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EDITORIAL

Experimental malaria in human volunteers: ethical aspects

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Infectious diseases are today – as in the past – a great threat to human health. They continue to cause considerable morbidity and mortality, especially in the less-developed countries. Great efforts are necessary to explore new methods of prevention and treatment, particularly new vaccines and antibiotics. In clinical research healthy volunteers are sometimes used to study the pathogenesis of infectious diseases and the efficacy of potential vaccines. Healthy volunteers have been exposed to influenza viruses, cholera and salmonella bacteria, and malaria parasites.
In this issue Verhage and coworkers from the Departments of Medical Microbiology and General Internal Medicine, Radboud University Medical Centre, Nijmegen, the Netherlands, describe the clinical outcome of experimental human malaria induced by infected mosquitoes.[1]
Such infection-inducing challenge experiments evoke serious ethical questions. They seem to be in conflict with the traditional guiding principle in medicine: *primum non nocere,* first of all, do not injure. However, on second thoughts, are they really different from phase 1 studies of vasoactive drugs administered intra-arterially in healthy volunteers?

There are now well-accepted international ethical guidelines for biomedical research involving human subjects, based on the Nuremberg code (1947), the Declaration of Helsinki (World Medical Association, 1964) and a number of other national and international guidelines. In the Netherlands the Medical Research involving Human Subjects Act came into force on 1 December 1999. Medical research involving human subjects may only be carried out if a recognised review committee has approved it, and the Dutch Act regulates this review in some detail.
The ethical aspects of infection-inducing challenge experiments have been very well discussed by Miller and Grady.[2] The most important questions that should be considered by the investigators and the institutional review committee are the following: Is there a scientific and/or societal rationale for the experiments? What is the research experience of the investigator(s)? What are the selection criteria for the volunteers? What are the risks and discomfort for them? Is there adequate information and do the volunteers have adequate (mental) capacity to really give informed consent? What is the financial compensation and will the insurance cover any harm to the subject caused by the research?

Let us now discuss these questions in more detail, in relation to the study by Verhage and coworkers as well as to the Dutch Medical Research involving Human Subjects Act.
Assessment of the study's scientific and/or societal rationale should include an assessment that the research will lead to the advancement of medical science and that this knowledge could not be achieved without the participation of human subjects or with a less loaded and less risky intervention. The main objective of the study was to evaluate the clinical safety of different (new) protocols for human experimental malaria, but a second objective was to measure the contamination and its reproducibility. The results can be used to validate the efficacy of potential malaria vaccines in human studies, to limit costly and unfeasible field trials in endemic malaria areas.
The methodology of the research has to be of the requisite standard and the research should be done by investigators with relevant research expertise.
It is clear that in this type of study healthy adult volunteers with an adequate mental capability should participate. Screening and exclusion criteria should be strictly defined.

Unexpected adverse events are always possible. Two volunteers developed psychiatric side effects after the onset of chloroquine treatment. The Dutch Act states that persons can not be included in research when their actual or legal relationship with the investigator(s) could interfere with the principle of free consent. In the study this problem was avoided by recruiting the volunteers through general advertisements in public places and local journals.
It is now well accepted that the risk and discomfort of nontherapeutic medical research studies should be minimal when the subjects are children or incapacitated persons who can not give free consent. Moreover, studies that can be done with similar profit in capacitated adults are not allowed in children or in incapacitated persons. However, when in such studies the subjects are well-informed healthy adults, who are capable of giving voluntary free consent, the risk and discomforts could be more than minimal. It is evident that the symptoms of experimental malaria in the volunteers are not 'minimal'. They require careful monitoring, not only to report adverse events but also to determine if an (acute) intervention is necessary and an effective treatment should be started. What degree of risk and discomfort is acceptable in such studies when there is no benefit for the participating subjects? The Dutch Act states that the risk and burden for the subject should be in proportion to the potential value of the research. The argument favouring this type of research is that the effect of vaccines on the malaria situation in the world (annually more than 300 million acute illnesses and one million deaths) justifies this type of research with considerable risk and discomfort for the participants. Should there be a limit to the extent of risk and discomfort in such studies when well-informed competent adult volunteers have the right to decide how much they are willing to accept? Where is the limit that it would be unethical to start such studies? Ultimately, the medical ethical review committee should make a decision after careful consideration.
The importance of information (informed consent process) should not be underestimated. There are several publications indicating that persons who participate in research studies do not fully understand the purpose of the study, the procedures, risks and discomfort. In general the investigators themselves should spend more time than they have done until now in the informed consent procedure to ensure that the volunteers really understand what is being told. Subjects who participate in research studies may withdraw at any time without consequences. This cannot be done in the malaria study: once you are in, you cannot withdraw, this should be explained to the participants.

