17%,[12] subsequent pregnancies in this group should be discouraged.

In conclusion, we present a patient who was diagnosed with peripartum cardiomyopathy, primarily by exclusion of other possible diagnoses. PPCM is a clearly defined entity of a yet unknown aetiology and a potentially lethal complication of pregnancy. Clinician familiarity increases the probability of prompt and appropriate treatment, offering patients the best possible prognosis.

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CASE REPORT

Hyperthyroidism as a cause of persistent vomiting

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ABSTRACT

A 32-year-old woman presented with persistent vomiting, epigastric pain and weight loss. A sinus tachycardia was the clue to the diagnosis of hyperthyroidism due to Graves' disease. On treatment with propylthiouracil and a β-blocking agent, her symptoms resolved within one day, even though her free thyroxine level was still high. Hyperthyroidism is an uncommon, but previously reported cause of persistent vomiting.

INTRODUCTION

Thyrotoxicosis is defined as the clinical syndrome that occurs when tissues are exposed to excess amounts of thyroid hormone. Causes of hyperthyroidism are autoimmune thyroid disease, toxic adenoma or toxic multinodular goitre, thyroiditis or overzealous exogenous thyroid hormone intake. In 1835, Graves published his account of 'violent and long-continued palpitations in females in each of which the same peculiarity presented itself, viz., enlargement of the thyroid gland'.[1] Graves' disease is the most common cause of hyperthyroidism; it occurs in up to 2% of women and is ten times less frequent in men. The disorder typically manifests between 20 and 50 years of age, although it also occurs in other age groups.[2] It is an autoimmune disorder resulting from thyrotropin (TSH)-receptor antibodies, which stimulate thyroid gland growth and thyroid hormone synthesis and release. The classical symptoms of thyrotoxicosis are dyspnoea on exertion, palpitations, tiredness, preference for cold, nervousness, excessive sweating and weight loss. However, these are nonspecific symptoms. For example, dyspnoea on exertion was found in 81% of patients with hyperthyroidism and in 40% of controls. Palpitations were found in 76% of patients and in 26% of controls in an older British study.[3] Specific signs of Graves' disease are ophthalmopathy (clinically obvious in approximately 25% of patients) and pretibial myxoedema, seen in 5% of cases of Graves' disease. Besides the classical, nonspecific symptoms, there are other ways in which thyrotoxicosis can present. In 1976, Rosenthal reported that vomiting can be an important presenting symptom of thyrotoxicosis.[4] Nevertheless, it is not a well-known phenomenon, as the following case demonstrates.

CASE REPORT

A 32-year-old woman was referred to the outpatient clinic because of persistent vomiting and epigastric pain. Her symptoms had started ten days earlier following a three-day course of prednisone given because of asthmatic symptoms. Metoclopramide, ranitidine, cisapride and domperidome prescribed by her general practitioner had given no relief. She was also taking an oral contraceptive, salbutamol and budenoside by inhaler, and paroxetine, which was started five months earlier because of a mild depression. Recently she had taken two courses of antibiotics for a suspected respiratory tract infection.

Physical examination showed a moderately ill woman who weighed 66.5 kg. Her blood pressure was 150/90 mmHg with a pulse rate of 96 beats/min. There was no orthostatic hypotension. There were white patches on the palate,

suspected to be thrush. Examination of the abdomen showed no abnormalities. Laboratory evaluation showed no electrolyte disorders and no signs of dehydration. She refused endoscopy of the upper gastrointestinal tract. Our hypothesis was that she was suffering from *Candida* oesophagitis following antibiotic treatment combined with corticosteroids or from duodenal ulcer or gastritis due to corticosteroid treatment. She was started on famotidine and fluconazol. *Helicobacter* serology turned out to be negative, as did a throat culture on yeast.

Three days later she reported dark stools. She was admitted to hospital for observation. She did indeed vomit after eating or drinking. There were no signs of gastrointestinal bleeding, i.e. no melaena or haematemesis. Again she refused endoscopic evaluation. Haemoglobin and calcium levels were normal. The only biochemical abnormality was a mild elevation of the aminotransferases. Pregnancy and hepatitis A and B were ruled out. Ultrasound examination of the abdomen showed no abnormalities. She was discharged still complaining of vomiting and epigastric pain.

Six days later she came to the emergency department with the same symptoms. She reported a weight loss of 7 kg. There was no diarrhoea. Her blood pressure and temperature were normal but she had a sinus tachycardia of 134 beats/min. At laboratory evaluation there were once again no signs of dehydration. The aminotransferase levels had normalised. An attempt was made to perform a radiographic examination of her stomach, but this proved to be impossible due to vomiting immediately after swallowing barium contrast.

