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EDITORIAL

Persisting challenges in plasma endocrinology: reference values and endocrine tests

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ABSTRACT

The analysis of plasma hormone concentrations is of fundamental importance for the diagnosis and treatment of endocrine diseases. Although hormone analyses are performed in huge numbers in all hospitals on a daily basis, the interpretation of the resulting plasma hormone concentrations can be difficult. In addition to the effects of the underlying disease, biological and analytical issues affect hormone concentrations. Therefore, adequate reference values and strict standardisation of sampling and analytical procedures are very important for the final interpretation of the results of hormone analysis.

The pretest likelihood of a test profoundly affects the sensitivity and specificity of tests. The pretest likelihood is based on interpretations of the symptoms and physical examination of the patient. Therefore, the experience of the doctor is of paramount importance in establishing an optimal pretest likelihood of the disease. Fortunately, in many cases the diagnosis of the underlying endocrine disorder is straightforward. However, in some patients the interpretation of the results of hormone measurements and endocrine tests may be very difficult. In this respect, it should be realised that many pathophysiological, biological and analytical issues affect hormone concentrations and endocrine tests. Failure to take into account the factors that affect hormone concentrations in addition to the effects of endocrine disorders may lead to great confusion. Therefore, adequate reference values and strict standardisation of sampling and analytical procedures are important for the final interpretation of the results of hormone analyses.

VARIATION IN HORMONE CONCENTRATIONS: PREANALYTICAL FACTORS

Biological variation

Many biological factors influence endogenous hormone secretion in addition to the effects of endocrine disease. These include gender, pulsatile and diurnal variation in hormone secretion, non-endocrine diseases, endocrine effects of non-endocrine drugs, nutritional status and age.

Gender

In addition to the effects of gender on oestrogens and progesterones, there are major effects on other endocrine systems. These include effects on the axis between the growth hormone and insulin-like growth factor-1 (IGF-1), and on leptin.

Pulsatile hormone secretion

Most hormones are secreted in a pulsatile fashion. As a consequence, a considerable variation can be present in hormone concentrations within a single subject. An example of a hormone with a manifold variation in hormone secretion is growth hormone.

Diurnal variation of hormone secretion

Most, if not all, hormones reveal diurnal changes in plasma concentrations. For example, plasma cortisol levels are higher in the early morning, in the afternoon they start to decrease to a nadir around midnight. Reference values for plasma cortisol in healthy subjects will, therefore, depend on clock hours.

Non-endocrine disease

Non-endocrine disease in general has profound effects on all aspects of endocrine regulation. These include changes

in thyroid hormone metabolism in otherwise euthyroid subjects (the so-called euthyroid sick syndrome), in the pituitary-adrenal axis (cortisol levels may increase) and in the pituitary-gonadal axis (decreased testosterone and oestrogen levels). Therefore, in patients with non-endocrine diseases, reference values of plasma hormone concentrations are different from those obtained in healthy subjects. If endocrine diseases are suspected in these subjects, this should be taken into consideration. For instance, the discrimination between the euthyroid sick syndrome and hyperthyroidism/hypothyroidism may sometimes be difficult. In the euthyroid sick syndrome both decreased (free thyroxine) FT_4 levels (in very ill subjects) and increased FT_4 levels (usually during recovery from euthyroid sick syndrome) may be found.[1]

Endocrine effects of non-endocrine drugs
A large number of drugs affect plasma hormone concentrations. In clinical practice it is helpful to consider these effects for every drug unless it has been proven otherwise. These effects of otherwise non-endocrine drugs complicate the interpretation of hormone concentrations. An important example is the diagnostic work-up of pheochromocytoma. These patients are often treated for their hypertension. Many antihypertensive drugs, however, increase plasma catecholamine levels and urinary catecholamine excretion. These drugs may, therefore, result in false-positive tests for pheochromocytoma. A second factor is the analytical interference between a drug and the assay, although in most of the newer methods this factor is of limited importance. Examples are the catecholamine measurement in the presence of methyldopa, sotalol and phenoxybenzamine.

Nutritional status
This is an important modulator of endocrine regulation. Both low (e.g. anorexia nervosa) and high body mass index profoundly affect hormone secretion. For instance, growth hormone secretion is increased in anorexia nervosa, but decreased in obesity. This effect of nutritional status affects the proper interpretation of endocrine tests aimed at diagnosing insufficient or excessive growth hormone secretion.

Age
The endocrinology of ageing is characterised by decreased plasma levels of several important hormones such as growth hormone, IGF-1 and sex steroids in both sexes. Therefore, age-adjusted reference values are required for such hormones.

Variation due to logistical factors
In addition to biological factors, logistical issues of sample collection, sample handling and storage may affect the final results of hormone measurements. These include the kind of tubes used to collect samples, temperature of the tubes, immediate versus delayed plasma collection, and duration and temperature of storage.

VARIATION IN HORMONE CONCENTRATIONS: ANALYTICAL FACTORS

The description of the radioimmune assay by Yalow and Bersow in 1959 has revolutionised the analysis of hormone analysis. Prior to their Nobel Prize winning discovery, the measurement of hormone concentrations by bioassays was very cumbersome. In the past forty years a continuing evolution of hormone assays has occurred. In recent years this has resulted in the widespread implementation of different kinds of robotic assays. Initially, clinical endocrinologists were able to base their career on the implementation of new (radioimmune) assays. Subsequently, hormone analysis was taken over by clinical chemists, at least in the Netherlands. Finally, with the introduction of robotic assays, there is a great danger that experience with the problems of the analysis of hormone concentrations is also waning not only in internists, but also in clinical chemists. In this respect there are several major problems. Each robot allows only a limited number of hormone assays, thus limiting the choice between the available assays. Frequently, choices for less optimal assays are based merely on the available types of robots. Another problem is that reference values are usually not determined within each laboratory, but derived from the description by the commercial producers of the assays. Importantly, it appears that different laboratories using the same commercial assays may yield different results for the same plasma samples. Moreover, continuous quality control within each laboratory remains necessary to ascertain that the intralaboratory variation remains within acceptable limits. Problematic assays with respect to large intralaboratory and interlaboratory variation are those of IGF-1 and of urinary cortisol. Within the Netherlands the LWBA, the national work group for bindings analysis, focuses on quality control of analytical variation of hormone analysis between and within laboratories.

PLASMA HORMONE CONCENTRATIONS AND DIAGNOSTIC TESTS OF ENDOCRINE DISEASES

The endocrinology department of the Academic Medical Centre of Amsterdam University is to be praised for their continuing efforts to evaluate the reference values for endocrine tests. By evaluation of a balanced group of healthy volunteers with respect to sex and age, they report

the normal reference values for plasma aldosterone concentration and plasma renin activity.[2] These values were determined in plasma and urine samples obtained during a strict protocol, limiting preanalytical variation as much as possible. The next step is to evaluate the sensitivity and specificity of these tests in patients with hypertension with and without primary hyperaldosteronism.

In the other publication,[3] the same group evaluated the value of the thyrotropin-releasing hormone (TRH) test in differentiating idiopathic hyperprolactinaemia and prolactinoma in 92 consecutive patients with hyperprolactinaemia. In a previous study the reference values of this test were established in healthy subjects. Remarkably, hyperprolactinaemia was not confirmed in 17% of the patients, which may be due to interlaboratory analytical variation and/or preanalytical biological variation. In addition, they show that the TRH test can be omitted in the evaluation of hyperprolactinaemia.[3] Previously, the TRH test was also omitted in the evaluation of thyroid dysfunction, because the thyroid-stimulating hormone (TSH) response is directly related to baseline TSH levels measured by ultrasensitive TSH assays. One of the few indications remaining for TRH testing in clinical endocrinology may be the follow-up of patients with cured acromegaly. During prolonged follow-up, recurrence of growth overproduction occurs in ~ 19% of the patients and in some of them the persistent, paradoxical response of growth hormone to TRH predicts recurrence of growth hormone overproduction.[4]

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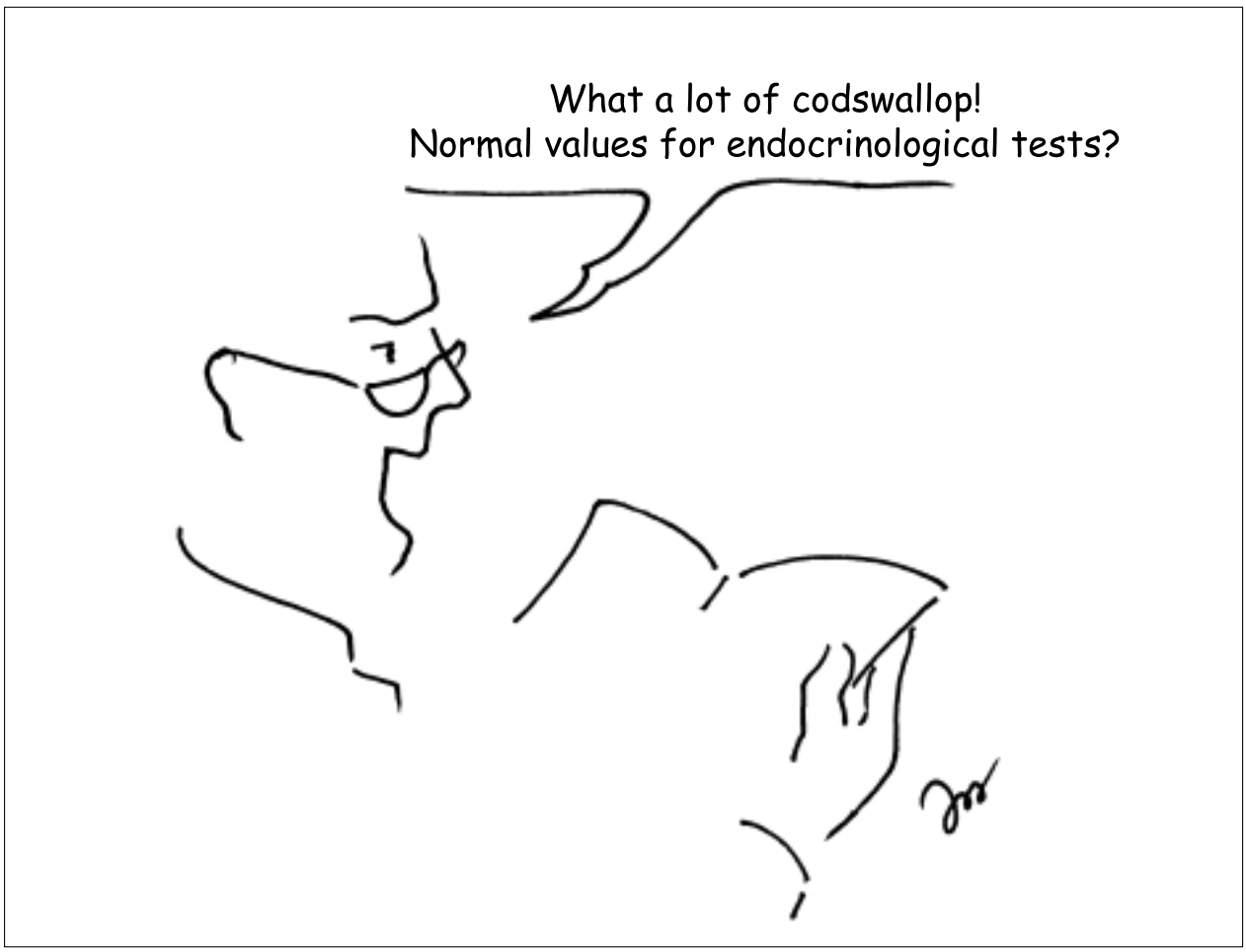

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Cardiovascular disease in sub-Saharan Africa: a disaster waiting to happen

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ABSTRACT

The looming threat posed by the emergence of cardiovascular disease (CVD) in sub-Saharan Africa (sSA) is underestimated and often denied. The health services and societies struggle to cope with the direct effects of poverty, war, fragile social and economic structures and AIDS. The threat of CVD seems less direct and few reliable data are available. This has resulted in neglecting serious warning signs on the emergence of CVD in sSA. This short review deals with the strong increase in a number of risk factors for CVD in certain areas, necessitating preventive measures to lighten the 'double burden of disease' in this part of the world.

HEALTH TRANSITION

Traditionally, attention for health problems in sSA was focused on infectious diseases. Changes in demographic and epidemiological determinants of health (such as ageing of a population and availability of vaccination and antibiotics to control infectious diseases) and changes in lifestyle associated with urbanisation have resulted in a so-called health transition.[1] This has led to a shift in the patterns of disease with the emergence of non-communicable diseases (NCDs) as a major cause of morbidity and mortality, which is comparable with changes in disease patterns that had occurred earlier in industrialised countries and more recently in some Asian countries. However, whereas prevalence and incidence of infectious diseases declined sharply in industrialised countries, this burden has remained high in sSA. The health transition has, therefore, led to what has become known as a 'double burden of disease' for the developing world: first the 'unfinished agenda' of infectious diseases (particularly among the young) and second the 'emerging agenda' of NCDs, in particular CVD and malignancies.[2]

The 1993 World Development Report 'Investing in Health' highlighted the need to address CVD in sSA.[3] The report acknowledged the continuous major health threat posed by infectious diseases, but expected the burden of CVD to increase rapidly in the near future. This shift in disease patterns is confirmed in a rare analysis of historical data on causes of death in a developing country. In the West-African capital Banjul the proportion of all deaths due to NCDs increased from 22 to 41% between 1942 and 1997, with CVD accountable for the majority of the NCD deaths (*figure 1*).[4]

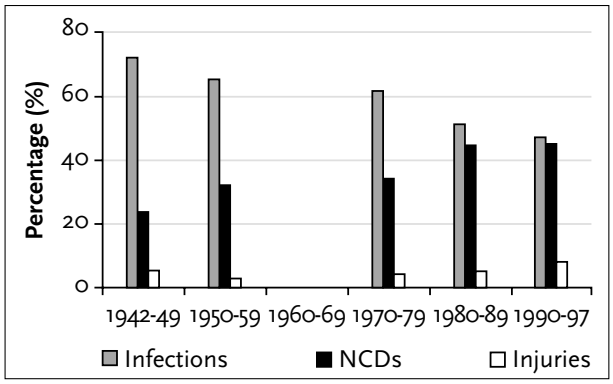

Figure 1

Causes of death in the West-African capital Banjul, 1942-1997[4]

No data were available on causes of death between 1960 and 1969. NCDs = non-communicable diseases.

Age-specific rates of many CVDs are currently higher in adults in sSA than in populations in industrialised countries.[5] Based mainly on case report studies, it is thought that the main CVD burden is caused by stroke, and cardiac and renal failure.[6,7] Nevertheless, in the absence of reliable mortality and morbidity registers in most of the subcontinent, it is not easy to obtain an accurate picture of the prevalence and incidence of major CVDs among the population. On the other hand, there is strong evidence on the increase in CVD risk factors, which suggests that CVDs will become more and more common.