In the study by Verhage and coworkers it is not mentioned if the volunteers were financially compensated. It is generally agreed that such a compensation is acceptable and should be based on the time and inconvenience of the participation. Influencing the volunteer's capacity to decide freely on his participation by offering him too much money should be avoided. According to the Dutch Act research cannot be conducted unless a contract of insurance has been closed covering liability for death or injury resulting from the research. Such insurance does not need to cover injury which is inevitable or almost inevitable.
The research study by Verhage and coworkers poses important ethical as well as research questions. The risks and the burden are justified by the consequences for public health and therefore the study is ethically acceptable. We should be grateful to the volunteers who were willing to participate in this study, which may help to test potential malaria vaccines in the future.

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Reduced-intensity conditioning regimens in malignant haematological diseases

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ABSTRACT

Allogeneic stem cell transplantation is a potentially curative procedure for patients with haematological malignancies. Conventional, myeloablative conditioning is, however, poorly tolerated by patients of advanced age, those receiving second transplants and those with concomitant diseases. Based on recognition of the importance of a graft-versus-disease (GVD) effect in curing malignant haematological disease, reduced intensity conditioning (RIC) as preparation for allogeneic stem cell transplantation has been developed for these patients. Although large prospective randomised clinical trials with significant follow-up are lacking, transplant-related morbidity and mortality of RIC transplants seem to compare favourably with conventional conditioning in this group of patients.

INTRODUCTION

High-dose myeloablative chemotherapy followed by allogeneic haematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for several haematological malignancies. Prior to the infusion of donor stem cells, high-dose chemotherapy and/or radiotherapy, collectively called 'conditioning', is used to eliminate the disease. In addition, immunosuppressive drugs are administrated to prevent graft rejection and extensive graft-versus-host disease (GVHD), which, in its acute form, is featured by potentially life-threatening allogeneic T-cell responses primarily affecting the immune system, skin, intestinal tract and liver. Patients undergoing HSCT therefore experience a prolonged period of profound immunodeficiency, which renders them highly susceptible to opportunistic, potentially life-threatening, infections. The risk of regimen-related toxicity and graft-versus-host disease rises with increasing age and poor performance status of the patient. Although allogeneic transplantation after myeloablative conditioning has been successfully performed in patients over the age of 60, this procedure is generally limited to younger patients (<55 to 60 years) in good health status.[1-7] Since the median age for many haematological malignancies to occur is over 50 years, only a minority of patients can benefit from myeloablative allogeneic HSCT.[2]

In the past years, it has become increasingly evident that the curative potential of HSCT is not primarily due to the myeloablative conditioning regimen. The graft-versus-disease (GVD) effect, i.e. allo-reactivity of donor immune cells against the host's tumour, plays a major role in controlling or even eradicating the patient's malignancy.[2,8] This phenomenon was first described in chronic myeloid leukaemia (CML), in which relapses were successfully treated with donor lymphocyte infusions (DLI). Responses to DLI and a GVD effect were not only seen in patients with CML, but also in patients with other haematological malignancies who relapsed after allogeneic transplantation.[9,10] This implicates that the intensity of the conditioning regimen may not be as important as previously believed and less aggressive preparative treatments may be suitable if the immunosuppressive effect of the regimen is sufficient to establish donor engraftment.[3] Recognition of the importance of this GVD effect for success in allogeneic HSCT for haematological malignancies has led to a new therapeutic strategy, reduced intensity conditioning (RIC) transplantation (synonyms: reduced

intensity stem cell transplantation (RIST), reduced intensity transplant (RIT), 'mini'-transplant).[4,5,11,12] The purpose of RIC is to enhance tolerance of the host to the graft while permitting the establishment of donor haematopoiesis and using antidisease properties of donor lymphocytes for disease eradication, at the same time avoiding the extensive early toxicity of standard myeloablative HSCT.[4,11] By exploiting the GVD effect and reducing the toxicity of the transplantation,[3,13] elderly patients, recipients of second transplants and patients with severe concomitant other organ disease, who are at high risk of transplant-related mortality (TRM), may be successfully treated by this new RIC approach.[8,14]

REDUCED INTENSITY CONDITIONING

The use of donor lymphocyte infusions (DLI) and recognition of a GVD effect have been central to the philosophy of RIC stem cell transplantation. Differences in susceptibility to GVD effects are seen among different malignancies, as shown in *table 1*.[15] Patients with chronic myeloid leukaemia are most likely to respond, but responses have also been seen in patients with acute myeloid leukaemia, chronic lymphocytic leukaemia, myeloma and lymphoma. Patients with acute lymphoblastic leukaemia (ALL) seem least likely to respond.[16-18] Recognition of this potency of DLI has driven the development of RIC regimens, which are increasingly being used for allogeneic HSCT.