Four days later she was seen at the outpatient clinic. She was carrying a bucket because of the continuous vomiting. She told us that she was eating hardly anything but was able to drink fluids. She denied excessive use of salbutamol. Again, there were no abnormalities on physical examination, except her pulse rate which was 100 beats/min. Because of the tachycardia and the weight loss we thought of hyperthyroidism. The thyroid gland was not enlarged and there was no exophthalmos. The thyroid stimulating hormone (TSH) level turned out to be < 0.01 mE/l, and her free thyroxine level was >75 pmol/l (normal 8-23 pmol/l). Also, the serum aminotransferase levels were increased to five times normal. She was admitted under the diagnosis of thyrotoxicosis and treated with a β-blocking agent (metoprolol 4 dd 100 mg) and propylthiouracil (3 dd 75 mg). Within 24 hours she was free of symptoms after 23 days of continuous vomiting. The free thyroxine level was still 50.7 pmol/l after five days of treatment. The aminotransferase levels normalised within a week. Scintigraphic imaging of the thyroid gland was compatible with the diagnosis of Graves' disease. Six months later she was doing well on thiamazole and levothyroxine without complaining of vomiting or epigastric pain again.

DISCUSSION

The persistent vomiting and epigastric pain of the patient described here were most likely caused by thyrotoxicosis. We cannot rule out other causes with certainty because of the lack of endoscopic and/or radiographic diagnostic imaging. However, she did not respond to treatment with antacids or prokinetic agents while all symptoms resolved completely after treatment of the thyrotoxicosis.

Vomiting is not a well-known symptom of hyperthyroidism. In *Harrison's Textbook of Internal Medicine* vomiting is not mentioned as a symptom of thyrotoxicosis (*table 1*).[2]

Table 1
Signs and symptoms of thyrotoxicosis (in descending order of frequency)[2]

Symptoms
Hyperactivity, irritability, dysphoria
Heat intolerance and sweating
Palpitations
Fatigue and weakness
Weight loss with increased appetite
Diarrhoea
Polyuria
Oligomenorrhoea, loss of libido
Signs*
Tachycardia; atrial fibrillation in the elderly
Tremor
Goitre
Warm, moist skin
Muscle weakness, proximal myopathy
Lid retraction or lag
Gynaecomastia

**Excludes the signs of ophthalmopathy and dermopathy specific for Graves' disease.*

Nevertheless, Rosenthal *et al.* reported on seven cases in 1976.[4] After that, several case reports have been published describing persistent vomiting due to hyperthyroidism. In a number of the reported cases Graves' disease was the cause of excess thyroid hormone production; however in some cases the cause of the hyperthyroidism was not specified (*table 2*).[5-12] Werner and Ingbar's *The Thyroid* mentions thyrotoxic vomiting when describing gastrointestinal symptoms in hyperthyroidism. Furthermore, it is stated that clinical and experimental data on the effect of thyrotoxicosis on gut motility provide some evidence that thyroid excess affects the orderly propulsion

Table 2
Case reports of thyrotoxic vomiting

AUTHOR	SEX	AGE	MAIN SYMPTOMS	CAUSE OF HYPERTHYROIDISM
Rosenthal *et al.*[4]	F	41	Vomiting, abdominal pain	Graves' disease
	F	35	Vomiting	Graves' disease
	F	49	Vomiting	Graves' disease
	F	66	Vomiting	Nodular thyroid disease
	F	57	Vomiting	Graves' disease
	F	66	Vomiting	Graves' disease
	M	54	Vomiting	Not specified
Dreyfuss[5]	F	53	Epigastric pain, vomiting	Graves' disease
Desai *et al.*[6]	M	54	Vomiting	Graves' disease
Muller *et al.*[8]	M	12	Vomiting	Not specified
Canslar *et al.*[10]	F	35	Vomiting, abdominal distention	Not specified
Chen *et al.*[11]	M	40	Vomiting	Graves' disease
Parkin *et al.*[12]	M	43	Vomiting, weight loss	Graves' disease
Hoogendoorn *et al.*	F	32	Vomiting	Graves' disease

of ingested materials through the gastrointestinal tract.[13] Harper carried out a retrospective chart review of 25 patients hospitalised for thyrotoxicosis and found that 44% reported vomiting and 20% complained of abdominal pain.[14] Of notion is that one or more of these abdominal symptoms were included as a chief complaint in 36% of cases reviewed.

The mechanism that causes vomiting in thyrotoxicosis is not clear. Suggestions are direct action of excess thyroid hormone on gastrointestinal motility or thyroid hormone stimulation of a chemoreceptor trigger zone in the central nervous system.[13] Of interest is the relationship with hyperemesis gravidarum, a syndrome of nausea and vomiting associated with weight loss of 5% or more during early pregnancy that occurs in 0.1 to 0.2% of pregnancies. Elevated serum FT4 and T3 concentrations are a common finding in women with hyperemesis gravidarum. The placenta secretes hCG, a glycoprotein hormone sharing a common alpha subunit with TSH but having a unique beta subunit, which confers specificity. It is known that hCG has thyroid-stimulating activity.[15] Women who develop hyperemesis gravidarum have higher serum hCG and oestradiol concentrations than do normal pregnant women. The hCG of women with hyperemesis seems to have even more thyroid-stimulating activity because more of it is desialyated.[16] Therefore, serum TSH concentrations are more often low in women with hyperemesis than in normal pregnant women[17]. A few of these women have high serum free T4 concentrations. The elevated FT4 could contribute to the vomiting of hyperemesis gravidarum in those women.