HYPERTENSION

The best documentation exists on the increasing prevalence of hypertension, the most common CVD risk factor in the world. Studies up to the 1970s showed a low prevalence or virtual absence of hypertension, and no increase in blood pressure with age,[8-10] but this contrasts with studies over the past 20 years.[11-14] Although it is difficult to compare studies due to different methodologies and differences in prevailing definitions of hypertension, estimates from recent studies suggest that about 8% of the rural population and 15% of the urban populations may have a BP ≥160/95 mmHg, with the highest prevalence found in southern Africa.[13,15,16] Cooper *et al.* estimated that at least 5% of all adult sSA deaths are related to hypertension.[17] A longitudinal study in rural Nigeria, in the early 1990s, found an excess mortality of 7% due to hypertension.[18] The WHO estimates that hypertension-associated mortality in sSA may rise to 20% by the year 2020 (unpublished).

There are phenotypical differences between hypertension in people of black and of white ethnic origin, which might be associated with different genetic susceptibilities. However, there is accumulating evidence that the driving forces behind the steep increase in the prevalence of hypertension are the same risk factors as identified in studies in industrialised countries: increased salt intake, body weight and stress, and decreased physical activity. The strong association with age, which now appears to be nearly universal, might reflect an accumulation of these and other lifestyle-related risk factors with time, rather than a biological phenomenon.[19] This was well illustrated by a longitudinal study in Kenya, which documented an almost immediate rise in blood pressure upon migration from a rural to an urban area.[20] The International Collaborative Study on Hypertension in Blacks (ICSHB) studied blood pressure and associated CVD risk factors among seven populations of West-African origin, including rural Nigeria, urban and rural Cameroon, three Caribbean sites and a site in the USA. They observed a clear increase in blood pressure along the gradient of urbanisation.[13,21]

OBESITY

Unlike hypertension, which is prevalent in all parts of society, overnutrition and (central) obesity appear currently limited to specific segments of sSA society, particularly those in which lifestyles have become most urbanised and westernised. A study in The Gambia showed that while overall prevalence of obesity (body mass index (BMI) >30) was low (2.3%), nearly a third (32.3%) of the urban women aged 35 and over were obese (*figure 2*).[22] This is similar to data obtained in Cameroon,[16] and in the earlier mentioned ICSHB sites, where BMI (as blood pressure) increased along the degree of urbanisation.[23] Abdominal or central obesity is considered particularly detrimental; increases in central obesity are correlated closely with urbanised lifestyle.[24]

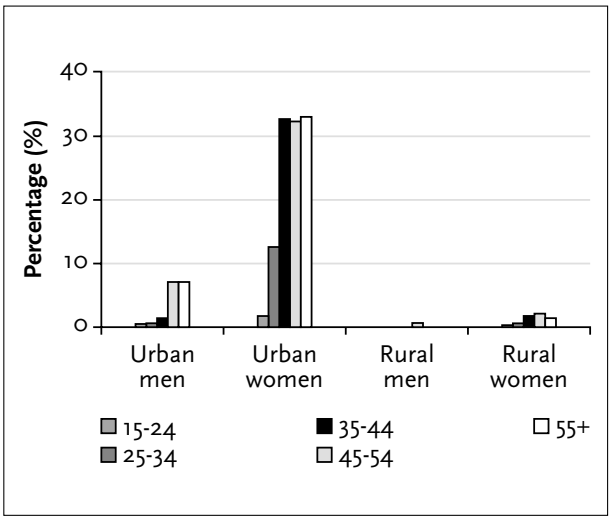

Figure 2
Prevalence of obesity (BMI >30) by sex and age group in urban and rural Gambia, 1996-1997[22]
BMI = body mass index = weight (kg) / (height (m)).[2]

It remains unclear why the association between urbanisation and obesity is more marked among women than men. This divergence strongly points to behavioural factors in aetiology, since men and women share most genetic and environmental factors. In contrast to the situation in many western countries, most people in sSA do not perceive obesity as a problem. Obesity, as well as the absence of a need for physical activity, is often considered a sign of prosperity (and protection for potential hard times to come), similar to perceptions in Europe in past centuries. While obesity is a new health problem, undernutrition remains a serious public health problem in sSA, often exacerbated by disasters such as famine and war. This illustrates the 'double burden of disease' faced in sSA.

INSULIN RESISTANCE

Central obesity is the most common cause of insulin resistance, which can lead to impaired glucose tolerance (IGT) and eventually to diabetes mellitus (DM). Apart from its direct metabolic effects, DM is a major risk factor for CVD. Data on community prevalence of diabetes are sparse, related to the considerable logistic challenges involved. Type 2 diabetes is the predominant diabetes form in sSA. Reported prevalences in rural populations are generally below 1%,[25,26] but higher in elderly urban populations, in which they vary between 2.4% in Sierra Leone[27] to 8.4% in The Gambia.[28]
IGT on the other hand is common in both urban and rural areas, reported prevalence ranging from around 8% in rural Tanzania[29] to over 20% in The Gambia.[28] Although there is some debate on the relative importance of IGT as a precursor of DM in sSA,[30] this high prevalence nevertheless suggests that diabetes can be expected to increase considerably in the near future. In some transitional societies, diabetes prevalence has surpassed rates found in industrialised countries.[25] A recent review predicts that prevalence of DM in sSA could triple in the next 25 years.[26]

SMOKING

Tobacco companies have been very successful at marketing in developing countries. In several studies prevalence of smoking among men was more than 50%; among women it is rarely more than 5%.[31,32] In many societies men start smoking at an earlier age than women, so this epidemic may still be in an early phase. The long-term harmful effects of smoking are often not known to the general population. There are no health warnings on most packages. There is a lack of alternative sources of affordable enjoyment or alternative image creation in poor communities. Absence of taxation results in relatively cheap products. Coupled with aggressive marketing, all this suggests that further increases in smoking in the near future are inevitable, unless well-coordinated counter efforts are made.

DYSLIPIDAEMIA

Whereas renal failure, cerebrovascular accidents and heart failure are frequent diagnoses, ischaemic atherosclerotic coronary disease is rarely reported.[7] This might be related to generally favourable lipid profiles (low cholesterol and high ratios of high-density lipoprotein) and low homocysteine values among the general population.[33]
Nevertheless, the few data available show that in spite of relatively low mean population levels, hyperlipidaemia is not rare. Elevated serum cholesterol (>5.2 mmol/l) is reported in up to a quarter of the population aged 35 and over in studies in rural Tanzania and The Gambia.[14,34] In Tanzania, hypertriglycerides (triglyceride ≥1.7 mmol/l) were also found among 15% of the over 35 group.[31] This suggests that within the next generation significant increases in coronary heart disease may occur as well.[35]

OUT OF AFRICA

Studies in the USA and Europe observed that among people of black African descent the prevalence of CVD risk factors, such as hypertension and obesity, is higher and that at a given blood pressure level the risk of target-organ damage, especially cerebrovascular accidents, is higher compared with the general population.[36] This suggests there may be a lower threshold for target-organ damage in African populations. It has been argued that certain genetic factors that could convey a selective survival advantage for common infectious diseases may put people at increased risk of CVD in a different environment; this hypothesis is known as the 'thrifty genotype'.[37] An alternative explanation is known as the 'thrifty phenotype'. This argues that early life events, in particular foetal undernutrition at critical periods of growth, lead to permanent adaptations in metabolic processes. In later life this increases the risk of hypertension, obesity, diabetes, CVD and premature death.[38] Neither of these theories is as yet proven, nor are they mutually exclusive. Both are consistent with the observation that the risk of CVD in the black African population increases with urbanisation, in and out of Africa.

CONCLUSION

Regardless of the underlying mechanisms of the potential higher susceptibility of people from sSA to develop CVD, this susceptibility only manifests itself when lifestyle changes associated with urbanisation occur. Efforts should focus on modifying such unhealthy changes as industrialisation and urbanisation can be expected to increase in the near future. The first step for policymakers (often at increased risk themselves) will be to acknowledge the current and projected magnitude of the problem. The next step needs to be implementation of intensive, interdisciplinary, preventive and therapeutic interventions.
As with many other health problems facing sSA, implementation of what already is known to work could have a huge impact. It is possible, for example, to modify lifestyle through national health policy: in Mauritius a

population-wide intervention programme promoting a healthy lifestyle resulted in a marked decrease of the prevalence of several cardiovascular risk factors (hypertension, smoking, inactivity and hyperlipidaemia).[39] To circumvent a 'prevention paradox'[40] a population prevention strategy should be complemented by a high-risk strategy.[41] In a pilot project in South Africa, care of CVD and CVD risk factors was successfully decentralised.[42] Optimising existing primary care services has been shown to have a marked impact on adherence to treatment and on adequate control of CVD.

A similar approach has resulted in a decline in CVD rates in much of the industrialised world; sSA should benefit from these experiences. Rather than waiting for a full epidemic to develop, resources must be mobilised now.

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Establishment of reference values for endocrine tests. III: primary aldosteronism

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ABSTRACT

Background: In our laboratory well-defined reference values for the screening test and confirmation test used in the diagnosis of primary aldosteronism were lacking. In this study we established the reference values of the plasma aldosterone concentration (PA), plasma renin activity (PRA) and PA/PRA ratio after a two-hour upright period, and of the urinary aldosterone excretion after oral sodium loading.

Methods: Fifty healthy volunteers, equally distributed according to sex and aged between 20 and 70 years, went through the screening and confirmation test of primary aldosteronism. PA, PRA and the PA/PRA ratios were measured after a two-hour upright period (screening test). Urinary aldosterone excretion was determined in two 24-hour urine samples after an oral suppletion of 6 g NaCl a day for five days (confirmation test).

Results: The following reference values were established: PA (after two-hour upright position) <0.03-1.05 nmol/l (mean: 0.47), PA/PRA ratio 0.05-0.47 (mean: 0.15) and urinary aldosterone excretion after sodium loading <3.0-47.0 nmol/24h (mean: 10.5). PRA showed a significant decrease with advancing age: median values in the 3rd to 7th decade are 3.9, 3.5, 2.5, 1.6 and 2.1 ng A1/ml/h respectively (p=0.04). PA was lower in subjects ≥50 years old. Age did not affect the PA/PRA ratio or the urinary aldosterone excretion. There were no significant differences between the sexes in any of the above-mentioned parameters.

Conclusion: In this study we established reference values for the screening and confirmation test used in the diagnosis of primary aldosteronism.

INTRODUCTION

Primary aldosteronism is a form of mineralocorticoid excess (MCE). Hypertension, an abnormally high plasma aldosterone secretion, suppressed plasma renin activity (PRA), increased urinary excretion of potassium, and hypokalaemic alkalosis are the characteristic findings in patients with primary aldosteronism.[1-3]
It is probably the most prevalent form of MCE. The most common causes of primary aldosteronism are the unilateral aldosterone-producing adenoma (APA; 64% of the cases) and bilateral idiopathic hyperaldosteronism (IHA; 32% of the cases).[4-6] It is important to diagnose primary aldosteronism in patients with hypertension because of the therapeutic implications: in APA the hypertension may be cured by surgery.[7] It has been advocated that all patients with resistant hypertension, whether normokalaemic or hypokalaemic, should be screened for primary aldosteronism.[1,3] The diagnosis of primary aldosteronism is divided into three phases according to Young *et al.* (1990): screening test, confirmation test and tests to determine the subtype of primary aldosteronism. This study concentrates on the first two phases. To distinguish patients with essential hypertension from patients with primary aldosteronism the plasma aldosterone/plasma renin ratio (PA/PRA ratio) is the screening tool of choice. This ratio has the highest accuracy according to the current literature.[1,4,8-12] In our institution a positive screening test is indicated by an increased PA/PRA ratio; a suppressed PRA; and hypokalaemia in combination with an inappropriate kaluresis. If two of the three criteria mentioned above are met, the screening test is considered positive. The diagnosis primary aldosteronism is confirmed by demonstrating an increased (unsuppressible) aldosterone excretion in a 24-hour urine sample after three

days of sodium loading. The sodium loading has to be sufficient, i.e. the urinary sodium concentration should exceed 200 mmol/24h. [6,12-14] According to Bravo *et al.* (1983) this is the single best test (sensitivity 96%, specificity 93%) to identify patients with primary aldosteronism.
As the diagnostic procedures used in the different studies of primary aldosteronism differ slightly, the resulting reference values may vary. In addition, PA and PRA can be influenced by many factors including pregnancy, age, the use of several drugs (NSAIDs, oestrogens, diuretics, spironolactone, ACE inhibitors, β-blockers and calcium antagonists), eating liquorice and race.[3,15-22] The aim of this study was to establish reference values for PA, PRA and the PA/PRA ratio after a two-hour upright period and for the urinary aldosterone excretion after oral sodium loading. The screening and the confirmation test were performed in fifty healthy subjects recruited from the general population of Amsterdam and surrounding area.

SUBJECTS AND METHODS

Subjects

Fifty subjects were recruited by advertisement in a local newspaper with a free house-to-house distribution in the Amsterdam region and by advertisements in 'Status', the biweekly information bulletin of the Academic Medical Centre of Amsterdam University. The subjects were screened for the inclusion and exclusion criteria by telephone and during the intake visit. Inclusion criteria were age between 20 and 70 years and a self-proclaimed general good health. Exclusion criteria consisted of hypertension exceeding a diastolic blood pressure of 100 mmHg, use of diuretics, spironolactone, ACE inhibitors, β-blockers, calcium antagonists, oestrogens or NSAIDs, and pregnancy. Altogether, 93 subjects satisfied the criteria mentioned above: fifty of them, selected at random, completed the two tests.
The subjects were divided into five age categories (20-29 years, 30-39 years, etc.) Each category consisted of ten persons: five men and five women. Categorising was done in order to find out if the values might be age-dependent. At the intake interview, information was given about the study and the tests, and questions were asked about race, diet (salt intake), use of liquorice, medication, smoking, use of alcohol and hypertension. The hospital's ethics committee approved the study and informed consent was obtained from all subjects.
Two subjects did not complete both tests: a 20-year-old man because of nausea and vomiting during the days of salt suppletion and a 26-year-old woman because of personal circumstances. They were excluded and replaced by subjects of the same sex, selected at random from the same age categories.