Several regimens have been investigated in an attempt to reduce procedure-related toxicity in elderly or heavily pretreated patients, or in patients with medical comorbidities precluding the use of myeloablative preparative regimens.[19] Most protocols for RIC regimens use fludarabine combined with low-dose total body irradiation (TBI),[20] low-dose cyclophosphamide[15,21,22] or high-dose alkylating agents such as melphalan.[8,12,23,24] Examples of different RIC regimens are shown in *table 2*. Reported TRM of these regimens varies between 15 and 20%,[19,25,26] which is low compared with conventional conditioning considering

Table 1

Complete response rates after donor lymphocyte infusion (DLI) in different haematological diseases

DIAGNOSIS		INCIDENCES OF COMPLETE RESPONSES AFTER DLI
Chronic myeloid leukaemia:	Overall	60%[9]
	Chronic phase	76%
	Accelerated phase	33%
	Blastic phase	17%
Acute myeloid leukaemia/myelodysplastic syndrome		15-26%[9,18]
Acute lymphoblastic leukaemia		3-15%[9,18]
Chronic lymphocytic leukaemia		29%[60]
Multiple myeloma		5-29%[18,67]

Table 2

Reduced intensity conditioning regimen

HOUSTON[23]	LONDON[36]
Fludarabine (25 mg/m²) x 5	Fludarabine (25 mg/m²) x 5
Melphalan (90 mg/m²) x 2; or (140 mg/m²) x 1	Cyclophosphamide (1 g/m²) x 2
PBSCT	PBSCT/BMT
GVHD prophylaxis: tacrolimus/methotrexate	GVHD prophylaxis: cyclosporine/methotrexate
HOUSTON[74]	**BARCELONA[78]**
Fludarabine (25 mg/m²) x 5	Fludarabine (30 mg/m²) x 5
Cyclophosphamide (1g/m²) x 3	Melphalan (70 mg/m²) x 2 or (80 mg/m²) x 1 or busulphan
ATG (20 mg/kg) x 3	(1 mg/kg x 10 doses) x 3
PBSCT/BMT	PBSCT
GVHD prophylaxis: tacrolimus or cyclosporine/methotrexate	GVHD prophylaxis: cyclosporine A/methotrexate
JERUSALEM[52]	**SEATTLE[20]**
Fludarabine (30 mg/m² daily, 5 days)	Fludarabine (30 mg/m²) x 3
Busulphan (4 mg/kg daily, 2 days)	TBI 200 cGy TBI (dual cobalt source or linear accelerator, 7 cGy/min)
ATG (10 mg/kg) x 4	
PBSCT	PBSCT
GVHD prophylaxis: cyclosporine/methotrexate	GVHD prophylaxis: cyclosporine/MMF

PBSCT = peripheral blood stem cell transplantation; GVHD = graft-versus-host disease; BMT = bone marrow transplantation; ATG = antihuman T-lymphocytes globulin; TBI = total-body irradiation; MMF = mycophenolate mofetil.

the advanced age and concomitant disease or previous treatments of the patients receiving these transplants. Fludarabine is an effective immunosuppressive, rather than myeloablative, agent. It eliminates T-cells and is used to augment pretransplantation immunosuppression in order to improve the engraftment of donor cells for a better exploitation of the GVD effect.[2,15] In addition, to reduce the frequency of acute GVHD, T-cell depletion (TCD) by anti-lymphocyte serums such as antithymocyte globulin (ATG) and CAMPATH-1H is used in some protocols.[14,19,24,27,28] A dose-dependent effect of ATG on acute GVHD was shown by Mothy *et al.* with a tendency toward better progression-free survival (PFS) for patients receiving a low ATG dose as compared with patients receiving a high ATG dose (25 and 22% respectively).[19] A disadvantage of TCD is the increased rejection rate seen in TCD RICs. However, a formal comparison between T-cell undepleted and depleted RIC as concerns, for example, overall survival has not yet been made.

The occurrence of moderate to severe GVHD increases the risk of life-threatening infections.[19,26,29,30] On the other hand, GVHD also has a beneficial role since it is associated with decreased risk of disease progression in several studies.[12,19,25,31,32] As GVHD is poorly tolerated by elderly or debilitated patients, this can explain higher rates of TRM after RIC in patients ≥60 years (TRM 18% <60 years *vs* 35% ≥60 years) as shown by Gómez-Núñez *et al.*[25] However, TRM appears to be unacceptably high (>50%), only in the presence of additional adverse factors, such as poor performance score and a previous autologous HSCT. Therefore, age itself should not preclude RIC transplants. A conditioning regimen based on a combination of the antitumour and immunosuppressive activity of melphalan and the immunosuppressive activities of both melphalan and fludarabine was developed by the MD Anderson group. They reported consistent engraftment and durable remissions in some patients with advanced haematological malignancies,[33] which was subsequently confirmed by other groups. In combination with ATG, it also leads to engraftment in recipients of matched unrelated donor grafts.[23]