Another possible mechanism behind thyrotoxic vomiting, besides direct action of thyroid hormone on gastric motility or on the chemoreceptor trigger zone, could be the increase in β-adrenergic activity in hyperthyroidism due to an increased number of β-adrenergic receptors.[18] The increase in β-adrenergic activity is responsible for many of the other symptoms associated with hyperthyroidism. It also explains the ability of β-blockers to cause a rapid improvement in many of the symptoms, including palpitations, tachycardia, tremulousness, anxiety, and heat intolerance. In support of the theory that vomiting is caused by β-adrenergic stimulation is the observation Dreyfuss made in a 53-year-old woman who suffered from epigastric pain and vomiting due to hyperthyroidism.[5] He noted that after 36 hours of treatment with propanolol and propylthiouracil, the symptoms had resolved completely, while the thyroxine level was still high. This was also seen in our patient.

Rosenthal *et al.* reported on seven patients with thyrotoxic vomiting.[4] Four of the seven patients showed elevated liver enzymes values. This was also the case in the patient described here. It is not clear by which mechanism this is caused. Possible thyroid-liver interactions include liver damage secondary to the systemic effects of thyroid excess or direct toxic effects of thyroid hormone on the liver.[13] The abnormal values were reversed to normal within a week of starting treatment.

CONCLUSION

Persistent vomiting and epigastric pain can be symptoms of thyrotoxicosis. The symptoms resolve quickly and completely with treatment with β-blocking agents and antithyroid drugs.

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Blood pressure measurement: we should all do it better![1]

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'Because blood pressure (BP) measurement is a simple procedure, it is taken for granted that all graduates from medical training programmes have the ability to record accurate, precise and reliable BP readings. However, research since the 1960s has shown this assumption to be false. Most health professionals do not measure BP in a manner known to be accurate and reliable. If you doubt this statement watch as BPs are taken in your own clinical setting to determine whether the guidelines discussed herein are followed and then examine recorded readings for signs of observer bias.' This citation is taken from a chapter by Carlene and Clarence Grim, both very experienced BP researchers and teachers, in a recent book.[2] Earlier, these authors published a curriculum for the training and certification of BP measurements for the healthcare providers.[3]

BP measurement is nowadays recognised as probably the most commonly performed clinical procedure. Nurses, physicians, medical students and even patients measure BP routinely. BP can be measured directly (intra-arterially) or indirectly. The first method represents the 'gold standard' for BP measurements but is invasive, requiring arterial cannulation and is therefore only used in particular (research) circumstances. The indirect method is widely used in both daily practice and research.

Many BP measuring devices have been developed in the last decades. However, measurement of BP using a mercury sphygmomanometer and a stethoscope according to the Korotkoff's auscultatory principle remains the cheapest and most accurate (when compared with intra-arterial readings), and is considered the noninvasive gold standard, providing that the measurement is performed correctly. The tendency to ban the use of mercury, a toxic substance, in clinical practice is leading to mercury sphygmomanometers being replaced by alternative instruments. Most of these devices are based on the oscillometric principle. However, only a limited number of them have already been validated. Several factors affect indirect BP readings.

FACTORS RELATED TO PATIENT

Some of the factors affecting the BP are related to the person in whom the BP is being measured, commonly referred in the literature as biological variation (*table 1*).

Rest period

It has been recommended that the BP should be measured after several minutes rest to allow the BP to stabilise.[4-7] However it is not exactly known how long the rest period should be. Average drops in the systolic BP (SBP) of 9 and 14 mmHg, respectively, have been reported after a rest period of four and eight minutes prior to the BP measurement. The decrease was less evident in the diastolic BP (DBP), amounting to 3 and 4 mmHg, respectively, for the same rest intervals.[8] These results are consistent with the results of other studies that also report a decrease of similar magnitude within the first five to ten minutes of rest.[9-13] A longer rest period of more than 25 minutes was found to further slightly decrease the BP values, especially the SBP, but the question remains whether such a long rest period is feasible in general practice.[9,13] On the other hand, clinical experience shows that in a few patients the BP increases if they have to wait to have their BP measured. These patients can be traced by measuring the BP both immediately after assuming supine or sitting position and after the rest period. Based on this data, it has been recommended that at least five minutes of rest should be allowed before the measurement of BP.[14]

Table 1
Factors that affect indirect blood pressure readings with a mercury sphygmomanometer and a stethoscope

FACTORS THAT INFLUENCE INDIRECT BLOOD PRESSURE READINGS
Related to the patient
Resting time
Diurnal variation
Seasonal variation (changing in temperature)
'White coat' effect
Pain, anxiety
Acute smoking or ingestion of alcohol or caffeine
Postprandial state
Distended bladder
Talking
Degree and type of activity
Between arm differences
Paretic arms
Special factors (arrhythmia, 'silent gap', soft Korotkoff sounds)
Related to the observer
Training
Terminal digit preference
Expectation bias
Impaired hearing
Related to the instruments and technique
Instrument accuracy (manufacture errors, maintenance, defects)
Noisy room
Cuff length and width
Tight, thick clothes under the cuff
Body posture (sitting, supine, standing)
Arm level with respect to the right atrium
Arm/wrist/finger cuff
Arm, back and feet support
Inflation and deflation rate
Parallax error