Tests

Screening test

Two consecutive 24-hour urine samples were collected separately at home. During this collection, the urine was kept refrigerated. Of the two 24-hour urine samples, total volume as well as the concentrations of sodium, potassium, creatinine and aldosterone were measured. At 9 am (t0), 15 minutes after inserting an intravenous canula, blood samples were taken in sitting position to measure the PA and PRA. At 11 am (t2), after a two-hour upright period, blood samples were taken in an upright position for the measurement of sodium, potassium, creatinine, PA and PRA. The diurnal and day-to-day variation of plasma aldosterone is high.[2,8] Therefore, the PA in all subjects was measured at the same time in the morning, when the concentration is at its peak. The values obtained after the two-hour upright period were of interest for the evaluation of the screening test. The completeness of the urine collections was assessed from the 24-hour urinary creatinine concentrations. If the creatinine excretion of the urine sample with the highest creatinine excretion was ≥150% of the creatinine excretion of the other sample, the urine collection was considered incomplete and the two samples were excluded.[23] Weight and height were recorded, and blood pressure was measured with the Dinamap® Compact monitor. The subjects were investigated after an overnight fast and smoking was not allowed.

Confirmation test

During five days before to the blood sampling the subjects took an additional 6 grams of salt: two tablets of 1 g NaCl, three times a day. During the last two days of this salt intake they collected their urine. Total volume as well as the concentrations of sodium, potassium, creatinine and aldosterone were measured per 24-hour sample. Blood sampling for the determination of plasma potassium was performed at the end of the five-day period at 9 am after an overnight fast. Smoking was not allowed. Before the blood sampling, weight and blood pressure were measured.

Analytical methods

PA was measured by a commercial RIA (radial immuno-assay) (coat-a-count, Diagnostic Products Corporation, Los Angeles, CA). The detection limit is 0.03 nmol/l, the interassay coefficient of variation (CV) 4.7 to 12.0% and the intra-assay CV 3.6 to 8.4% (at 1.53 to 0.13 nmol/l).
The PRA was determined by RIA as described previously (Hollemans *et al.*, 1969). The detection limit is 0.3 ng A1/ml/h, the interassay CV 6.0 to 11.0% and the intra-assay CV 4.0 to 6.0% (at 10.6 to 1.4 ng A1/ml/h). Urinary aldosterone was determined after extraction with ethylacetate by the same RIA. The interassay coefficient of variation is less than 18%. Plasma sodium, potassium and creatinine were measured by standard clinical chemical methods and reagents

on a Hitachi 747 analyser (Roche Diagnostics, Almere, the Netherlands). For the determination of urinary sodium, potassium and creatinine concentrations a Hitachi 911 analyser (Roche Diagnostics, Almere, the Netherlands) was used. The pregnancy test used was the ABBOT TestPack®Plus™.

Statistical methods

Use of alcohol was defined as the intake of at least two units a day, smoking as daily smoking and liquorice use as the consumption of more than two pieces of liquorice a day. The subjects were questioned about their daily salt intake. They could choose from categories varying from a low, low/normal, normal, normal/high and high salt intake. For the body mass index (BMI) the mean value between the day of the screening test and the day of the confirmation test could be used, as there was no significant difference between the two time points. The urinary concentrations of aldosterone, creatinine, sodium and potassium were calculated as the mean of the values of the two subsequent 24-hour urine samples, as there were no significant differences between the two samples. Values below the detection limit of the assays were included in the analyses as having a value of 50% of the detection limit. Differences between groups were analysed with the Wilcoxon signed-rank test, with the exception of the plasma potassium concentration and the systolic and diastolic blood pressure, for which the paired t-test was used. Sex differences, influence of smoking, alcohol, race, liquorice and history for hypertension were analysed with the Mann-Whitney U test. Effects of age were tested by the Kruskal-Wallis test, possible correlations with the Spearman's rank correlation. We used SPSS 10.1 for the statistical analysis. In all tests, p values below 0.05 were considered statistically significant. Reference values are given as mean ±2 SD. PA, PA/PRA ratio and urinary aldosterone excretion showed a non-parametric distribution. After logarithmic transformation the distribution appeared to be normal, allowing for the assessment of mean ±2 SD of these parameters. Age-dependent reference values are given as the observed range in view of the small number of observations.

RESULTS

Table 1 gives some characteristics of the subjects. During the screening test three subjects had a diastolic blood pressure between 90 and 100 mmHg. A similar blood pressure was found in two subjects during the confirmation test.

Screening test

The urine samples of four subjects were excluded because of incomplete/incorrect urine collection. In addition, one PA assay could not be included, because of a preanalytical mistake.

PRA, PA and PA/PRA

PRA increased significantly between t0 (sitting position) and t2 (after a two-hour upright period) (*figure 1a* and *table 2*). The increase in the PRA between t0 and t2 varied from 0 to 11.0 ng A1/ml/h with a median of 1.1 ng A1/ml/h. The PRA at time t2, used for the formal assessment of the screening test, declined (p=0.04) with advancing age (*table 3*). Subjects of 50 years and older had significantly lower reference values (range: <0.3-7.5 ng A1/ml/h, median: 1.8 ng A1/ml/h) than subjects under 50 years (range: 0.3 to 20 ng A1/ml/h, median: 3.4 ng A1/ml/h) (p<0.01).

PA also increased significantly in upright posture (*table 2* and *figure 1b*). The increase varied from -0.10 to 0.73 nmol/l (median: 0.15 nmol/l). Especially after the two-hour upright period the range was rather wide. PA tended to decline with advancing age (p=0.05) (*table 3*). Subjects of 50 years and older showed a significantly lower PA value while upright as compared with younger subjects (p=0.03). *Figure 2* shows the strong correlation between PA and PRA (p=0.72, p<0.01) on time t2.

Values for the PA/PRA ratio are given in *figure 1c* and *table 2*. Although the mean values of the PA/PRA ratio were quite similar at time t0 and t2, the range was smaller at t2.

Table 1

Subject characteristics (n=50) (median; range)

Age (years)		44	(20-69)
Body mass index	Women (n=25) Men (n=25)	25.2 24.9	(19.7-39.6) (18.5-33.0)
Race	Caucasian Non-Caucasian	41 9	(82%) (18%)
Blood pressure (mean)	Screening test Confirmation test	127/72 122/68	mmHg mmHg
Smoking		13	(26%)
Alcohol use		8	(16%)
Liquorice use		5	(10%)

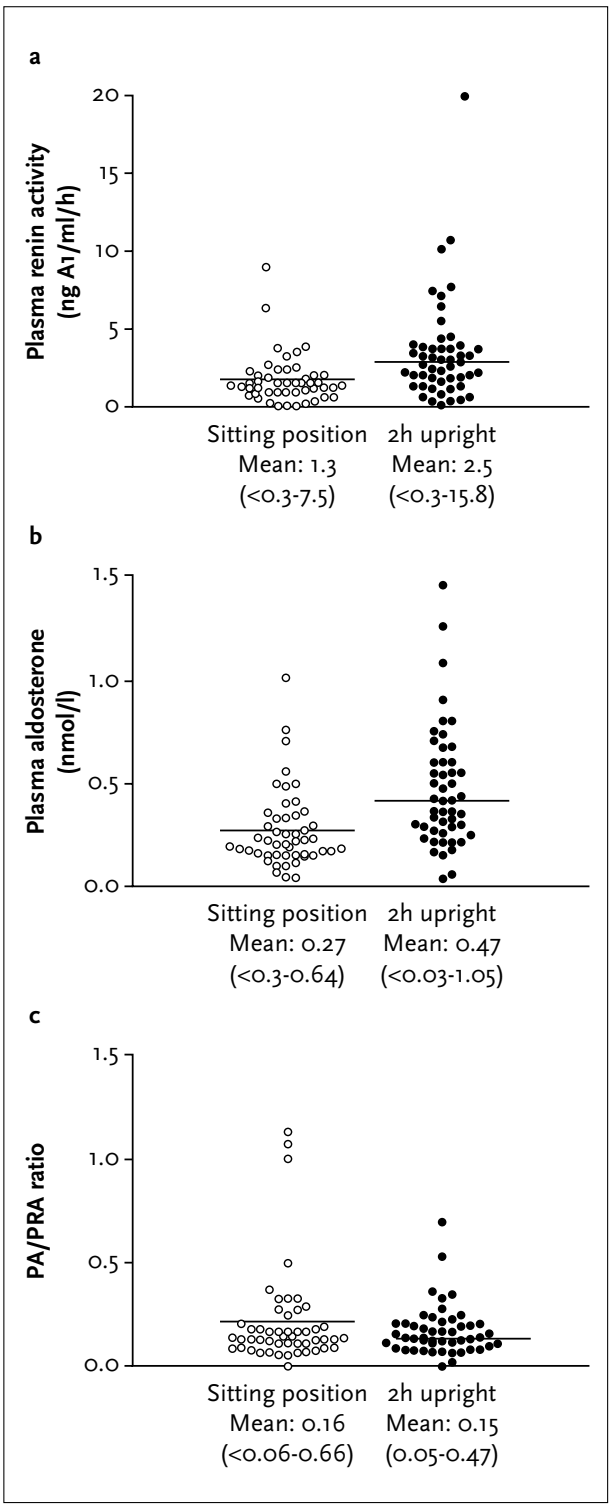

Figure 1

The plasma aldosterone concentration (PA), plasma renin activity (PRA) and PA/PRA ratio during the screening test in sitting position (t0) and after a two-hour upright period (t2) (mean values indicated by horizontal lines)

The PRA is significantly lower (p<0.01) at t0 (n=50) in comparison with t2 (n=50) (a). The PA is significantly higher on t2 (n=50) in comparison with t0 (n=49) (p<0.01) (b). The mean of the plasma aldosterone/ plasma renin activity ratio at t0 (n=49) is not significantly different from to the ratio on t2 (n=49) (c).

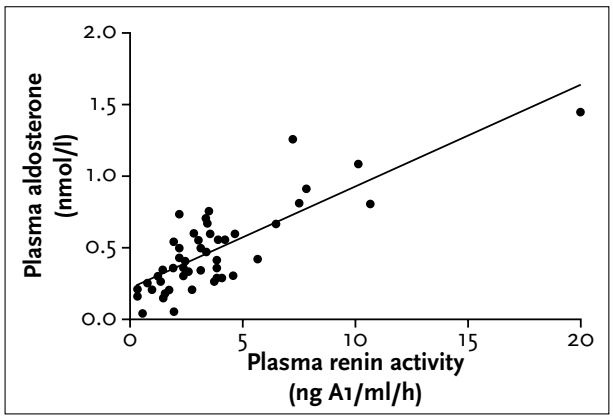

Figure 2

Correlation between plasma aldosterone concentration (PA) and plasma renin activity (PRA) after a two-hour upright period (11.00 h, t2) (r=0.72, p<0.01)

Age did not affect the ratio. All other factors, i.e. race, BMI, smoking, use of alcohol, use of liquorice, history for hypertension, salt intake and sex, did not affect PA, PRA and PA/PRA ratio.

Potassium, sodium and creatinine in plasma and urine

Men had a higher urinary potassium excretion than women (p<0.01), but plasma potassium did not differ between sexes (*table 2*). During the screening test as well as during the confirmation test the urinary and plasma concentrations of creatinine were higher for men than for women (p<0.01). The values for the plasma and urine sodium concentrations were not affected by age, BMI, race, smoking, use of alcohol, use of liquorice, salt intake and a history of hypertension.

Confirmation test

Urine samples of eight persons were excluded because of incomplete/incorrect urine collection. The mean 24-hour volume of the collected urine after oral salt loading increased significantly as compared with those collected in the screening test: the mean increased from 1569 ml to 1900 ml (p<0.01). The urinary aldosterone concentration was significantly lower during the confirmation test (p<0.01) (*table 2* and *figure 3a*). The difference varied from -58 to +7 nmol/24h with a median -6 nmol/24h. Neither sex nor age effected the urinary aldosterone. The mean urinary sodium excretion was normally distributed and increased significantly after salt suppletion (p<0.01) (*figure 3b*). The mean difference of the sodium excretion between the screening test and the confirmation test was +79.5 mmol/24h (range: -18.4 to +226.9 mmol/24h). The plasma potassium concentration decreased significantly after administration of NaCl (p<0.01) (*table 2*). The urinary potassium excretion differed slightly with sex; men showed a higher urinary potassium excretion than women (p=0.04) (*table 2*).

Table 2
Results of the screening and confirmation tests for primary aldosteronism

		SCREENING TEST			CONFIRMATION TEST		
PA (nmol/l) (total)	Sitting position (t0)	0.27	<0.03-0.64	n=49			
	2 hours upright (t2)	0.47	<0.03-1.05*	n=49			
PRA (ng Al/ml/h)	Sitting position (t0)	1.31	<0.3-7.49	n=50			
	2 hours upright (t2)	2.47	<0.3-15.84*	n=50			
PA/PRA	Sitting position (t0)	0.16	0.04-0.66	n=49			
	2 hours upright (t2)	0.15	0.05-0.47	n=49			
Plasma potassium (mmol/l)		4.1	3.6-4.7	n=50	3.9	3.5-4.3†	n=50
Plasma creatinine (mmol/l)	Men	71	49-94	n=25			
	Women	59	43-76‡	n=25			
Urinary aldosterone (nmol/24h)		18.8	4.6-76.8	n=46	10.5	<3.0-47.0†	n=42
Urinary sodium (mmol/24h)		149.7	47.3-251.1	n=46	225.6	106.3-344.9†	n=42
Urinary potassium (mmol/24h)	Men	87.5	32.7-142.3	n=22	82	34.4-129.6	n=23
	Women	64.9	27.2-102.6‡	n=24	82	29.8-104.4§	n=19
Urinary creatinine (mmol/24h)	Men	14.3	8.4-20.2	n=22	13.9	8.6-19.2	n=23
	Women	9.8	6.9-12,7‡	n=24	9.4	5.8-13.0‡	n=19

*Values are mean ±2 SD. * Significant difference from sitting posture, p<0.01; † significant difference from the screening test, p<0.01; ‡ significant difference from the men, p<0.01; § significant difference from the men, p=0.04.*

Table 3
Reference values for tests in the evaluation of primary aldosteronism

			REFERENCE VALUES	
Screening test	PRA	20-29 years	0.3-2.0	ng A l/ml/h
(after a two-hour upright period)		30-39 years	1.9-7.8	ng A l/ml/h
		40-49 years	0.5-7.2	ng A l/ml/h
		50-59 years	<0.3-4.5	ng A l/ml/h
		60-69 years	0.3-7.5	ng A l/ml/h
	PA	20-29 years	0.21-1.45	nmol/l
		30-39 years	0.26-0.90	nmol/l
		40-49 years	0.04-1.25	nmol/l
		50-59 years	0.15-0.55	nmol/l
		60-69 years	0.16-0.80	nmol/l
	PA/PRA		0.05-0.47	
Confirmation test	Urinary aldosterone after sodium loading		<3.0-47.0	nmol/24h

DISCUSSION

For the evaluation of the screening test, PA and PRA measurements are essential. As could be expected, the upright posture causes an increase in PRA through stimulation of the renin-angiotensin-aldosterone system, compared with time t0 (in sitting position). We observed a decrease in PRA with advancing age, in accordance with the literature. In a study by Hegstad (1983), PRA of older subjects (upright, normal salt intake) was lower than that of younger subjects.[17] PRA of persons over 50 years fell by 50%.[2] In our study one outlying value of 20 ng A1/ml/h in the age category 20-29 years occurred in a subject with a normal blood pressure (103/78 mmHg); this PRA value thus still belongs to the normal spectrum. PA in our study increased after a two-hour upright period as well and declined with advancing age. According to the literature PA concentrations in subjects older than 50 years are lower than those in subjects younger than 30 years.[17] This was also evident in our study: the subjects of 50 years and older had a lower PA than those younger than 50 years.