DLI can be used in addition to RIC regimens to enhance the GVD effect.[11] Indications for DLI after RIC allografts are mixed donor and recipient chimerism, disease progression, failure of the transplantation to achieve a complete remission, and as pre-emptive treatment against disease relapse or on the assumption that they may eliminate undetectable minimal residual disease.[5,19,34-36] Complications of DLI are acute and chronic GVHD and especially pancytopenia,[9] probably due to depletion of host-derived normal haematopoiesis from the marrow. Timing and dosage of DLI should therefore be adapted accurately to chimerism and tumour response.[5]

TOXICITY OF REDUCED INTENSITY CONDITIONING REGIMENS

Toxicity of treatment can be divided into regimen-related toxicity and toxicity associated with GVHD. In general, short-term regimen-related toxicities are mild after RIC treatment,[25] but they may still be significant, depending on the conditioning regimen that is used. As the dose of melphalan in the fludarabine-melphalan regimen is 140 to 180 mg/m^2, significant mucosal, pulmonary, renal, hepatic and cardiac toxicity is to be expected[33,37] and was, in fact, not different from that observed in parallel studies by Besien *et al.* in patients treated with conventional regimens including TBI.[33]

Martino *et al.* showed no significant differences in the probability of infection-related mortality between a standard conditioning regimen and a RIC regimen consisting of fludarabine in combination with busulphan or melphalan, 19 and 17% respectively, although less *Streptococcus viridans* septicaemias and CMV infections were seen in the RIC group.[26] Similar rates of infection between RIC and HSCT are contradictory to the assumption that a nonmyeloablative regimen in RIC should lead to fewer infections. A possible explanation could be the profound immunodeficiency due to immunosuppressive treatment against GVHD. Moreover, median age of patients receiving conventional HSCT was lower compared with patients receiving RIC, 38 and 54 years respectively.

Toxicity of the gastrointestinal (GI) tract is currently one of the most important dose-limiting factors for high-dose treatment with autologous or allogeneic, haemopoietic stem cell support.[38] Clinical GI toxicity of RIC transplants has been reported as being very moderate, depending on the regimen used.[28,38] Also, preclinical studies offer good reasons for the assumption that allogeneic transplantation with RIC causes less damage to the gut mucosa barrier than myeloablative conditioning.[22] Therefore, Johansson *et al.* investigated the intestinal barrier function in patients undergoing HSCT with RIC.[38] A significant increase in intestinal permeability during transplantation was measured in patients who received conventional, myeloablative conditioning, while patients receiving RIC did not develop any significant increase in intestinal permeability. All patients receiving myeloablative therapy were in need of therapy against GI toxicity (nausea/vomiting, oral pains, and/or diarrhoea) during transplantation, while only two out of nine RIC transplant patients needed this therapy. Most patients receiving RIC were able to continue enteral feeding during the transplant course.[38]

A frequent and often lethal complication of bone marrow transplantation is veno-occlusive disease (VOD). VOD is a

clinical syndrome resulting from hepatic toxicity, appearing shortly after bone marrow transplantation and is characterised by hyperbilirubinaemia, fluid retention, and painful hepatomegaly.[39,40] Results of previous studies have shown incidences of VOD ranging from 0 to 70% and mortality of VOD ranging from 20 to 50%, depending on the diagnostic criteria used in each study.[41] After RIC, the incidence of VOD is clearly reduced. In a study of 21 patients by Mothy *et al*. VOD was observed in one out of 21 patients (5%) with haematological malignancies receiving RIC and Picardi *et al*. observed no VOD of the liver in their study of 22 patients receiving RIC.[42,43] These data are especially impressive since patients who were given RIC allo-transplants had often already been extensively pretreated.

A common complication of conventional HSCT with myeloablative conditioning regimens is haemorrhagic cystitis (HC), which may result from cyclophosphamide in the conditioning regimen or from viral infection. Yamamoto *et al*. investigated HC following RIC.[44] HC was defined as two or more episodes of macroscopic haematuria in sterile urine with normal coagulation status, without any history or evidence of renal stones or genitourinary malignancy. HC was associated with immunosuppression, which can be brought about by GVHD prophylaxis. Also, busulphan use in the preparative regimen increased the risk of HC. The incidence of HC after RIC was not significantly different from that following conventional HCST (11.7% following RIC and 9.7% following conventional HSCT). However, HC after RIC tended to be milder, with lower blood transfusion requirements and the duration was shorter compared with conventional HCST.[44]

Although there is a beneficial GVD effect associated with GVHD, toxicity of acute and chronic GVHD remains a major problem, also after RIC. Incidences of acute and chronic GVHD are comparable between RIC and conventional HSCT;[20,23] however, a delayed onset of acute GVHD is frequently seen after RIC.[45,46] Moreover, patients receiving RIC often show clinical features of acute and chronic GVHD simultaneously,[47] questioning the usefulness of the standard definitions of acute and chronic GVHD, where chronic GVHD was categorised as all GVHD occurring after more than 100 days. GVHD severity partly depends on GI toxicity, since translocation of bacteria and/or endotoxin to the systemic circulation is a potent stimulator of release of inflammatory cytokines, which are important mediators of GVHD. Reduced dose intensity of conditioning caused less intestinal toxicity and a subsequent reduction of acute GVHD.[48] To improve safety and outcome of transplantation, prevention and treatment of GVHD should be further explored without abrogating the GVD effect, possibly by modulation of immunosuppressive schedules or manipulation of T-cell subsets in the stem cell graft.