Daily variability *per se*
It has been proven in the literature that there is a substantial diurnal BP variation with a clear fall in BP during the night of up to 15%, as a result of both sleep and inactivity, reflecting the decrease in sympathetic tone.[15-17]
Various daytime activities induce increases in BP of different magnitude. Activities accompanied by a large increase in BP of between 10 and 20 mmHg include meetings, physical work, transportation, walking and dressing. Activities accompanied by increases in BP of up to 5 mmHg include deskwork, reading and watching television. Talking results in approximately a 7 mmHg increase in BP and should thus be avoided during BP measurement.[15] Even reduced muscular activity such as inflating one's own cuff during self-recording of BP produces an increase of approximately 12 mmHg in SBP. The BP returns to its initial level within on average seven seconds but this can take up to 21 seconds. Thus the SBP may be overestimated during self-measurement if the subject does not inflate the cuff to a high enough pressure or deflates it to quickly.[18]

Seasonal variability
A seasonal variation of the BP has also been suggested in the literature, showing on average 3 to 8 mmHg higher BP values during the winter than during the summer, even in patients living in a stable environmental temperature.[19-25] These differences seem to be inversely associated with the body mass index, possibly due to the increased thermoregulatory requirements of leaner individuals.[25] Extrapolating these observations to clinical practice, hypertensive patients may require a lower dose of antihypertensive medication during periods of fever or if they move to (sub)tropic countries (holidays, business).

Office *vs* home BP measurement
Some patients have higher BP levels when taken in the physicians' office than at home. Also the BP can be higher when measured by a physician than by a nurse or a medical student. This phenomenon is known as the 'white coat' effect. When this phenomenon is suspected, nurses rather than physicians should measure the BP and 24-hour ambulatory BP monitoring (ABPM) could be performed,[26-28] or self-measurement at home.

Various sympathetic stimulators
Pain and anxiety also acutely increase the BP, probably due to increased sympathetic activity.[29,30] The procedure should therefore be explained adequately beforehand, especially in nervous patients. Patients should also been told that there may be some minor discomfort caused by the inflation of the cuff. A distended bladder is also reported to increase the BP,[31,32] thus patients should be advised to empty their bladder before the BP measurement.

Various stimulants
Smoking the first cigarette of the day may acutely induce a rise in BP that lasts for 15 to 30 minutes, which is likely due to the acute release of norepinephrine.[33] On the other hand, chronic smoking induces tolerance.[34]
Ingestion of caffeine-containing beverages may induce an acute rise in BP; however, also here a certain degree of tolerance may occur with repeated consumption and it is also dependent on individual plasma half-life of caffeine.[35-39] Other ingredients in coffee apart from caffeine may also be responsible for the cardiovascular activation.[35-38] Eating as an activity increases BP by 8 to 9 mmHg;[15] however a postprandial decrease in BP can also be noted, especially in elderly patients.[40-43]

Ingestion of alcohol can also acutely increase BP.[44] Thus smoking, eating, consuming of alcohol or caffeine-containing beverages and chocolate should be avoided for at least an hour before the measurement of BP. Otherwise, a note should be made that this is a possible confounder.

Anatomy

There has been much controversy in the literature as to whether there is a difference between the BP readings in the two arms. Some authors recommend that BP should initially be measured in both arms and if, after at least three readings significant systematic difference (>10 mmHg) is found, the BP should routinely be measured in the arm with the highest value.[45-49] A special remark should be made with respect to the BP measurement in hemiplegic patients. In one study, the BP was higher in the paretic arms of patients with a spastic stroke and lower in the affected arm if the tone was flaccid.[50]

Other factors

The BP measurement can be particularly difficult in patients with arrhythmias, especially atrial fibrillation, soft Korotkoff sounds or a 'silent gap'. These factors must be taken into account especially when the BP is measured using electronic devices as many of these instruments are not validated for use in such cases.[51-53]

FACTORS RELATED TO OBSERVER

The person who is measuring the BP (the observer) requires meticulous and repeated theoretical and practical training and validation of his/her ability to measure the BP accurately (*table 1*).[51,54-57] However, even when the technique of measurement is correct, a number of factors can cause a bias in the BP reading. Observers may interpret the Korotkoff sounds differently. Also, observers very often have a terminal digit preference, which in the majority of cases is 0 (75%) or 5 (25%). Moreover, observers may be influenced by the knowledge of previous BP values during serial readings (expectation bias). They may also tend to read higher or lower values at threshold levels.[54-63] At least older (above 55 years) physicians and nurses should have their hearing tested regularly if they routinely measure BP using a stethoscope.