Factors other than posture and age may affect PA and PRA. The production of renin increases under the influence of oestrogens and reference values for PA can vary per sex.[2,3] In our study, however, we did not find sex differences for PA, PRA and the PA/PRA ratio. PA and PRA were not dependent on sodium intake, which corresponds with the

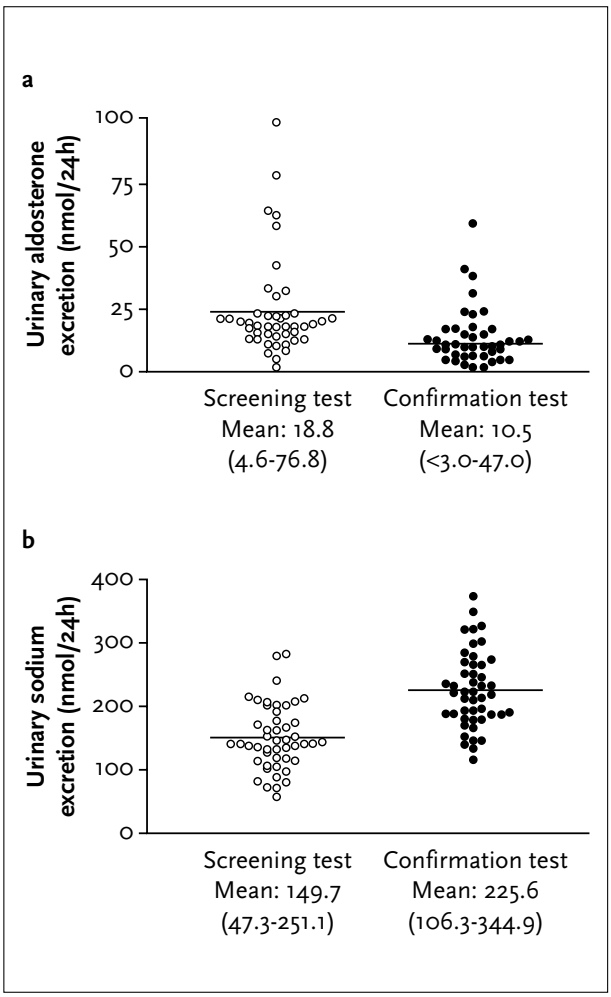

Figure 3

The urinary aldosterone and the urinary sodium excretion during the screening and confirmation test (mean values indicated by horizontal lines)

The urinary aldosterone excretion during the screening test (salt intake ad lib) (n=46) is significantly higher than during the confirmation test (after administration of 6 g NaCl per day for five days) (n=42) (p<0.01) (a). The urinary sodium excretion increased during the confirmation test (after administration of 6 g NaCl a day for five days) (n=46) compared with the screening test (salt intake ad lib) (n=42) (p<0.01) (b).

findings of Hiramatsu (1981) and Loh (2000). Although PA and PRA are suppressed by the active components of liquorice (glycyrrhizic acid and glycyrrhitic acid), liquorice did not emerge in our study as a factor influencing PA and PRA, probably due to the small number of subjects (10%) eating liquorice regularly.[19-21] PA and PRA in blacks are lower than in whites.[22] The non-Caucasian subjects in our study, however, were of Asian origin, and race had no effect on the reference values.

The PA/PRA ratio can be calculated on basis of the PA and PRA in the sitting position or after a two-hour upright period. For evaluation of the screening test the PA/PRA ratio after a two-hour upright period is preferred in view of its smaller range as compared with the sitting position. Likewise, the urinary aldosterone excretion before oral salt loading seems to be less useful for the confirmation of the diagnosis of primary aldosteronism, because the range is much wider than after salt loading.

As expected, the urinary aldosterone excretion decreased after salt suppletion as a result of the inhibition of the renin-angiotensin-aldosterone system. The sodium loading has to be sufficient for a proper evaluation of the confirmation test. The loading is considered sufficient if the sodium concentration exceeds 200 mmol/24h urine.[6] The mean concentration was indeed higher than 200 mmol/24h, but in 18 subjects (43%) it remained below this value. This cut-off value may be too high. It does not seem appropriate either to use the difference between the sodium concentration before and after salt loading as a cut-off value, because the sodium excretion in some of our subjects declined after salt suppletion (mean change: +79.5 mmol/24h, range: -18.4 to +226.9 mmol/24h). A decrease in sodium excretion could be caused by reduction of dietary sodium intake during the confirmation test, when subjects believe that their total salt intake is too high. The sodium excretion should at least be 103 mmol, if the subjects take all the NaCl tablets (6 g NaCl corresponds with 103 mmol NaCl).

The plasma potassium concentration before oral salt loading was higher than after salt loading. A possible explanation is the increased availability of sodium after salt loading. Because of the sodium suppletion the reabsorption of sodium diminishes in the proximal tubules. Therefore, the sodium availability increases in the distal tubules, in which the exchange of sodium and potassium takes place. As a result, more potassium can be exchanged for sodium, decreasing the plasma potassium concentration. This also occurs in normokalaemic patients with primary aldosteronism who develop hypokalaemia after salt loading.[6] The decreasing potassium plasma after salt loading does indicate that it is essential for patients with a suspicion of primary aldosteronism to be supplemented with potassium. The urinary potassium excretion of men was higher than that of women, which is in accordance with the study by Watenpaugh.[24]

In summary, we have established reference values for both the screening and the confirmation test in the diagnosis of primary aldosteronism. These values are presented in *table 3*. However, the most appropriate cut-off values should be ascertained in a study of patients suspected of having primary aldosteronism, who pass the same diagnostic protocol and finally can be labelled as having primary aldosteronism or not. In this respect, the cut-off value of urinary aldosterone excretion in the confirmation test might well be 39 nmol/24h as indicated in the literature:[5] in fact, only one of our healthy volunteers had a value above 39 nmol/24h (*figure 3a*).

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Evaluation of endocrine tests. A: the TRH test in patients with hyperprolactinaemia

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ABSTRACT

Background: In a previous study, we determined reference values for basal and thyrotropin-releasing hormone (TRH)-stimulated plasma concentrations of prolactin (PRL). The aim of the present study was to determine the clinical usefulness of the PRL response to TRH in the work-up of patients with hyperprolactinaemia.

Methods: We studied 92 consecutive patients referred for evaluation of hyperprolactinaemia. Patients with confirmed hyperprolactinaemia were divided into three groups: group A (pharmacological hyperprolactinaemia; n=2), group B (pathological hyperprolactinaemia; n=6) and group C (all other patients). Patients in group C underwent MRI of the pituitary and were subdivided into C1 (normal pituitary on MRI; n=6), C2 (slightly abnormal MRI; n=21), and C3 (evident microadenoma or macroadenoma on MRI; n=25 and 12, respectively). The MRI was technically insufficient in four patients. Basal PRL as determined by fluoroimmunometric assay and the PRL response to 400 μg TRH were determined in all patients.

Results: Hyperprolactinaemia was confirmed in 83% of the referred patients. Non-response, defined as a <2.5-fold PRL increase after TRH, occurred in one patient (50%) in group A, in 66% of patients in group B and in 99% of patients in group C. Within group C, basal PRL was not different between group C1 and C2, but higher (p=0.06) in group C3. The absolute PRL increase after TRH did not differ between the three subgroups. The relative PRL increase was smaller (p=0.03) in group C3 but overlapped considerably with groups C1 and C2. All patients except one in group C were so-called non-responders. Basal PRL and absolute PRL increases after TRH correlated with the adenoma diameter on MRI (r=0.66, p=0.0002 and r=0.49, p=0.008, respectively).

Conclusion: In patients referred for elevated serum PRL, hyperprolactinaemia should be confirmed under standardised conditions. The absolute or relative PRL increase after 400 μg TRH does not help to differentiate between patients with prolactinoma or idiopathic hyperprolactinaemia. Therefore, the TRH stimulation test is not useful in the work-up of hyperprolactinaemia.

INTRODUCTION

Hyperprolactinaemia may be physiological (during pregnancy and lactation), pharmacological (for example by use of neuroleptics or oestrogens) or pathological. Among the pathological causes of hyperprolactinaemia are primary hypothyroidism, renal failure, hypothalamic or pituitary disease interfering with the secretion of dopamine to the pituitary, and prolactinomas. In a substantial number of patients with mild hyperprolactinaemia (between 25 and 100 μg/l) no cause can be found; this situation is usually referred to as idiopathic hyperprolactinaemia. The condition is reversible in a substantial percentage of patients and only occasionally develops further into a detectable pituitary adenoma.[1] In many patients, a detailed history and physical examination will reveal the cause of hyperprolactinaemia. In others, ancillary investigations may be necessary. In the older literature, a thyrotropin-releasing hormone (TRH)

stimulation test was advised since a diminished response of plasma prolactin (PRL) to intravenous TRH (<2.5-fold increase in plasma PRL after TRH) supported the presence of a prolactinoma, whereas a normal response was highly unusual.[2] However, a blunted response of PRL to TRH is not specific for prolactinoma and is also seen with other types of hyperprolactinaemia. Therefore, dynamic testing of PRL secretion may not add to basal PRL levels alone in the differential diagnosis of hyperprolactinaemia.[3] Furthermore, the advent of high-resolution imaging techniques such as magnetic resonance imaging (MRI) has made the TRH test obsolete in the work-up of hyperprolactinaemia, according to many authors.[4] In a previous study, we established reference values for the plasma concentration of PRL and its response to TRH.[5] As part of an ongoing project aimed at standardising diagnostic procedures in our department, we proceeded and questioned the clinical usefulness of the TRH stimulation test in the work-up of patients with hyperprolactinaemia. Although indications for a TRH test are few[4] and some authors agree that TRH testing is not at all helpful, there is a paucity of studies clearly providing the evidence for this statement. To this end, we measured basal and TRH-stimulated plasma PRL under standardised conditions in 92 consecutive patients with hyperprolactinaemia, and analysed the results in relationship with the results of the pituitary MRI scan.

PATIENTS AND METHODS

Patients

We evaluated the clinical usefulness of the PRL response to TRH in the work-up of hyperprolactinaemia. Included were consecutive patients in whom clinical suspicion of hyperprolactinaemia was aroused by the existence of galactorrhoea, amenorrhoea, decreased libido or erectile dysfunction, or in whom hyperprolactinaemia had already been documented by the referring physician. Excluded were pregnant women (by assay of hCG in the urine) and breastfeeding women. Volunteers recruited by advertisements in a local newspaper served as controls.[5] Patients with confirmed hyperprolactinaemia according to the protocol and criteria described earlier[5] were divided into three groups. Group A had pharmacological hyperprolactinaemia. Group B had pathological hyperprolactinaemia caused by either renal insufficiency (plasma creatinine >200 μmol/L), severely impaired liver function, primary hypothyroidism or well-defined hypothalamic pituitary disorders clearly distinct from prolactinomas. Group C was composed of all the remaining patients and subdivided further based on pituitary MRI findings into group C1 (no abnormalities on MRI), group C2 (some abnormalities on MRI such as inhomogeneous pattern, pituitary asymmetry or partial empty sella but no apparent mass lesion), and group C3 (evidence of pituitary microadenoma or macroadenoma).

TRH test

A TRH stimulation test was performed in all patients in the postabsorptive state and in recumbent position, starting between 8.30 and 9.30 am. Weight, height and blood pressure were recorded. An indwelling venous catheter was inserted (at t=-30 min) in an antecubital vein and a blood sample was taken at t=-15 min for measurement of PRL, creatinine, OT, PT and thyroid-stimulating hormone (TSH). At t=0 min, a second blood sample was taken for measurement of PRL. Additional blood samples were taken at t=20, t=60, t=120 and t=180 min after administration of 400 μg of TRH intravenously (TRH Relefact, Hoechst) at t=0 min. A subnormal PRL response was defined as an increase of less than 250% over the basal PRL concentration according to Assies *et al.*[2] Sera were stored at -20°C until assay.

Analytical and statistical methods

PRL was measured by a solid phase, two-site, time-resolved fluoroimmunometric assay (DELFIA Prolactin, Wallac Oy, Turku, Finland). The intra-assay coefficient of variation (CV) was 4 to 6% (5-24 μg/l); the interassay CV was 5.5 to 7.2% (4-50 μg/l). We calculated basal PRL as the mean of PRL at t=-15 min and t=0 min, the absolute PRL increase as peak PRL – basal PRL, and the relative PRL increase as [peak PRL – basal PRL]/basal PRL x 100%. The upper normal limit of basal PRL was taken as 25 μg/l for females and 19 μg/l for males as determined previously using precisely the same preanalytical and analytical methods.[5] Group differences were evaluated by non-parametric tests, i.e. the Kruskal-Wallis and Mann-Whitney U test. Correlations between basal PRL and PRL increases, and between PRL levels and prolactinoma size were evaluated by linear regression analysis. We used the SPSS 8.0 statistical package. In all tests, p values below 0.05 were considered statistically significant.

RESULTS

Sixteen of the 92 consecutively included patients had normal basal PRL values and were not analysed any further. From the remaining 76 patients (65 females and 11 males), two had pharmacological hyperprolactinaemia caused by penfluridole and ethinyl estradiol (group A), six had pathological hyperprolactinaemia caused by primary hypothyroidism (n=3), acromegaly (n=1), meningioma (n=1) and astrocytoma (n=1) (group B), thus leaving 68 patients for group C (*table 1*).