CLINICAL RESULTS

Chronic myeloid leukaemia

Since very recently, imatinib, a tyrosine-kinase inhibitor, is considered the first-line treatment for chronic phase chronic myeloid leukaemia (CML). However, a small number of patients prove to be resistant to the drug or present in advanced stages of the disease, where its activity is clearly reduced. For these patients allogeneic HSCT is still a therapeutic option.

The existence of a graft-versus-disease (GVD) effect was first clinically identified[49] and later confirmed by the results of donor leucocyte infusions (DLI) in patients with CML. More than 70% of patients with CML can be curatively treated with allogeneic HSCT if they are less than 55 to 60 years of age and in the first chronic phase of the disease. Unfortunately, in older patients, and in patients with advanced disease, results remain poor.[50] Based on the recognition of the GVD effect, RIC regimens have been developed and given to patients who are not eligible for conventional allogeneic HSCT. Patients with CML seemed good candidates to evaluate RIC protocols because CML is a rather indolent disease, at least in the chronic phase.[51]

Or *et al*. reported a study of 24 patients in the first chronic phase of CML who underwent nonmyeloablative HSCT with a RIC regimen consisting of fludarabine and busulphan.[52] Recipients of matched unrelated donors also received ATG. This protocol was well tolerated, and all patients were alive at day 100 after transplantation. After a follow-up period of up to 70 months (median 42 months), three patients died as a consequence of GVHD, at day 116, 499 and 726. Both overall survival and disease-free survival were 85%, within an observation period of 7 to 63 months (median 37 months), with no patients relapsing during this period.[52]

Giralt *et al*. showed that 19 out of 27 patients with CML who were too old for conventional HSCT achieved complete remission, defined by standard morphological criteria and/or conventional cytogenetic analysis, after treatment with fludarabine and melphalan, with a probability of disease-free survival after one year of 34% for all patients.[23] Bornhäuser *et al*. described 44 patients with CML after allografting using RIC with fludarabine and busulphan.[51] They demonstrated that this treatment provided durable engraftment and low relapse rates. Although conventional conditioning remains the standard in advanced or imatinib-resistant disease, on the basis of these limited studies it can be concluded that reduced intensity conditioning should be considered in elderly patients with CML or in patients with poor performance status.

Acute myeloid leukaemia and myelodysplastic syndrome

One important option for curative treatment for myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) is allogeneic haematopoietic stem cell transplantation, in which the efficacy of allografting is partly due to the GVD effect.[49] Sibling donor transplantation for patients with AML or MDS who are older than 60 years, which is the median age for AML, has been shown to induce complete remission after RIC regimens in small series.[20,23] For this reason, several RIC regimens have been investigated in patients with MDS or AML who were considered poor candidates for conventional HSCT. In a study by Taussig *et al.* the use of a RIC regimen consisting of fludarabine and cyclophosphamide allowed allografting in patients (median age 54 years) with MDS or AML, who were not eligible for conventional allogeneic HSCT.[36] After a median follow-up of 26 months, 11 out of 16 patients were still alive, and low incidences of acute GVHD and TRM were seen after HSCT following the RIC regimen, which compares favourably with survival data of standard AML treatment in this age group.

A combination of melphalan and fludarabine was tested in 34 patients with high-risk AML and nine patients with MDS, receiving a matched unrelated donor (MUD) or a fully matched or one-antigen-mismatched related donor, by Giralt *et al.*[23] Of these, 26 achieved complete remission, with a probability of disease-free survival after one year of 26% for all patients. This study demonstrates that this RIC treatment allowed engraftment of unrelated and mismatched donors with acceptable levels of toxicity in older patients with associated comorbidities. The risk for grade III to IV GVHD was 19% for transplants from related donors and 39% for transplants from unrelated donors in this patient group, whose median age was more than 50 years. However, in most studies, disease recurrence and GVHD continue to be important causes of treatment failure.