FACTORS RELATED TO INSTRUMENTS AND TECHNIQUE

Instrument

The measuring device used and the measurement technique can induce large variations in BP.[54,64] There is a wide range of BP measuring devices on the market but unfortunately only a few of these devices have been validated according to official standards.[65] With the traditional mercury sphygmomanometers the regular control concerned three points: 1) adequate filling of the mercury reservoir; 2) replacement of the glazed tube in case of mercury precipitation; 3) replacement of the rubber connections in case of leak. But now the banning of mercury has been accepted worldwide, aneroid devices are usually chosen as substitutes, and those devices should regularly be checked against mercury (or an adequate substitute).[66,67] There is no clear evidence about what the interval should be between the check-ups, since the interval is dependent on the situation. For example, instruments used by midwifes need to be checked every six months, whereas devices used in hospitals could be initially checked after two years and then yearly. In general, the maintenance of mercury sphygmomanometers or alternatives available in many hospitals is not optimal.

When the newer devices, mostly based on the oscillometric principle, are used, it should be realised that these devices measure the mean arterial pressure and calculate the systolic and diastolic BP, based on an (industrially secret) algorithm which may vary in different devices or in newer types. The significance of the principle or algorithm is clearly demonstrated in diabetic patients. In a group of normoalbuminuric type I diabetes patients the BP measured with an oscillometric device (Dinamap) was overestimated in comparison with the same BP measured using a mercury sphygmomanometer.[68]

Environment

The measurements should be performed in a quiet environment, as noisy rooms make it difficult for the patient to relax and the observer to concentrate and adequately hear Korotkoff sounds. Room temperature should be not too high or too low either.

Bladder size

The inflatable bladder size also requires attention. The length of the bladder should be enough to encircle at least 75 to 80% of the arm and the width of the bladder should be equal to about two-thirds the distance from the axilla to the antecubital space. The BP may be overestimated by using too narrow or too short bladders. The former standard bladder was 12 x 26 cm, making it possible to measure BP in subjects with an arm circumference up to 31 cm. Bladders of 13 x 36 cm were shown to be adequate for patients with arm circumferences up to 48 cm. The use of large cuffs in lean patients has yet to be clarified.[69,70] The cuff should be placed on the bared arm, tight or thick clothes under the cuff should be avoided.

Position of body and arm

Other factors of clinical relevance for the BP measurement are the position of the patient during blood pressure measurement and the arm level with respect to the reference level of the right atrium. Most recent official guidelines for the BP measurement[5,7,71] recommend that BP should be routinely measured with the patient in the sitting position with the arm supported at the level of the right atrium. To detect orthostatic hypotension the BP should be measured in the supine position and then in the upright position but again, the arm in which the BP is measured should be placed at the level of the right atrium in all positions. The level of the fourth intercostal space[72] or the midsternum have been proposed as practical approximation of the right atrium level in the sitting and standing positions.[73] It has previously been shown that supporting the arm of the patient below the right atrium results in an overestimation of BP of approximately 0.7 mmHg for each cm deviation from the right atrium level.[74-87] Such errors can occur, for instance, in a patient who is standing with his arm hanging parallel to the body (along the side) or in a sitting patient whose arm is supported by the arm rest of the chair or by a regular office desk. Vice versa, placing the patient's arm above the level of the right atrium results in lower BP readings by the same order of magnitude as mentioned above.[81,82] Furthermore, the assumption that the BP readings taken in the sitting and supine positions are equivalent proved to be inadequate.[74,88-93] Especially when the patient's arm is carefully placed at the correct right atrium level, a significantly higher BP (about 9 mmHg for the SBP and about 5 mmHg for the DBP) was found in the supine than in the sitting position.[74,93]

The arms, the back and feet of the patients should be supported to avoid any isometric physical exercise that might increase the BP.

OTHER FACTORS

The bladder should be rapidly inflated to avoid prolonged discomfort for the patient, but slowly deflated at a rate of 2 to 3 mmHg per beat or per second, to accurately record the BP to the nearest 2 mmHg. Failure to do this may result in either too high or too low BP readings.[94] On the other hand, deflation can be speeded up in second and third readings, especially when there is an increase in pulse pressure (e.g. 224/62 mmHg) since otherwise the procedure may become too painful and pain may increase the BP further.

The centre of the sphygmomanometer scale should be placed at the same level as the eyes of the observer to avoid the parallax effect. According to this effect, higher BP will be read when the observer is watching from below the scale and vice versa, lower BP will be read when watching from above the scale.[57]

In the last decades, the attention of clinicians and researchers has been very much focused on the development of new BP measuring techniques, with complicated expensive devices operating on various principles. Measuring the BP according to the 100-year-old Korotkoff's auscultatory principle, with a mercury sphygmomanometer and a stethoscope, is sometimes regarded as obsolete. However, when performed in a correct and standardised manner, this technique provides us with one of the best predictors of the patient's cardiovascular status and future events. We subscribe to the point of view of Messerli *et al.*[1] that we should respect and treasure this simple clinical tool. More efforts should be made to standardise the procedure, to implement this standard in practice, to intensively train all medical professionals in correctly measuring the BP and raise awareness of its possible pitfalls.