Basal PRL and the absolute PRL increase after TRH did not differ between groups A, B and C, although a tendency was

Table 1

Basal PRL and TRH-stimulated PRL response in 50 healthy controls and in 76 hyperprolactinaemic patients (median values and range)

GROUPS†		CONTROLS	GROUP A	GROUP B	GROUP C	P VALUE
		n=50	n=2	n=6	n=68	group C vs group A+B
Sex (F, M)		25F, 25M	2F	6F	57F, 11M	
Age (years)		41 (22-66)	33 (32-35)	42 (27-58)	34 (19-79)	
BMI		24 (19-45)	26 (23-29)	21 (19-26)	25 (16-46)	
Basal	PRL (µg/l)	9 (4-25)	54 (42-66)	40 (26-225)	79 (23-13,000)	0.06
	PRL absolute (µg/l)	36 (2-120)	129 (84-173)	49 (10-103)	30 (-2-1250)	0.23
	PRL relative (%)	437 (18-1375)	273 (127-418)	74 (14-322)	29 (-8-345)	0.03
Non-responders§		24%	50%	66%	99%	

† *Controls derived from Le Moli et al.; group A = pharmacological hyperprolactinaemia, group B = pathological hyperprolactinaemia caused by primary hypothyroidism, acromegaly, meningioma, astrocytoma, group C = remaining patients including prolactinomas,* § *defined as relative PRL increase after TRH smaller than 250%.*

noted for higher basal PRL levels and lower absolute PRL increases in group C patients. The relative PRL increase after TRH was clearly lower in group C patients, giving rise to 99% of so-called TRH non-responders defined as a relative PRL increase after TRH smaller than 250%.
In group C, MRI scans of four patients could not be assessed properly for technical reasons. Of the remaining 64 patients (53 females, 11 males), six had a normal pituitary MRI (group C1), 21 had slight MRI abnormalities (group C2) and 37 had clear evidence of pituitary adenomas (group C3, microadenomas n=25, macroadenomas n=12). Peak PRL levels after TRH were predominantly reached at t=20 min, but occurred at t=60 min in two patients from group C1, in three patients from group C2, and in five patients from group C3. Basal PRL was not different between group C1 and C2 but significantly higher in group C3 (*table 2* and *figure 1*). The absolute PRL increase did not differ between the three groups, but the relative PRL increase after TRH was smaller in group C3.

Interestingly, all group C patients except one were so-called non-responders, a significant difference with the 24% TRH non-responders in the healthy controls (p<0.001).
In the patients with definite microprolactinomas or macroprolactinomas (group C3), a significant relationship was observed between the adenoma diameter on MRI in millimetres and basal PRL (r=0.66, p=0.0002) and absolute PRL increase (r=0.49, p=0.008), but not with relative PRL increase (r=0.06, ns).
Body mass index (BMI) was not related to basal PRL or relative PRL increase in group C patients, but we did observe a negative relationship between BMI and the absolute PRL increase after TRH in patients of groups C1 and C2 (*figure 2*), which was absent in group C3 patients.

Table 2

Basal PRL and TRH-stimulated PRL response in 64 hyperprolactinaemic group C patients, subdivided according to pituitary MRI readings (median values and range)

GROUPS†		GROUP C1	GROUP C2	GROUP C3	P VALUE*	
		n=6	n=21	n=37	(C3vsC1)	(C3vsC2)
Sex (F, M)		4F, 2M	18F, 3M	31F, 6M		
Age (years)		38 (35-43)	32 (21-66)	33 (19-73)	ns	ns
BMI (kg/m²)		26 (23-41)	25 (16-36)	26 (19-46)	ns	ns
Basal	PRL (µg/l)	54 (34-71)	60 (24-170)	132 (23-13,000)	0.000	0.002
	PRL absolute (µg/l)	21 (6-93)	33 (3-252)	25 (-3-1250)	ns	ns
	PRL relative (%)	58 (12-130)	61 (9-345)	18 (-8-166)	0.09	0.001
Non-responders§		100%	95%	100%		

† *Group C1= normal pituitary MRI, group C2 = slight abnormalities on pituitary MRI but no mass lesion, group C3 = definite microadenoma or macroadenoma on pituitary MRI,* § *defined as relative PRL increase after TRH smaller than 250%,* * *no differences were observed between groups C1 and C2.*

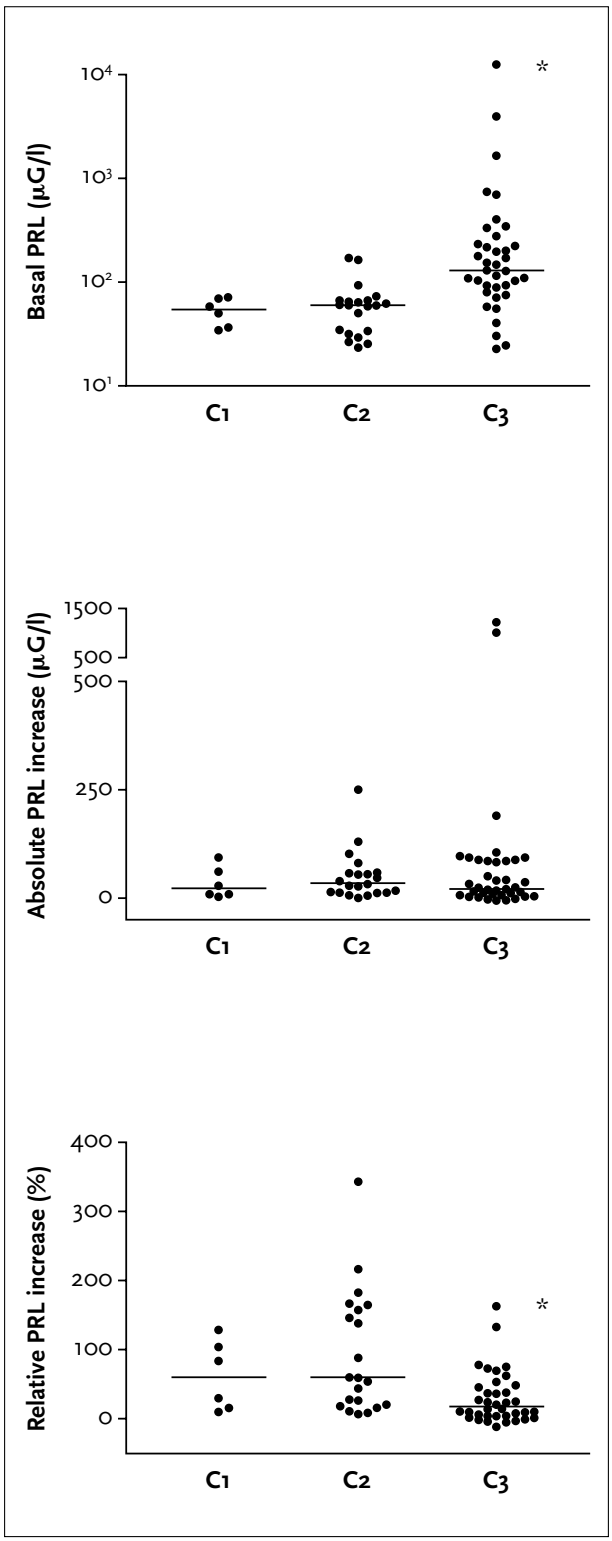

Figure 1

Basal PRL, absolute PRL increase after TRH, and relative PRL increase after TRH in 64 hyperprolactinaemic group C patients (for definition of groups see table 2)

Horizontal lines indicate median values. Basal PRL is not different between groups C1 and C2, but significantly higher in group C3. The absolute PRL does not show significant differences among the groups, but the relative PRL increase after TRH is significantly smaller in group C3. * Significantly different from groups C1 and C2.

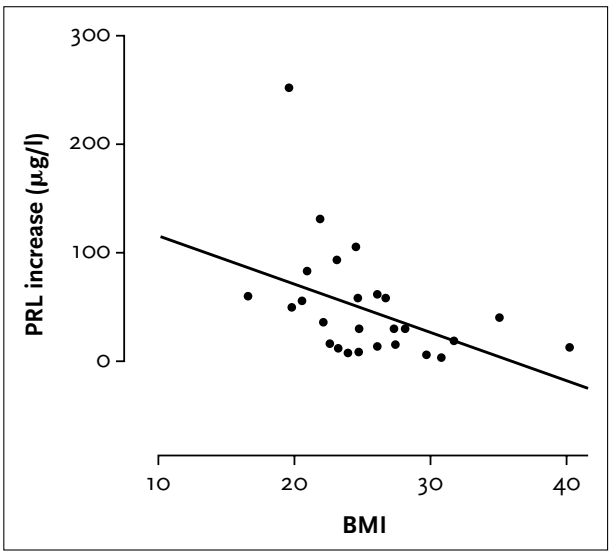

Figure 2

Relationship between BMI and the absolute PRL increase after TRH in hyperprolactinaemic group C patients with no or slight abnormalities on pituitary MRI (r=-0.43, p=0.02)

DISCUSSION

The present study was undertaken to determine the clinical usefulness of the TRH stimulation test in the setting of hyperprolactinaemia. Since many causes of hyperprolactinaemia are clear from the history, physical examination and routine laboratory tests (for example, pharmacological hyperprolactinaemia, renal failure), the question is whether the TRH stimulation test has any value in distinguishing idiopathic hyperprolactinaemia from prolactinoma. This differentiation has clinical relevance since idiopathic hyperprolactinaemia is a relatively benign and often self-limiting disease,[1] whereas patients with prolactinoma often require dopaminergical treatment as well as monitoring of tumour size in case of a macroprolactinoma.

In the present study, we used the protocol and reference values for basal plasma PRL described in our previous study.[5] Interestingly, hyperprolactinaemia was confirmed in only 83% of the referred patients. Since stress of any kind can cause a mild increase in serum PRL, our study reinforces the need to confirm hyperprolactinaemia under standardised conditions using an indwelling venous catheter before the patient is considered to have hyperprolactinaemia. Our present series of consecutive patients with confirmed hyperprolactinaemia contained only two patients with pharmacological hyperprolactinaemia and six patients with pathological hyperprolactinaemia. Five of these patients showed a <2.5-fold relative PRL increase after TRH, which is in accordance with other studies reporting a subnormal PRL response to TRH in more than 50% of patients with pharmacological and

pathological hyperprolactinaemia (e.g.).[6] In the remaining patients (group C), we found a <2.5-fold PRL increase after TRH in 99% of patients irrespective of the presence of a pituitary tumour on the MRI. Apparently, a subnormal PRL response does not help to differentiate between idiopathic hyperprolactinaemia and prolactinoma, since group C1 consisted of six patients with a normal pituitary MRI. Responders were absent in group C1 even after lowering the threshold for a subnormal response to 150% as can be seen from *figure 1*. Shangold *et al.*[7] reported a subnormal PRL response to TRH (defined as a <2.0-fold PRL increase after 500 µg intravenous TRH) in 37 out of 49 patients with hyperprolactinaemia without signs of a prolactinoma as shown by polytomography or CT. Also Assies *et al.*[2] found subnormal PRL responses to TRH to occur as frequently in hyperprolactinaemic patients without signs of a pituitary adenoma as in patients with definite prolactinoma. Since the latter studies were performed before the availability of MRI, the possibility of undetected small microprolactinomas in these patients could not be excluded. The results of our present study favour the alternative explanation of subnormal PRL responses to TRH in the majority of patients with idiopathic hyperprolactinaemia. In addition, we found 24% of subjects recruited from the general population to show a <2.5-fold PRL increase to TRH in our earlier study.[5] The majority of patients reached peak PRL levels after TRH at t=20 min, with only ten group C patients reaching peak PRL at t=60 min. Therefore, it is not necessary to extend the TRH stimulation test to t=120 or t=180 min before the maximal PRL response can be assessed. In accordance with earlier studies,[8] there was a positive and highly significant correlation of prolactinoma diameter with basal PRL and also with absolute PRL increase after TRH. In addition, hyperprolactinaemic patients without a clear adenoma on the MRI (group C1 and C2) showed a significant and negative correlation of absolute PRL increase after TRH and BMI. In obese women without hyperprolactinaemia, Donders *et al.*[9] showed a decreased PRL and increased TSH response to TRH as compared with normal weight women, possibly related to changes in serotonergic function. However, since a significant relationship between BMI and PRL response to TRH was absent in our controls with similar BMI (*table 2*), this cannot be the only explanation. On the basis of the results of the present study, the TRH stimulation test can be omitted in the work-up of patients with hyperprolactinaemia. However, our study reinforces the need to confirm hyperprolactinaemia using standardised procedures for the assessment of basal PRL.

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Bilateral adrenal tumour

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A 40-year-old homosexual Dutch man presented to the outpatient clinic with progressive malaise and pain in the left flank. He had been coughing for four months with fever up to 39°C accompanied by night sweats.
Over the last two months his weight had dropped by 12 kg. He experienced exertional dyspnoea, malaise and a progressive pain in the left flank. On physical examination the body temperature was 38.5°C, blood pressures were repeatedly around 120/65 mmHg and he had a tachycardia of 144 beats/min with signs of peripheral vasoconstriction.
The spleen was enlarged and palpation of the left flank was painful. Laboratory examination showed ESR 22 mm/h (n < 15 mm/h), haemoglobin 9.8 mmol/l (n 8.5-10.7 mmol/l), leucocytes 8.4 x 10^9/l (n 4.0-10.0 x 10^9/l), thrombocytes 263 x 10^9/l (n 150-300 x 10^9/l), lactate dehydrogenase 625 IU/l (n 175-400 IU/l), sodium 135 mmol/l (n 136-145 mmol/l), potassium 5.0 mmol/l (n 3.6-5.2 mmol/l) and creatinine 102 µmol/l (n 65-115 µmol/l). The chest X-ray was normal. In the search for a malignancy, such as a lymphoma, computer tomography (CT) of the abdomen showed bilateral enlargement of the adrenal glands (10 x 10 cm), spleen enlargement and multiple lymph nodes around the aorta (*figure 1*).
Consequently, a Synacthen test was carried out, which showed a blunted response of cortisol.

WHAT IS YOUR DIAGNOSIS?

See page 62 for answer to photo quiz.

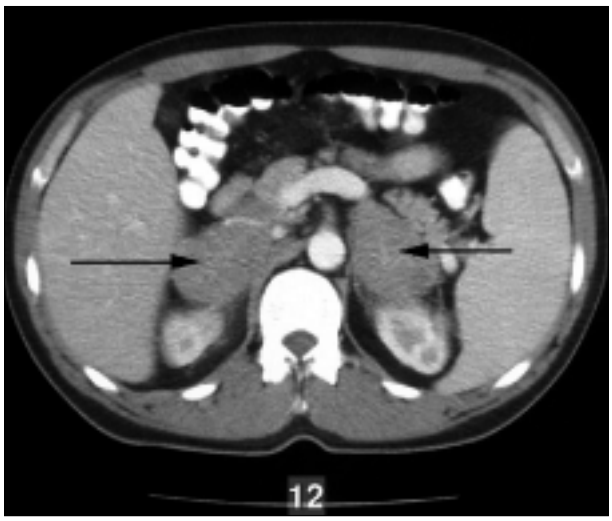

Figure 1
CT scan of the abdomen, showing bilateral enlargement of the adrenals

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CASE REPORT

Bleomycin and scuba diving: to dive or not to dive?