Acute lymphoblastic leukaemia

Five-year survival of adults with acute lymphoblastic leukaemia (ALL) is less than 40%.[53] To improve survival rates of these patients, high-dose therapy followed by autologous or allogeneic HSCT has been investigated. Of all leukaemias treated with allo-HSCT, ALL was shown to be one of the least susceptible for GVD[10,15,54] probably due to the rapid kinetics of disease relapse.[55] Also, ALL was found to be unresponsive to adoptive immunotherapy with DLI.[10,55] However, Passweg *et al.* postulated a GVD effect in ALL patients, based on the finding that lower relapse risks were seen in patients with clinically manifest acute and/or chronic GVHD.[56] Also, Arnold *et al.* showed a GVD effect in their study of 22 high-risk (relapsed or Philadelphia-chromosome positive) ALL patients treated with nonmyeloablative HSCT.[57] The RIC regimen used in this study consisted of fludarabine combined with busulphan and ATG, which was more intensive compared with other protocols with reduced intensity. Four of 22 patients were alive in complete remission 5, 14, 19 and 30 months after transplant. They conclude from their study that nonmyeloablative HSCT is feasible in adult ALL patients, however only in a subgroup of patients. Promising results have been shown by Martino *et al.* in a study of 27 adult high-risk ALL patients who were ineligible for conventional allogeneic HSCT.[58] After a median follow-up of 809 days, nine patients were still alive, of whom eight were alive without disease and one with a relapse ALL on day 321. Although ALL patients may not be optimal candidates for RIC regimens and larger studies are lacking, for elderly patients or patients with severe comorbidity, RIC may be considered a therapeutic option. However, especially in ALL patients with uncontrolled disease, the chance for cure by using RIC treatment probably remains very low.

Chronic lymphocytic leukaemia

Chronic lymphocytic leukaemia (CLL) is the most frequent leukaemia in Western countries. It is characterised by clonal proliferation and accumulation of neoplastic B lymphocytes in the blood, bone marrow, lymph nodes and spleen. The median age of patients at diagnosis is 65 years. Only 10 to 15% of the patients are less than 50 years at time of diagnosis. With a median survival of about ten years, CLL is often indolent.[59] The prognosis of patients with progressive CLL is however unfavourable, with a median survival of 24 to 72 months.[60] Reports of remission after DLI or after withdrawal of immunosuppressive drugs after allogeneic HSCT were evidence of existence of a GVD effect in CLL.[60] However, TRM after conventional myeloablative allogeneic HSCT in patients with CLL turned out to be almost 50%.[61] By introducing RIC regimens, transplant-related mortality may be substantially lowered in these patients. Slow kinetics of tumour cell growth in CLL allows enough time for the graft to exert an antileukaemic effect. A regimen consisting of fludarabine, busulphan and ATG was studied in 30 CLL patients by Schetelig *et al.*[62] Twelve patients achieved complete remission and 16 patients achieved a partial remission. After a median period of 24 months, 23 patients were alive. Death occurred because of disease progression or TRM. However, TRM after RIC followed by HCST was low. Dreger *et al.* reported results of 77 CLL patients receiving RIC.[3] After a median follow-up of 18 (1 to 44) months, event-free survival and overall survival were 56 and 72% respectively. Hence, although few studies have been performed in patients with CLL, and randomised controlled trials are lacking, RIC may be beneficial in CLL patients with poor prognostic characteristics.[62]

Multiple myeloma

Multiple myeloma is a B-cell malignant disorder characterised by the expansion of plasma cells producing a

monoclonal immunoglobulin.[2,63] Although response rates, disease-free and overall survival improved after high-dose chemotherapy followed by autologous transplantation of haematopoietic cells compared with standard chemotherapy in patients with multiple myeloma, the chance for cure remains low.[63,64] Because of high TRM associated with conventional allograft procedures, no improvement in overall survival compared with those achieved by autologous HSCT was seen.[16,65] Therefore, allogeneic HSCT has not been considered a routine treatment for most patients. This high treatment-related mortality is related to the median age at diagnosis of multiple myeloma, which is greater than 55 years. For the 15 to 20% of the patients who are below 50 years, allogeneic HSCT may be a better treatment, particularly because of the existence of a graft-versus-myeloma effect.[63] Low intensity conditioning regimens have been developed to avoid the high procedure-related mortality of conventional allogeneic transplants. A study by Einsele *et al.* showed that long-term disease control can be attained in patients with multiple myeloma by allogeneic HSCT following a RIC regimen consisting of fludarabine, cyclophosphamide, antithymocyte globulin and low-dose total body irradiation.[66] Moreover, in this study, the occurrence of chronic GVHD seemed to improve tumour control post-transplant, further supporting a graft-versus-myeloma effect. Lokhorst *et al.* included in their study 54 patients with relapsed myeloma who initially received T-cell depleted transplants after conventional myeloablative conditioning.[67] A response on DLI was observed in 28 patients, of whom 19 were partial and nine were a complete response. This study confirms the potential of DLI to induce responses by means of a GVD effect, which can therefore be an effective treatment for patients with relapsed myeloma. Shaw *et al.* showed that RIC protocols using CAMPATH were associated with faster engraftment, less severe acute GVHD and lower nonrelapse mortality at day 100 compared with myeloablative regimens.[68] A significantly higher overall survival after the RIC regimen compared with the myeloablative regimen was observed (54 and 18% respectively), showing the importance of further optimising the RIC regimen for myeloma patients. The Dutch HOVON cooperative study group is currently exploring a strategy in which multiple myeloma patients are sequentially treated with autologous HSCT followed by RIC allogeneic HSCT. Conditioning consists only of low dose TBI (2Gy). Results are expected within several years.