CONCLUSIONS

BP measurement can be learned by every doctor, nurse, technician and vascular trainee. But one of them, usually the doctor, should be well informed about all the pitfalls, shortcomings, algorithms, and about the validation status of the devices. The doctor should also organise the maintenance procedures of the devices in his/her department. Every co-worker should be controlled at regularly intervals and every newcomer should be trained to become a certificated BP observer.

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A case of multiple aortic thrombi

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CASE REPORT

A 50-year-old woman was admitted to our hospital with a ten-day history of fever, vomiting and dehydration. Her medical history revealed hypertension, hypercholesterolaemia, pyelonephritis and renal insufficiency for which peritoneal dialysis had been started seven months earlier. On physical examination no further clues as to the cause of her illness could be found. Chest X-ray showed no abnormalities. After blood cultures were taken antibiotic treatment was started with cefuroxim and gentamicin. Because of progressive jaundice abdominal ultrasonography was performed, which showed no abnormalities. A CT scan of the abdomen showed a large thrombus in the right ventricle (*figure 1*), which was confirmed by echocardiography. Also two sites of thrombi were found in the aorta: at the height of the renal arteries and just above the aortic bifurcation (*figure 2*). The last thrombus appeared to contain gas.

WHAT IS YOUR DIFFERENTIAL DIAGNOSIS?

See page 306 for the answer to this photo quiz.

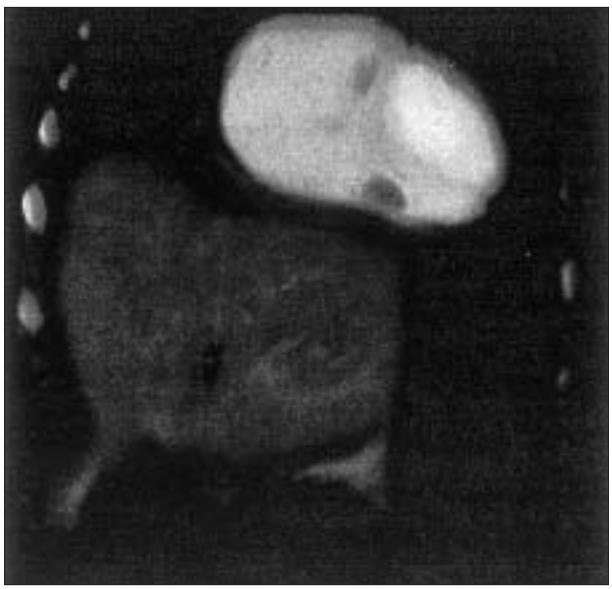

Figure 1
Abnormality in the right ventricular cavity suggestive of thrombus, which was later also confirmed by echocardiography

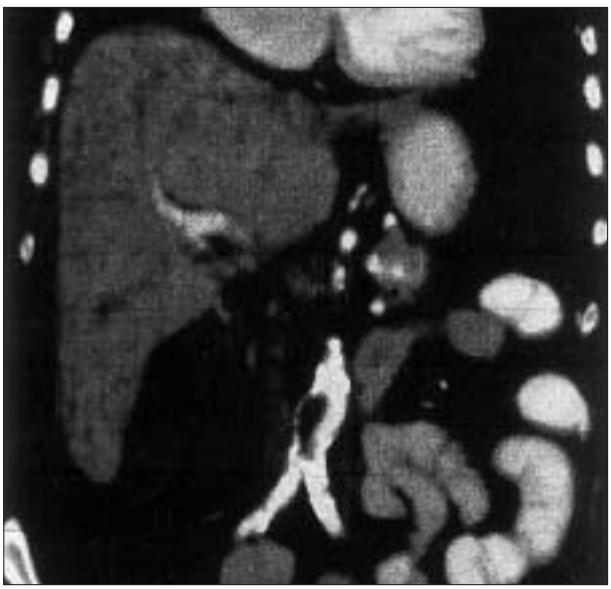

Figure 2
Gas-containing thrombus, localised at the aortic bifurcation

A colour version of these figures is available on www.njmonline.nl

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BOOK REVIEW

Emerging Infections 5

Edited by W. Michael Scheld, William A. Craig, James M. Hughes. 242 pp, illustrated. ASM Press, Washington DC, USA, 2001. $ 84.95. ASM members $ 69.95. ISBN 1-55581-216-3

A.M.L. Oude Lashof

As is stated in the preface, this fifth volume in the series of *Emerging Infections* is based primarily on presentations given the 2000 Interscience Conference on Antimicrobial Agents and Chemotherapy in Toronto, Canada. The 14 chapters contain various topics ranging from short descriptions of outbreaks to extensive reviews. Some parts focus on clinical aspects, others are mainly microbiologically oriented.

The first two chapters discuss emerging viruses. The 1998 Taiwanese Enterovirus 71 epidemic describes the clinical picture (hand-foot-and-mouth disease, encephalitis and pulmonary manifestations) and molecular epidemiology. The 1999 West Nile Virus outbreak in New York City is highlighted from a public health point of view. The authors remind the reader that the presence of dead crows may be the first sign of an outbreak; this might also occur in Europe.