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ABSTRACT

Bleomycin is to treat patients with testicular cancer and lymphoma. Bleomycin can bind to DNA and chelate iron. The resulting complex can form an intermediate capable of interacting with oxygen to produce reactive oxygen species, particularly superoxide. Administrating high-inspired oxygen concentrations (e.g. during anaesthesia or acute illness) has been reported to exacerbate pulmonary injury. The duration of risk after bleomycin chemotherapy is unknown. Here we discuss our advice to a young male patient, who was successfully treated with bleomycin for testicular cancer, concerning the safety to return to scuba diving. Since scuba divers are exposed to high partial oxygen pressures (depending on the depth of the dive) we discouraged this patient from resuming scuba diving.

INTRODUCTION

Bleomycin is given in the standard regimen for treating testicular cancer. The incidence of fatal pulmonary toxicity in this low-risk population of young male patients is approximately 2 to 3%. Patients treated with bleomycin are sensitive to oxygen-mediated lung injury. Here we discuss a question patients frequently ask concerning the safety of returning to scuba diving following bleomycin therapy.

CASE REPORT

A 35-year-old man was admitted to our hospital because of a painless enlargement of the left testis, without further symptoms. Ultrasonography demonstrated a solid mass in the left testis. Laboratory examination showed an elevated serum chorionic gonadotropin (β-hCG) of 1100 IU/l (normal: <2) and a serum lactate dehydrogenase of 520 IU/l (normal: 160-320); serum alfa-foetoproteine was normal. Chest X-ray was normal. CT scan of the abdomen showed retroperitoneal lymphadenopathy (maximal diameter 6 cm). The patient proceeded to inguinal orchidectomy with removal of the affected testis. Histopathologically the removed testis consisted of choriocarcinoma. Since this patient had a stage IIC carcinoma with a good prognosis, he was treated with three courses of BEP (bleomycin, etoposide and cisplatin). No pulmonary toxicity was observed. Subsequent radiographic evaluation showed no residual disease and tumour markers normalised. At this time our patient, who used to be an active scuba diver, asked whether it was safe to return to scuba diving after having undergone chemotherapy containing bleomycin. We will discuss our considerations based on a literature search.

BLEOMYCIN

The bleomycins are a family of cytotoxic glypeptide antibiotics isolated from *Streptomyces verticullis*, with a molecular weight of approximately 1500 D. All contain a unique structural component, bleomycinic acid, and differ only in their terminal alkylamine group. Bleomycin A2, the predominant peptide, and a series of analogues are prepared by total chemical synthesis.[1] The primary biochemical action of the A2 peptide is to produce single- and double-strand breaks in DNA. This breakage is reflected in the chromo-

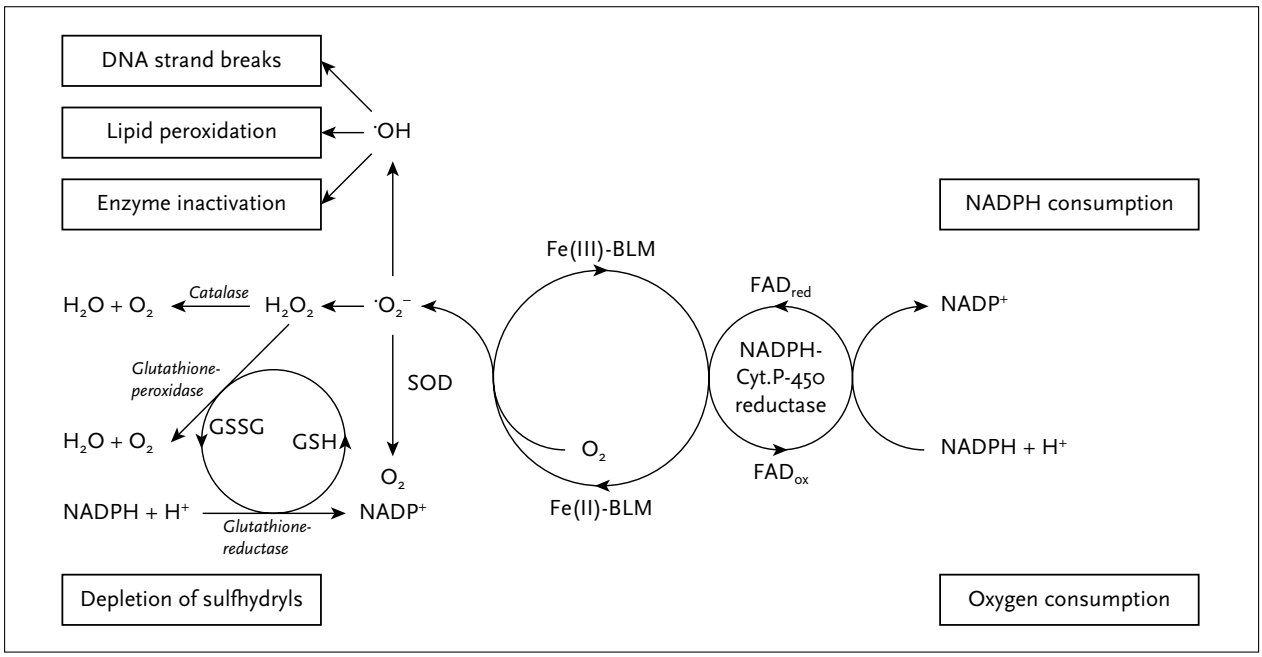

Figure 1

Redox cycling of the iron bleomycin complex with subsequent 'oxidative stress' caused by the formation of reactive oxygen species (superoxide radical $\cdot O_2$-, hydroxyl radical $\cdot OH$, hydrogen peroxide H_2O_2), potentially toxic reactions and enzymatic detoxification mechanisms

SOD = superoxide dismutase, GSSG = oxidised glutathione, GSH = glutathione, NADPH = nicotinamide adenine dinucleotide phosphate (reduced form), NADP+ = nicotinamide adenine dinucleotide phosphate (oxidised form), FADred = flavin adenine dinucleotide (reduced form), FADox = flavin adenine dinucleotide (oxidised form), BLM = bleomycin.

somal gaps, deletions and fragments seen in cytogenetic studies of whole cells. The mechanism of DNA breakage has been clarified by investigation of the action of bleomycin on both viral and mammalian DNA and results from the production of free radicals by an Fe(II)-bleomycin complex intercalated between opposing strands of DNA (see *figure 1*). The initial step in this reaction sequence seems to be the production of an activated bleomycin-Fe(II)-O_2 complex. The activated complex then binds to DNA. At saturating concentrations of bleomycin, one molecule of drug is bound per four to five base pairs of DNA.[2] The second step in the action of bleomycin is the induction of DNA breaks, mediated by free radicals produced by the activated bleomycin-Fe(II) complex. The importance of Fe(II) is indicated by the observation that iron-chelating agents inhibit the DNA scission reaction.[3] The enzyme-like bleomycin-Fe(II) complex induces the reduction of molecular oxygen to superoxide and hydroxyl radicals.[4] In this process, Fe(II) undergoes oxidation to Fe(III). The hypothesis that oxygen radicals participate in the DNA cleavage mediated by bleomycin is based on several observations. First, strand breakage requires the presence of O_2 and ceases in an anaerobic environment.[5,6] Second, the oxidation of bleomycin-Fe(II) requires oxygen consumption.[3] Finally free-radical scavengers and superoxide dismutase (which inactivate O_2 radicals) inhibit DNA strand breakage in *vitro*[7] and pulmonary toxicity *in vivo*.[8]

BLEOMYCIN AND PULMONARY TOXICITY

Intracellular bleomycin is inactivated by an aminohydrolase that is found in both normal and malignant cells.[9] The enzyme cleaves the carboxamide amine from the β-aminoalaninamide, yielding a weakly cytotoxic deamido-bleomycin. Interestingly, this enzyme is present in relatively low concentrations in lung and skin, the two normal tissues most susceptible to bleomycin damage.[10] Several distinct pulmonary syndromes have been associated with the use of bleomycin, such as bronchiolitis obliterans with organising pneumonia (BOOP), eosinophilic hypersensitivity and, most commonly, interstitial pneumonitis.[11] In its later stages interstitial pneumonitis can be complicated by progressive interstitial fibrosis, hypoxia and death. Pulmonary toxicity, usually manifesting with cough, dyspnoea and bibasilar pulmonary infiltrates on chest X-ray film, occurs in 3 to

5% of patients receiving a total dose of less than 450 units of bleomycin, increasing significantly to a 10% incidence in those treated with greater cumulative doses.[12] Although the risk of lung toxicity increases with cumulative doses greater than 450 units, severe pulmonary sequelae have been observed at total doses below 100 units. In the standard regimen for treating testicular cancer, bleomycin is given in doses of 30 units weekly for 9 to 12 doses, and the incidence of fatal pulmonary toxicity in this low-risk population of young male patients is about 2 to 3%.[13,14] Pulmonary function tests, particularly the carbon monoxide diffusing capacity, are of possible value in predicting a high risk of pulmonary toxicity. However, most patients treated with bleomycin show a progressive (10 to 15%) deterioration in diffusion capacity with increasing total dose and a more marked increase in changes above 240 units total dose. It is not clear whether the diffusion capacity test can be used to predict which patients will subsequently develop clinically significant pulmonary toxicity.[15] In advanced stages in the evolution of bleomycin pulmonary toxicity, the diffusion capacity as well as arterial O_2 saturation and total lung capacity become markedly abnormal. Besides the total dose of bleomycin given, various other factors have shown to increase the pulmonary toxicity of bleomycin: prior radiation of the lung parenchyma,[16,17] administration of high fractional-inspired oxygen concentration,[18-22] the age of the patient[23] and renal insufficiency (bleomycin is cleared by the kidneys).[24] The sensitivity of bleomycin-treated patients to high concentrations of inspired O_2 is intriguing in view of the molecular action of bleomycin, which is dependent on, and mediated by, the formation of oxygen-derived free radicals. Goldliner *et al.* observed five testicular tumour patients treated with 135 to 595 units of bleomycin 7 to 12 months earlier who underwent retroperitoneal lymph node dissection or resection of pulmonary metastases while receiving an intraoperative fractional concentration of inspired oxygen (FIO_2) ranging from 0.35 to 0.42.[18] All five developed respiratory failure postoperatively and died.[18] A reduction in inspired O_2 to an FIO_2 between 0.22 and 0.25, and a decrease in fluids administered during surgery, prevented mortality in subsequent patients.[18] It has been shown that a greater degree of experimental lung injury with oxygen was found at 8 versus 21 days following intratracheal bleomycin.[25] Nevertheless, in the above-mentioned report by Goldiner *et al.* the mean time between bleomycin administration and surgery was 9.6 months.[18] Thus, the period of time when oxygen administration appears to be safe following bleomycin has not been established. Therefore, current safeguards for anaesthesia of bleomycin-treated patients (both with a history of bleomycin toxicity and even those with previous drug exposure without clinical toxicity) include the use of the minimal tolerated concentration of inspired oxygen and modest fluid replacement to prevent pulmonary oedema.[26]

SCUBA DIVING

Very few scuba divers, and even fewer sport scuba divers, use oxygen in their tanks. The vast majority of sport divers use compressed air (21% oxygen). The partial inspiratory oxygen pressure (PIO_2) is a function of the fractional concentration of inspired oxygen (FIO_2), the barometric pressure (PB), and the partial pressure of water vapour (PH_2O) in humidified gas; that is $PIO_2 = FIO_2 (PB - PH_2O)$.[27] So the partial pressure of oxygen in the inspired (compressed) air is a direct function of the depth of the dive. For every 9.9 m depth of a seawater dive, the ambient barometric pressure to which the diver is exposed increases by 1 atm. At a dive depth of 19.8 m of seawater (3 atm total pressure), the partial pressure of inspired oxygen in a scuba diver breathing compressed air is 0.63 atm, equivalent to breathing 63% oxygen on the surface. At a dive depth of 29.7 m of seawater, not an unusual depth for many sport divers, the partial pressure of oxygen is 0.84 atm, equivalent to breathing 84% oxygen on the surface.

ADVICE REGARDING PREVIOUS TREATMENT WITH BLEOMYCIN AND SCUBA DIVING

Several clinical and animal studies strongly support the relationship between bleomycin toxicity and oxygen therapy. However, there are no data on what time interval is safe between the last dose of bleomycin and oxygen therapy. Bleomycin is especially successful in the BEP (bleomycin, etoposide, cisplatin) regimen against testicular cancer. These young men frequently ask whether it is safe to resume scuba diving. However, the partial pressure of inspired air is dependent on the barometric pressure, which is a direct function of the depth of the dive. Therefore, these patients should be advised that theoretically there is a risk of developing pulmonary damage due to exposure to a higher partial pressure of inspired oxygen while scuba diving. However, published data regarding the safety of exposure to high concentration oxygen during recreational activities such as scuba diving are limited and patients should be counselled that safety cannot be assured during these activities.[28] Since the period of time when oxygen administration appears to be safe following bleomycin has not been established (see above) we think scuba diving should be discouraged, even several years after bleomycin treatment.

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CASE REPORT

Tension pneumopericardium caused by positive pressure ventilation complicating anaerobic pneumonia

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ABSTRACT

A 22-year-old man was admitted with pneumonia. He was immediately intubated and positive pressure ventilation was initiated. Blood and sputum cultures showed *Bacteroides fragilis* and *Corynebacterium* sp., which were treated with metronidazole and clindamycin. Three weeks later his blood pressure suddenly dropped with an elevation of the central venous pressure. Chest X-ray revealed a pneumopericardium. A parasternal mediastinotomy with partial pericardiectomy was immediately performed. On opening the pericardium his blood pressure normalised. The patient gradually recovered and six weeks after admission he was extubated. Two weeks later he was discharged. A pneumopericardium without previous thorax trauma is very rare and early recognition is imperative because a tension pneumopericardium with cardiac tamponade may develop, as happened in this case. A tension pneumopericardium has to be treated with immediate pericardiocentesis followed by partial pericardiectomy to avoid recurrence.

INTRODUCTION

A pneumopericardium is an uncommon disorder that is most often caused by blunt or penetrating chest trauma or iatrogenic trauma related to pericardiocentesis, cardiac surgery or positive pressure ventilation. Spontaneous pneumopericardium is very rare and can be caused by direct extension of infectious or neoplastic processes of the lungs or by pericardial infection with gas-forming bacteria. Anaerobic bacteria are relatively common pulmonary pathogens, most often causing infection after aspiration or in periodontal disease. Lung abscesses and empyema are well-known complications of anaerobic pneumonia, but pneumopericardium is extremely rare in these patients. We describe a patient with a tension pneumopericardium during positive pressure ventilation, complicating pneumonia caused by *Bacteroides fragilis* and *Corynebacterium* sp.