Lymphoma

Depending on the stage of the disease, up to 80% of patients with relapsed Hodgkin's disease (HD) can be cured with chemotherapy and/or radiotherapy. Patients who fail to enter complete remission after the initial treatment are increasingly being treated with high-dose chemotherapy or a combination of chemotherapy and radiotherapy to achieve long-term disease control.[69] Findings that relapse rates after allo-HSCT seemed lower than after auto-HSCT,[70] and that patients developing acute GVHD showed lower relapse rates,[71] may implicate the possibility of a GVD effect. Also, in low-grade non-Hodgkin's lymphomas (NHL), curative potential of allo-HSCT, due to GVD effect, was seen.[72,73] Based on the existence of this GVD effect, patients with Hodgkin's disease and non-Hodgkin's lymphoma were treated with myeloablative allogeneic transplants. Because of high TRM and adverse effects of GVHD, results have however been disappointing, possibly related to extensive pretreatment of patients eligible for allo-HSCT.[71,73] For this reason, the feasibility of RIC regimens was explored. Small studies have been performed on allo-HSCT after RIC in patients with HD,[27,61,74-76] and low-grade NHL.[77] RIC allo-HSCT clearly shows reduced TRM (20 to 25%) in extensively pretreated patients compared with conventional HSCT (50 to 85%).[78] In addition, chances of relapse seem to compare positively with autologous transplants.[73] Although results seem promising, the number of patients included in most studies is still small.

The role of allogeneic transplants in intermediate and high-grade lymphoma has not yet been established in large clinical trials. A small number of these patients have been treated with RIC, but progression was seen shortly after transplant.[14] No studies with larger patient groups have been performed, but according to the kinetics of the tumour growth, it seems likely that RIC regimens may not allow a curative GVD effect in patients with active disease.

CONCLUSIONS

As no prospective randomised trials have been performed comparing conventional *vs* RIC HSCT no definitive answers can be given to questions as to which conditioning regimen is optimal for patients with haematological malignancies. Up to now, most of the patients who received allogeneic transplants after RIC were those who were ineligible for conventional conditioning, making formal comparisons concerning overall survival and transplant-related mortality extremely difficult to interpret. GVHD is an ongoing problem that may initially be less severe after RIC, but eventually no advantage is attained compared with conventional conditioning in this respect. Relapses continue to be a great problem and may be more frequent after RIC. Many different regimens for RIC are being explored and at present it is unclear which of them is most appropriate for the disease the transplant is being performed for. However, it is evident that allogeneic transplants can now be performed with acceptable toxicity in patients who would have been ineligible for this potentially curative treatment only a few years ago, before RIC regimens were introduced. Therefore, in principle, in all patients with haematological

malignancies who are not candidates for conventional transplants, RIC HSCT should be considered, although for older patients after previous autologous transplants and with poor performance status even this still leads to unacceptable toxicity. Whether RIC has the potential to replace conventional conditioning in younger patients and in those without concomitant diseases is still unknown, but should be a topic of future studies. Adequate trials and longer follow-up are needed to optimise protocols, to determine the optimal timing of the procedure in the course of the disease and to evaluate the long-term outcome and toxicity of this treatment.

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Clinical outcome of experimental human malaria induced by *Plasmodium falciparum*-infected mosquitoes

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ABSTRACT

Background: Human experimental malaria infections have been safely carried out previously. The objective of this study was to evaluate infection rates and clinical safety of different protocols for human experimental malaria induced by *Plasmodium falciparum*-infected mosquitoes.
Methods: Thirty nonimmune volunteers were infected by bites of 1-2 or 4-7 *Anopheles stephensi* mosquitoes infected with the NF54 strain of *P. falciparum*.
Results: A 100 or 50% infection rate was obtained after bites of 4-7 and 1-2 infected mosquitoes, respectively. Median prepatent period was 8.8 days. The most common symptoms after a median incubation time of eight days were headache, malaise/fatigue and fever. There was no significant difference in clinical and parasitological presentation between groups infected by 4-7 or 1-2 mosquitoes. Delay of treatment by maximally 48 hours after the first positive thick smear was generally well tolerated but fever was higher and more frequently observed. The most prominent laboratory abnormality was uncomplicated thrombocytopenia. Two volunteers with parasitaemia developed psychiatric side effects after chloroquine treatment.
Conclusion: With stringent inclusion criteria, close monitoring and immediate administration of treatment upon detection of parasitaemia, experimental human malaria challenges can be considered safe and generally well tolerated.