The reviews on Q-fever and *Mycoplasma pneumoniae* are excellent. Especially informative are the sections on the chronic form of Q-fever and the extrapulmonary complications of *M. pneumoniae* infections. The parts on *Staphylococcus aureus* and *Clostridium difficile* show that well-known pathogens are able to transfer into more pathogenic species. For example, the emergence of community-acquired oxacillin-resistant *S. aureus* (ORSA) is a problem in the USA. One chapter deals with infections in patients on haemodialysis due to the close contact of water with the patient's bloodstream. The effects of contact with products of microorganisms (such as endotoxins and microcystins) leading to acute disease and mortality are probably underestimated in daily practice.

An interesting chapter is the one on Buruli ulcer disease caused by *Mycobacterium ulcerans*, the third most common mycobacterial disease among immunocompetent people in the tropical world. A great deal of research remains to be done to understand this destructive disease. It is a well written and balanced chapter dealing with the clinical features and microbiological background.

Lyme disease-like illnesses and non-Lyme disease erythema migrans have recently been discovered and may be caused by other *Borrelia* species than *B. burgdorferi*. Vaccination is only mentioned in the discussion and it would have been preferable to describe this elsewhere in the chapter. The section on babesiosis, another tick-borne disease, teaches us that the expanding population with animal contact will result in more zoonoses.

Accurate laboratory-based tests to identify *Entamoebe histolytica* from the nonpathogenic *E. dispar* have improved the diagnosis of amebiasis. Due to changing travel behaviour amebiasis may well be an emerging infection. It would have been appropriate to mention the treatment strategies for this disease.

The exciting chapters on bioterrorism, a hot topic and a real threat according to the American authors, describe the clinical symptoms of anthrax and plague that may be of use to recognise an attack in an early stage. The list of possible agents that could be used as a weapon is also illustrative.

Emerging Infections 5 is easy to read, discussing a large number of different pathogens and illnesses occurring in the developing and developed world. It is interesting for clinicians who want to broaden their knowledge on emerging infections and microbiological techniques. And the clinical parts and public health subjects will be of use for microbiologists to get a picture of what is currently happening in the field of infectious diseases.

I am looking forward to reading *Emerging Infections 6*.

ANSWER TO PHOTO QUIZ (ON PAGE 304)
A CASE OF MULTIPLE AORTIC THROMBI

Blood cultures grew *Salmonella* serogroup D (not *Salmonella typhi*). *Salmonella* bacteria are capable of producing gas (*figure 2*). This case is therefore an example of septic thrombi caused by *Salmonella* infection. The antibiotic regimen was subsequently changed to amoxicillin and ciprofloxacin intravenously.

Salmonella bacteraemia can result in endovascular infection. *Salmonellae* seem to have a predeliction for diseased vascular walls and mural thrombi.[1,2] In the literature most case reports focus on infected thrombi localised in left ventricular aneurysms. Only 13 cases of patients with infected mural thrombus in left ventricular aneurysms were described between 1966 and 2000.[1] Aneurysmectomy and removal of infected thrombi seemed to give the best chance of survival in these patients.

In our patient the right ventricular thrombus was no longer present on echocardiography ten days later. No signs of pulmonary embolism occurred. A mycotic aneurysm developed at the aortic bifurcation, for which an aortic prosthesis was successfully placed.

DIAGNOSIS

Septic thrombi associated with *Salmonella* infection.

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'Gaper'

Peter Becks

Peter Becks' favourite medium is woodcarving. In his work you will find basal forms such as circles, triangles and squares. He also includes real-life figures as animals, plants and cars. In his own words: 'My art is built up from figures, colours, forms and signs. I build, mix and write these ingredients in a way that results in an associative, emotional and intuitive science.'

Becks (Veghel 1969) graduated at the Academy of Arts in Arnhem in 1993, the same year he was nominated for the 'Javaanse Jongens' graphic award. In 1994 he received the Dutch Graphic Award. His work has been shown in group as well as solo exhibitions as Prent '95 and Prent '97 in Nijmegen, Plaatsmaken in Arnhem in 2003 and 2004 and at a group exhibition in Moers, Germany.

This month's print entitled 'Gaper' is part of a series of 11 prints. These series (30 in edition) are available at a price of € 600 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.

INFORMATION FOR AUTHORS

Aims and scope
The Netherlands Journal of Medicine publishes papers in all relevant fields of internal medicine. In addition to reports of original clinical and experimental studies, reviews on topics of interest or importance, case reports, book reviews and letters to the Editor are welcomed.

Manuscripts
Manuscripts submitted to the Journal should report original research not previously published or being considered for publication elsewhere. Submission of a manuscript to this Journal gives the publisher the right to publish the paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

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Language
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.

Preparation of manuscripts
Type all pages with double spacing and wide margins on one side of the paper. To facilitate the reviewing process number the pages; also we would appreciate seeing the line numbers in the margin (Word: page set-up - margins - layout - line numbers). Divide the manuscript into the following sections: Title page, Abstract, Introduction, Materials and methods, Results, Discussion, Acknowledgements, References, Tables and Figures with Legends.