CASE REPORT

Patient A, a 22-year-old man, had been ill for three days with a fever of 40°C, unproductive cough and progressive shortness of breath. He was admitted to another hospital with severe dyspnoea and cyanosis. His previous medical history was remarkable due to a spontaneous right-sided pneumothorax one year before, which was treated conservatively. A tall, thin, very ill young man in respiratory distress was seen with a temperature of 40.2°C, a blood pressure of 82/56 mmHg, a pulse of 106 beats/min and an oxygen saturation of 46%, breathing 10 litres oxygen/min. Heart sounds were normal and bibasilar pulmonary rales were heard. The physical examination was also notable for many carious teeth and gingivitis. A chest X-ray revealed bilateral pulmonary infiltrates in the lower lobes. The patient was immediately intubated and mechanical ventilation was initiated. Initially his pneumonia was treated with amoxicillin-clavulanic acid and gentamycin. One sputum culture and two blood cultures taken in the first two days showed *Bacteroides fragilis* and *Corynebacterium* sp.

Antibiotic therapy was then changed to clindamycin and metronidazole. His pneumonia was complicated by a left-sided pneumothorax on the second day after admission and a right-sided pneumothorax on the fifth day, which were both adequately drained percutaneously. On the seventh day pleural fluid culture still showed *Bacteroides fragilis*. Because of increasing problems with mechanical ventilation caused by the adult respiratory distress syndrome (ARDS) on the sixth day, he was transferred to our hospital. He was ventilated in the prone position for 48 hours and needed high ventilation pressures. Both lungs were ventilated separately through a double-lumen tube for three days.

Three weeks after admission, he was slowly recovering and being ventilated with decreasing pressures. Suddenly his blood pressure dropped, his heart rate and central venous pressure increased, and his tidal volumes decreased. On physical examination heart sounds were barely audible and bibasilar rales were heard. Chest X-ray revealed a recurrent partial right-sided pneumothorax and a large amount of gas surrounding the heart, compatible with a pneumopericardium (*figure 1a*). A parasternal mediastinotomy was performed immediately. When the pericardium was opened, a large amount of gas escaped after which his blood pressure, heart rate and tidal volumes normalised almost immediately. A partial pericardiectomy was performed and a new drain was placed in the right hemithorax. A postoperative chest X-ray showed that the pneumopericardium had disappeared (*figure 1b*). After this episode the patient gradually recovered, showing no signs of recurrent pneumopericardium. Six weeks after admission he was extubated, his thorax drains were removed and he was discharged two weeks later. His cellular and humoral immunity proved to be normal and he tested negative for HIV. His carious teeth most probably caused his initial anaerobic pneumonia and he was urgently advised to get dental treatment.

DISCUSSION

A pneumopericardium is a rare disorder most often caused by an abnormal connection between the pericardium and a nearby air-containing structure. In our patient two possible causes for the pneumopericardium were present. In the first place direct extension of the infection with *Bacteroides*, a gas-forming micro-organism, from the pleural space to the pericardium could have occurred, although no fluid or pus was found in the pericardium. Secondly the patient had been ventilated with high positive airway pressures

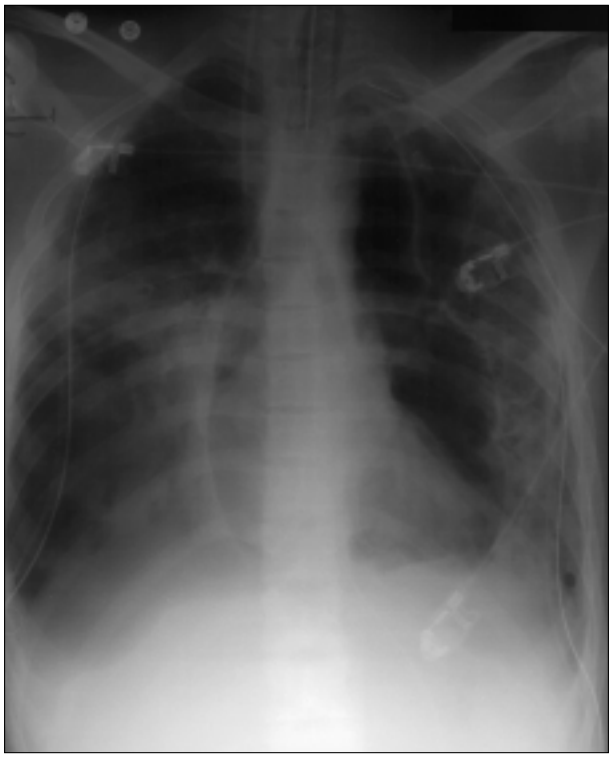

Figure 1a
Chest X-ray showing a partial right-sided pneumothorax, thorax drains on both sides and a large radiolucent rim around the heart and large blood vessels: a pneumopericardium

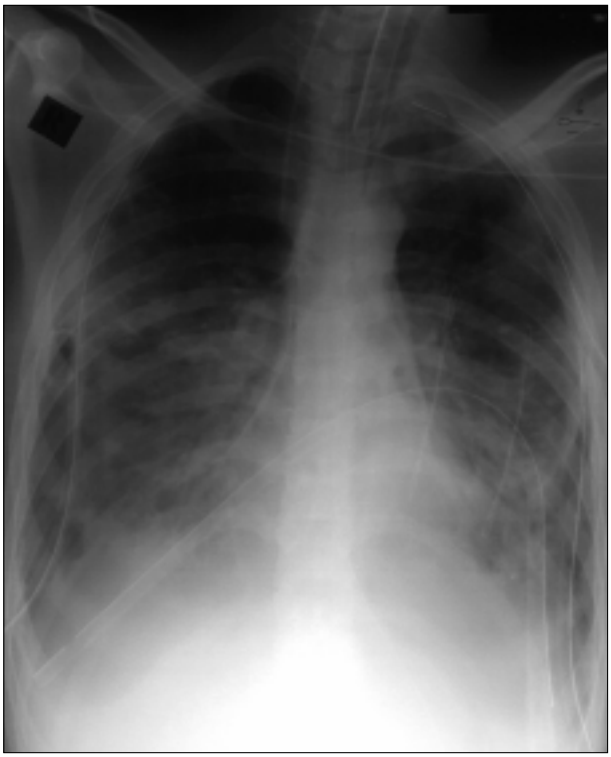

Figure 1b
Chest X-ray after pericardiocentesis, partial pericardiectomy and insertion of a new thorax drain on the right side: the pneumopericardium has completely disappeared

for several weeks, but these pressures were considerably diminished when the pneumopericardium appeared. We speculate that in this case the pneumopericardium was most probably caused by long-term positive pressure ventilation, because there were no signs of infection in the pericardium at the time of pericardiectomy. Also, the apparently adequate antibiotic treatment leading to the gradual recovery made infection less likely as the major cause of this complication. The extensive infection of the lungs and pleural space with *Bacteroides fragilis* and *Corynebacterium* sp. could, of course, have been a contributing factor. Patients with a pneumopericardium caused by *Bacteroides fragilis*, massive *Aspergillosis, Staphylococci, Klebsiella* and *Escherichia coli* have been described before.[1-5] Barotrauma after mechanical ventilation, especially when large tidal volumes or high end-expiratory pressures were used, has been described as a cause of pneumopericardium, especially in neonates.[3]

Clinically, pneumopericardium typically presents with dyspnoea and precordial chest pain.[3] On physical examination heart sounds are usually 'distant' and precordial tympany may be elicited.[1,3] The cardiac examination classically shows a typical auscultatory finding called Hamman's sign. This is a loud gurgling or splashing metallic sound in the precordial area, synchronous with the heartbeat. This sound does not disappear when breathing is stopped and is often heard by the patient. Hamman's sign is not only noticed in pneumopericardium, but also in some cases of pneumomediastinum and very rarely in left-sided pneumothorax.[6] On the chest X-ray pneumopericardium appears as a continuous radiolucent rim of air around the heart and the large blood vessels, and is outlined by a fine line representing the pericardial sac. The air surrounding the heart gives an appearance referred to as the 'halo' sign.[3] Radiologically a pneumopericardium can be reliably distinguished from the more common pneumomediastinum. Pneumomediastinum usually manifests as multiple streaks of air that do not completely surround the heart, and which usually extend into the superior mediastinum and neck while pneumopericardium virtually always consists of a single continuous band of air extending from the hemidiaphragms to the ascending aorta and pulmonary arteries.[6] Air in the pericardial space shifts to the non-dependent side, whereas air in the mediastinum stays fixed.[7] It has been suggested that a decreasing cardiac size on serial chest X-rays, in the presence of a pneumopericardium, strongly supports the diagnosis of tension pneumopericardium.[8] Electrocardiography shows low voltages in patients with pneumopericardium.[3]

CONCLUSION

A tension pneumopericardium is rarely seen, usually occurs after blunt chest trauma or in positive pressure ventilation, and has a very high mortality rate without early recognition and acute intervention.[9,10] Tension pneumopericardium clinically presents with cardiac tamponade, leading to decreased cardiac output, hypotension, increased central venous pressure, tachycardia and pulsus paradoxus, as was seen in our patient. Immediate percutaneous or surgical pericardiocentesis to relieve the tamponade is essential and is usually lifesaving.[1,3] In most cases a (partial) pericardiectomy is necessary to avoid recurrence and prevent pericardial constriction from occurring later.[1] In the absence of tamponade, pneumopericardium can probably be safely observed while treating the patient's primary condition.[11]

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CASE REPORT

Cardiac failure following group A streptococcal infection with echocardiographically proven pericarditis, still insufficient arguments for acute rheumatic fever: a case report and literature update

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ABSTRACT

We recently encountered a 49-year-old female who developed fever due to group A streptococcal (GAS) bacteriaemia spreading to an abscess in the iliac muscle and a bacterial monarthritis of the right knee with a sterile arthritis of her left knee. Treatment was started with a six-week course of intravenous penicillin. She developed a mitral valve insufficiency and pericarditis on the tenth day of admission. In the third week heart failure developed with, on echocardiograph, a high output left ventricular failure without signs of valvulitis or myocarditis. Using a diuretic regimen she was recompensated. Because of the pericarditis with mitral valve insufficiency corticosteroids were given, which had a rapid beneficial effect. A discussion follows on the position of acute rheumatic fever versus post-streptococcal reactive arthritis in this clinical picture and the literature is updated.

INTRODUCTION

The last decades have witnessed a striking resurgence of sequelae of infections with β-haemolytic streptococci and, in particular, group A streptococci (GAS). GAS infection may lead to both pyogenic and non-purulent sequelae. Classically, a non-purulent migratory polyarthritis secondary to GAS infection is attributed to acute rheumatic fever (ARF).[1] The body of evidence has grown, however, that in the developed world several different poststreptococcal syndromes can be recognised, including poststreptococcal reactive arthritis (PSRA).[2-6] ARF differs from PSRA with regards to clinical characteristics, but also to levels of humoral immunity,[7-9] and to genetics.[10] We describe here a patient who interestingly shows a simultaneous purulent and non-purulent sequel due to GAS; this patient does not meet the Jones criteria for ARF, but more or less fulfils the preliminary criteria for PSRA as proposed by Ayoub and Ahmed.

CASE REPORT

A 49-year-old female was admitted because of high fever and malaise. She recalled generalised malaise for 12 days prior to admission without a sore throat or flu-like symptoms. The last two days before admission she had been feeling cold and shivery. Nobody in her family had been ill recently. Several times a week she looked after two small children in her neighbourhood. Physical examination revealed generalised illness with some pharyngitis and fever of between 38 and 39°C. Her heart appeared normal on percussion and auscultation: no murmurs or symptoms of a pericarditic crepitus were heard. On palpation the left upper abdomen was painful, without a clear palpable tumour. Both knees were arthritic. On puncture 60 ml purulent fluid was drawn from the right knee: the leucocyte count was 30 x 10^9/l, a direct preparation showed Gram positive cocci and culture revealed GAS. Puncture from the left knee produced 40 ml clear synovial fluid with a leucocyte count of <0.1 x 10^9/l, a direct preparation showing no micro-organisms and a

negative culture. Laboratory investigation revealed the following: ESR 64 mm/hr (normal: <12 mm/hr), CRP 436 mg/l (normal: <10 mg/l), haemoglobin 6.3 mmol/l (normal: 7.2-9.8 mmol/l), leucocyte count 23.6 x 10^9/l (normal: 4.0-11.0 x 10^9/l) with left-shift, serum creatinine 81 µmol/l (normal: <95 µmol/l), ASAT 76 U/l (normal: <45 U/l), ALAT 90 U/l (normal: <45 U/l), alkaline phosphatase 237 U/l (normal: <80 U/l), gamma glutamyltranspeptidase 140 U/l (normal: <100 U/l), IgG type kappa paraprotein 9.4 g/l with residual gamma globulin 7.0 g/l, and normal IgA/IgG/IgM levels, without Bence Jones proteinuria. Serologically she was negative for rheumatoid factor but had an elevated antistreptolysin O (ASO) >3600 U/l (normal: <200 U/l) and antideoxyribonuclease B (anti-DNase B) >1200 U/l (normal: <200 U/l). Bacteriological culture of blood and purulent synovial fluid were positive for GAS, M serotype 11, T serotype 11 with exo-enzymes A and C.

A chest X-ray, abdominal ultrasonography and echocardiography performed on admission were all normal. Abdominal CT scan on the second day after admission showed thickening of her left iliac muscle suggestive of an abscess in statu nascendi. Drainage of this abscess was impossible. Thoracoabdominal CT scan excluded a generalised lymphadenopathy.

It was concluded that the patient had a sterile left-sided gonarthritis, a bacterial right-sided gonarthritis and an incipient iliac abscess, all due to GAS. She also had a monoclonal paraproteinaemia, which was not thought to be a predisposing factor for the aforementioned septic sequelae, as the total gamma globulin level was adequate for normal humoral immunity.

During a six-week period she was treated intravenously with penicillin 24 x 10^6 U/24hrs. On the tenth day of admission echocardiography revealed a pericarditic fluid and a mitral valve insufficiency. Several days later she gradually developed congestive heart failure. During this third week of admission, congestive heart failure progressed, which echocardiographically was primarily characterised by severe dysfunction of the left ventricle.

Recompensation was reached by using intravenous bumetanide. The severe cardiac dysfunction was echocardiographically not proven to be due to a myocarditis, but it cannot be excluded completely; a cardiac biopsy was not performed as it was thought to be a too risky procedure. As echocardiography had revealed a myocardial dysfunction with pericarditis and a secondary mitral valve insufficiency, a diuretic regimen was combined with a course of prednisone 50 mg daily. She made a quick recovery. After six weeks of intravenous penicillin the iliac muscle abscess had vanished and daily intravenous penicillin was switched to an intramuscular depot of 1.2 x 10^6 U benzathine-benzylpenicillin every three weeks (later every four weeks). She was send home eight weeks after admission on prednisone 5 mg a day, in a tapering dose regimen, and benzathine-benzylpenicillin 1.2 x 10^6 U every three and later every four weeks, for a period of two years. During this treatment, the gammopathy resolved (semi)spontaneously within six months: IgG type kappa 9.4 g/l at the beginning, 7.8 g/l after one month, 1.6 g/l after four months and not detectable after six months.

Findings of sequential echocardiography

An echocardiogram on the third day after admission revealed normal left and right ventricular function without further abnormalities. One week later there was a mild mitral valve insufficiency with some pericarditic fluid. Gradually a congestive heart failure developed. Two weeks later echocardiography revealed severe dysfunction of the left ventricle, whereas the right ventricle was functioning nearly normally. There were no symptoms compatible with endocarditis, nor were valvulitis or valvular vegetations found; however, a myocarditic component cannot be excluded completely. Five weeks after admission echography revealed normal cardiac functioning without pericarditic fluid: a complete resolution had occurred.

DISCUSSION

Lancefield GAS account for about 3 to 17% cases of septic arthritis.[11] The number of serious invasive streptococcal infections has increased over the last decade,[12] possibly due to spreading of more virulent clones, higher numbers of patients with conditions interfering with immunity, and/or alterations in patterns of child care. Common routes of entry for GAS are the nasopharynx, surgical wounds and the skin; in many patients, however, the portal of entry cannot be ascertained, as was the case in our patient. Next to pyogenic sequelae, GAS infections are known for their non-pyogenic, sterile but sometimes devastating sequelae such as in ARF. The GAS strains epidemiologically associated with epidemics of ARF tend to belong to a limited number of M serotypes, fail to synthesise the alpha-lipoproteinase known as opacity factor, and are often heavily encapsulated. After several decades of a steadily declining frequency of ARF, the past decades have witnessed a striking resurgence of PSRA in the developed world.[6] Next to bacterial factors, host factors play a role, primarily in an individual's susceptibility for developing PSRA or ARF.

Genetically, there are differences between hosts with ARF and hosts with PSRA. ARF is significantly more associated with HLA DRB1*16, and PSRA with HLA DRB1*01.[10] This may at least partly explain a difference in genetic susceptibility between individuals. Immunologically, ARF is associated with a cellular and humoral overstimulation. A number of B lymphocytic antigens have been associated

with ARF.[7] Most promising is the B lymphocytic stimulation of the allogenic cellular surface marker D8/17. Using a selected cut-off level of reactivity with D8/17 positive B cells, 100% of ARF patients but only 14% of controls are positive.[7] This humoral hyper-responsiveness following streptococcal infection appears to only occur in part of the GAS-infected population. *In vitro* elevated D8/17 binding to B lymphocytes has therefore been proposed as a susceptibility marker for developing ARF.[7,8] In PSRA markers of humoral responsiveness have not yet been studied to our knowledge. A pilot study, which we recently performed in Dutch PSRA patients, revealed elevated D8/17 positive B lymphocytes in a minority (28%) of the PSRA patients, suggesting that most Dutch PSRA patients may lack this major risk factor for serious organ involvement as occurs in ARF.[9] This may be due to an absence of humoral disturbance of B lymphocytic hyper-responsiveness, as has been demonstrated to persist *in vitro* in ARF for at least two years after the initial attack.[13] It then becomes questionable whether long-term penicillin prophylaxis is still merited in all patients with GAS-induced PSRA. A five-year monthly penicillin prophylaxis as indicated in ARF would probably mean overtreatment in PSRA. A two-year prophylactic course of monthly penicillin appears to be sufficient to prevent carditis in PSRA in the Netherlands.[14] Whether prophylaxis with a monthly penicillin course is really needed in GAS-induced PSRA warrants further investigation in a randomised controlled trial.

In the patient presented here, heart failure developed concurrently with an intravenous physiological saline infusion together with a penicillin infusion consisting of an extra 43 mmol sodium, comparable with only 280 ml physiological saline infusion extra a day. There were no echographic symptoms of myocarditis, nor was valvulitis present to explain the heart failure. These cardiac sequelae occurred together with arthritis following an M-type 11 GAS infection, which is a type not known from the ARF literature. Clinically, a migratory polyarthritis,[1] or a myocarditis or valvulitis, would have been essential for a diagnosis of ARF meeting Jones criteria. The patient, however, does not meet the Jones criteria but does fulfil the preliminary criteria of PSRA as proposed by Ayoub and Ahmed (*table 1*).[15]

First attacks of ARF are accompanied by carditis in >30%.[16-19] Moderate to severe carditis is usually an indication for corticosteroids, which are generally thought to be superior to salicylates in rapidly resolving acute manifestations. It is suggested that the incidence of carditis in ARF may be somewhat lower in elderly than in younger patients.[20,21] Nowadays, ARF and PSRA are both known to occur sporadically with pericarditis but they also have some dissimilarities, which may be helpful in categorising a patient (*table 2*). PSRA occurs predominantly in adults, whereas ARF predominates in young children.[22] ARF is almost invariably found between 5 to 20 years of age, with a peak incidence at 8 years, contrary to the PSRA patient group in which the mean age is around 32 to 42 years.[4] The predominant type of arthritis differs: ARF is known for its migratory type of polyarthritis occurring in 50 to 100%,[1,13,23,24] whereas PSRA is known for its non-migratory type of monarthritis, pauciarthritis or polyarthritis. A monarthritic or pauciarticular presentation as in the presented patient appears to be another distinction from ARF.

Table 1

Guidelines for the diagnosis of ARF[1] and proposed criteria for the diagnosis of PSRA[15]

SET OF CRITERIA		SCORE OF PRESENTED PATIENT
Modified Jones criteria for ARF		
Major	Carditis	No valvulitis
	Migratory polyarthritis	No
	Sydenham's chorea	No
	Erythema marginatum	No
	Subcutaneous nodules	No
Minor	Fever	Yes
	Arthralgia	No
	Elevated acute phase reactants	Due to septicaemia?
	Prolonged PR interval	No
Proposed criteria for PSRA		
Arthritis: acute onset		Yes
Arthritis: non-migratory		Yes
Arthritis: protracted/recurrent		Possibly
Arthritis: poor response to salicylates/NSAIDs		Yes
Evidence of antecedent streptococcal infection		Yes
No other major Jones manifestation present		Yes
Not fulfilling modified Jones criteria		Yes

ARF = acute rheumatic fever, PSRA = poststreptococcal reactive arthritis. The presence of two major or one major and two minor manifestations indicates a high probability of ARF, if supported by evidence of preceded Group A streptococcal infection.

Table 2
Overview of major differences between GAS-induced ARF and PSRA

	ARF	PSRA
Bacterial causative trigger		
GAS M serotypes	1,3,5,6,18,19,24	9,28[5]
Genetics		
HLA association[10] DRB1*01	No	Yes
DRB1*16	Yes	No
Humoral immunology		
D8/17 elevation[7]	63-100%[7,8]	29%[9]
Clinical sequelae		
Highest prevalence	Developing world	Developed world
Patient age	Young: 5-20 years[24]	Adult: 16-75 years[2-6]
Carditis risk	>30%[16,20]	±0%[2-6]
Pericarditis	Rare[27]	Rare[27]
Myocarditis/valvulitis	50%[27]	6%[27]
Arthritis	Migratory: 50-100%[16,23,24]	Non-migratory: 95%[2-6]
Erythema nodosum/multiforme	<1-7%[20]	33-52%[4,5]
Hepatitis	Sporadic[23]	7-17%[4,5]
Treatment		
Penicillin prophylaxis	5 years/age>18 years[7,8]	1-2 years[9,26]

From the literature we know that socioeconomic environments differ: ARF is still common in developing parts of the world, whereas PSRA occurs sporadically in developed parts of the world. All these factors plea for categorisation of the present patient into the PSRA group.
PSRA may not only develop secondary to GAS, but also secondary to group C and G streptococci (GCS, GGS).[5,25] Antibiotic prophylaxis is not indicated in PSRA secondary to GCS/GGS: non-group A streptococci (NGAS). Therefore, the more benign NGAS-induced PSRA and GAS-induced PSRA should be differentiated.[4-6] ARF can only occur following infection with GAS, particularly the M serotype 1,3,5,6,18,19 and 24, so-called rheumatogenic serotypes.[6,21] In PSRA, the as yet non-rheumatogenic M serotypes 9 and 28 have been described.[5]
If PSRA is diagnosed secondary to GAS, a two-year period of monthly penicillin prophylaxis is given similar to that used in ARF. Although not proven in a randomised controlled trial, a monthly penicillin prophylaxis appears to be safe;[4-6,14] in GAS-induced PSRA it may therefore be justified to advocate penicillin prophylaxis for a one-year period and then discontinue it if carditis has still not occurred.[26] In ARF penicillin prophylaxis should be continued for a minimum of five years or until the age of 21 years, whichever is longer.[26] Any patient with sequelae due to streptococci should be appropriately categorised into one of the poststreptococcal disorders so that proper advice can be given on penicillin and/or corticosteroids. The presented patient clearly shows the dilemmas of diagnosis and treatment in such cases and underscores the necessity of randomised controlled trials into the treatment options of PSRA patient groups.

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ANSWER TO PHOTO QUIZ (ON PAGE 49)

BILATERAL ADRENAL TUMOUR

The clinical presentation and normal urinary excretion of catecholamines ruled out pheochromocytoma.
The final diagnosis was made from a CT-guided biopsy of the adrenal gland, showing granulomas, lymphohistiocytic aggregates (*figure 2*) and micro-organisms that were identified as yeasts (*figure 3*).

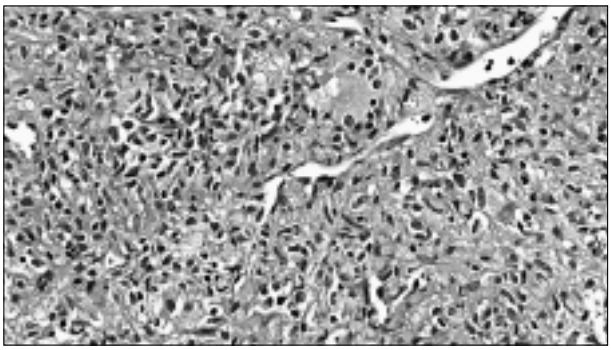

Figure 2
Adrenal biopsy showing clusters of histiocytes with a few multinucleated giant cells (H&E, 200x)

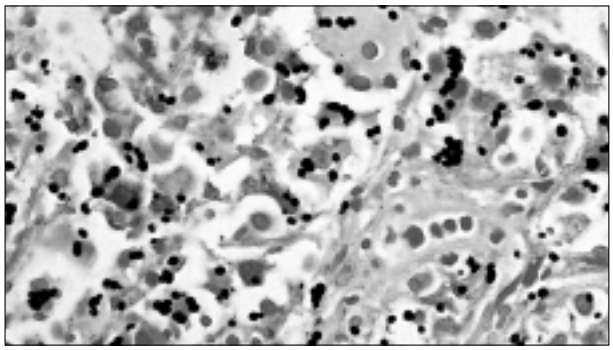

Figure 3
Multiple yeasts within histiocytes, consistent with Histoplasma capsulatum (Grocott, 400x)

Initially the tissue was not sent in for culture. The snap frozen material was cultured, but the cultures remained negative. Leishmaniasis was ruled out morphologically and based on the presence of budding organisms. Morphology and measurements of the organisms (2-5 μm) left a differential diagnosis of *Candida albicans, Candida glabrata* and *Histoplasma capsulatum*. Since the organisms were mainly present within histiocytes a diagnosis of generalised *C. glabrata* infection was unlikely. *C. albicans* was thought to be less likely, because of the absence of pseudohyphae and the mainly intracytoplasmatic localisation of the micro-organisms. Therefore, the preferred diagnosis was that of a disseminated *Histoplasma capsulatum* infection.

Repeated questioning revealed that this patient frequently travelled to areas in Indonesia with many bat caves, although his last visit was two years ago. He was tested HIV negative and his cellular immunity was normal. After suppletion of cortisol and treatment with itraconazol his clinical condition improved rapidly and his symptoms resolved. After six months of treatment the adrenal glands were reduced to their normal size.

CONCLUSION

Adrenal tumours larger than 4 cm are highly suspect for malignancy.[1,2] Although disseminated histoplasmosis with bilateral adrenal gland involvement and adrenal insufficiency is very rare in western Europe, this case shows that bilateral enlargement of the adrenal glands of more than 4 cm is not by definition malignant, and hormonal testing and histology are imperative.

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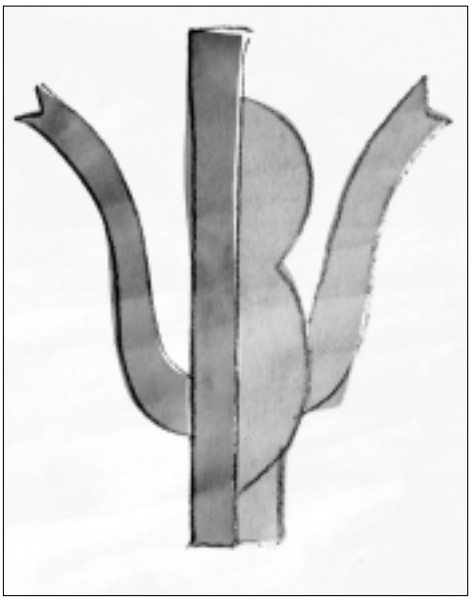
Netherlands
The Journal of Medicine

'Doorgezaagd'

Klaas Gubbels



Klaas Gubbels (1934) first attended the Academy of Art in Rotterdam. When he moved to Arnhem he pursued his studies at the Academy of Art there.
In his work as painter and graphic artist, Klaas limits his motifs to a small range which he cherishes: tables, coffee pots, pipes, teapots and bottles. Usually these motifs – often completed with supporting forms, his half-figurative metaphors, symbols of a mood or situation – are only for the artist to interpret.
A special feature of his work is that it is painted 'flat', meaning not spatial, without 'escape routes' to a third dimension. Klaas calls himself a 'brewer'; everything he sees or that goes through his mind leads to imagination of flattened utensils, which can be lonely, sad, happy or wanton.
His characteristic figurative language have made him an international well-respected pictorial artist.
This recently made print entitled 'Doorgezaagd' is a block printing subscribed by hand, printed on beautiful hand-dipped paper. A very limited edition of these original prints is available at a price of € 350. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573 CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

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