A *Covering letter* should accompany the manuscript, identifying the person (with the address, telephone and telex numbers, and e-mail address) responsible for negotiations concerning the manuscript: the letter should make it clear that the final manuscript has been seen and approved by all authors. Conflicts of interest, any commercial affiliations, consultations, stock or equity interests should be specified. In the letter 1-3 sentences should be dedicated to what this study adds. All authors should sign the letter.

The *Title page* should include authors' names, degrees, academic addresses, address for correspondence including telephone, fax and e-mail, and grant support. Also the contribution of each author should be specified.
The title should be informative and not exceed 90 characters, including spaces. Avoid use of extraneous words such as 'study', 'investigation' as well as priority claims (new, novel, first). Give a running title of less than 50 characters. If data from the manuscript have been presented at a meeting, list the name, date and location of the meeting and reference and previously published abstracts in the bibliography. Give a word count (including references, excluding tables and legends) at the bottom of this page.

Subheadings should not exceed 55 characters, including spaces.

Abbreviations: Measurements should be abbreviated according to SI units. All other abbreviations or acronyms should be defined on the first appearance in the text. Use a capital letter for proprietary names of substances and materials. At first mention of a chemical substance, use the correct chemical designation as well as the generic name.

The *Abstract*, not exceeding 200 words, should be written in a structured manner and with particular care, since this will be the only part of the article studied by some readers. In original articles, the abstract should consist of four paragraphs, labelled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed and which measurements were carried out, the most relevant results, and what the authors conclude from the results.

The *Introduction* should be brief and set out the purposes for which the study has been performed.

The *Materials and methods* should be sufficiently detailed so that readers and reviewers can understand precisely what has been done without studying the references directly. The description may be abbreviated when well-accepted techniques are used.

The *Results* should be presented precisely without discussion.

The *Discussion* should directly relate to the study being reported. Do not include a general review of the topic, but discuss the pertinent literature.

Acknowledgement: All finding sources should be credited here. Also a statement of conflicts of interest should be put here.

References should be numbered consecutively (in square brackets) as they appear in the text. Type the reference list with double spacing on a separate sheet. References should accord with the system used in Uniform requirements for manuscripts submitted to biomedical journals (N Engl J Med 1991;324:424-8).
Examples:

[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.

[2.] Kaplan NM. Clinical Hypertension. 7th Edition. Baltimore: Williams & Wilkins; 1998.

[3.] Powell LW, Isselbacher KJ. Hemochromatosis. In: Harrison's Principles of Internal Medicine, 15th Edition, Braunwald E, Fauci AS, Kasper DL, et al. (eds). New York: McGraw-Hill; 2001. p. 2257-61.

Please note that the first six authors should be listed; when seven or more, list only the first three and add *et al*. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised.

Tables should be typed with double spacing each on a separate sheet, numbered consecutively with Arabic numerals, and should contain only horizontal lines. Provide a short descriptive heading above each table with footnotes and/or explanation underneath.

Figures must be suitable for high-quality reproduction. Submit line drawings made in Word or other computer programmes but not in a PowerPoint file. India ink drawings or sharp, strongly contrasting photographic prints on glossy paper are also acceptable. Lettering should be complete, of professional quality, and of a size appropriate to that of the illustration of drawing, with the necessary reduction in size taken into account. Figures should be no larger than 12.5 x 18 cm. Submit half-tone illustrations as black-and-white prints on glossy paper, with as much contrast as possible. Identify each figure on the back with a typed label, which shows the number of the figure, the name of the leading author, the title of the manuscript and the topside of the figure. Colour figures are occasionally possible and will be charged to the authors.
Legends for figures should be typed, with double spacing, on a separate sheet.

Brief reports
Brief reports containing concise reports on original work will be considered for publication. Case reports which are relevant for understanding the pathophysiology or clinical presentation of disease may also be accepted under this heading. Articles published in this section should be no longer than 1000 words, and be supplied with a summary of about 60 words, preferably no more than two figures and/or tables, and no more than 15 references.

Letters to the editor
Letters to the editor referring to articles previously published in the journal will be considered by the editors; letters should be no more than 500 words and sent both on disk or e-mail and in hard copy.

Submission
Manuscripts should be sent to the Editor in chief, Prof. J.W.M. van der Meer, University Medical Centre St Radboud, Department of General Internal Medicine, PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 04 59, e-mail: g.derksen@aig.umcn.nl. They should be submitted in four complete copies, which include four sets of the figures; authors should retain one copy of the manuscript. Rejected manuscripts will not be returned to the author unless specially requested at the time of submission.

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After external and editorial review of the manuscript, the authors will be informed about acceptance, rejections or revision. Unless stated otherwise in our letter, we require revision within three months.

Acceptance
After acceptance we prefer electronic submission of text and figures, either by e-mail to g.derksen@aig.azn.nl or on floppy disk. A disk plus two final and exactly matching printed versions should be submitted together. It is important that the file saved is in the native format of 'Word' or any other computer programme used. Label the disk with the name of computer programme used, your name, and the name of the file on the disk.

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