INTRODUCTION

Malaria is one of the most important infectious diseases worldwide. The number of infected people is increasing due to human migration, climate changes, failure of programmes for malaria control and the global spread of drug resistance. Today, malaria is found throughout the tropical and subtropical regions of the world and causes more than 300 million acute illnesses and at least one million deaths annually.[1] The potential effect of vaccines on the devastating malaria situation worldwide justifies the highest priority for its development. Pre-erythrocytic vaccines are partly developed to prevent disease in people travelling to malaria-endemic countries and for children in endemic countries. Asexual vaccines, which contain blood stage antigens, are developed to reduce the severity and lethality of malaria in endemic countries.[2] Preclinical studies have proven to be useful to test malaria vaccine candidates, but the ultimate validation of efficacy depends on human studies.[3] Due to limited resources, only the most promising vaccines can be tested in elaborate field trials in endemic areas. In addition, human challenges have shown to be safe, reliable and ethically acceptable for testing the efficacy of potential malaria vaccines.[4] Hundreds of volunteers have been experimentally infected by bites of generally five infected mosquitoes.[4-8]
The objective of this study was to evaluate infection rates and clinical safety of different protocols for human experimental malaria induced by *Plasmodium falciparum*-infected mosquitoes.

MATERIALS AND METHODS

Production of infected mosquitoes

Culture of *P. falciparum* parasites and the infection of *Anopheles stephensi* mosquitoes has been a routine procedure for the past ten years.[9] The chloroquine-sensitive NF 54 strain was used in all challenge studies. Batches with more than 90% infected *Anopheles stephensi* mosquitoes were used with a mean of at least 10,000 sporozoites per paired salivary gland. A small cage containing the desired number of mosquitoes was placed between the forearms and the mosquitoes were allowed to feed for ten minutes. Blood engorged mosquitoes were dissected to confirm the presence of sporozoites in the salivary glands. If this was not the case, another feeding session followed (maximum of three) until the desired number of infected mosquitoes had fed.

Recruitment

Thirty healthy volunteers (18 to 45 years) were included. Exclusion criteria were 1) previous history of malaria or travel to malaria endemic areas, 2) previous history of dermatological, central nervous system, renal, cardiac, pulmonary, hepatic, and splenetic disease, splenectomy, pregnancy and lactation, 3) need for medication, and 4) known allergy to antimalarial agents. Volunteers were recruited through general advertisements in public places and local journals. All volunteers had to live in the vicinity of our hospital. Screening included a physical examination, complete blood count, liver and renal function tests, urinalysis for glucosuria, protenuria and pregnancy test, and serological testing for antimalarial antibodies, HIV, and hepatitis B and C. The protocol was adapted to more stringent criteria of <10% for risk of coronary heart disease. Risk was calculated according to the Framingham Heart Study Coronary Heart Disease Risk Prediction Chart.[10] The volunteers were informed about the expected adverse events and risks before inclusion. An informed consent form was signed by all subjects. An independent specialist in internal medicine could be consulted by the subjects to obtain information on the studies. The subjects' general practitioners were asked to mention any conditions known to them that could increase the risk of an adverse outcome. The studies were approved by the institutional ethical board (CWOM numbers 0004-0090, 0011-0262, 2001/203, and 2002/170).

Experimental set-up

The studies were conducted from 1999 to 2003 at the Centre for Clinical Malaria Studies in the Radboud University Medical Centre, Nijmegen, the Netherlands. In Group A, 15 (three groups of five) volunteers were challenged by bites of 4-7 infected mosquitoes. Thick smears were taken following the World Health Organisation's standard procedure. Smears were screened for parasites in 200 fields at high-power magnification. Standard chloroquine (base 100 mg, salt 136.3 mg, Aventis) treatment, 10 mg/kg initially followed by 5 mg/kg after 6, 24 and 48 hours, was started immediately after detection of parasitaemia by thick smear.

In group B (5 volunteers), curative treatment was delayed for maximally 48 hours after the first microscopic detection of parasitaemia, to monitor parasite multiplication. To ensure maximal safety, we admitted the volunteers to the hospital as soon as the thick smear was positive. They were closely monitored with review by a physician unrelated to the study. Treatment was immediately initiated when parasitaemia was >500/μl, in case of severe laboratory abnormalities, or on development of clinical symptoms that required prompt treatment according to either the investigator, the independent physician, or the volunteer. In Group C, ten (two groups of five) volunteers were challenged by bites of 1-2 infected mosquitoes.

Follow-up

Follow-up of volunteers in group A and C was on an outpatient basis with close monitoring. Ear temperature was measured, and all symptoms were recorded on a case report form at every visit. Volunteers were requested to measure their temperature twice daily, and note their symptoms in a booklet. At the end of the study the subjects were asked to complete a questionnaire on their perception on inconveniences and severity of disease during the study. Thick smears were done twice daily from day 6 after infection until they were positive and treatment had been initiated. Chloroquine treatment was provided to all volunteers including the ones whose thick smears remained negative to the end of the study. Standard blood and urine laboratory tests were done once daily in the three days post-treatment including haemoglobin, platelet count, white blood cell count with differentiation, creatinine, blood urea nitrogen, sodium, potassium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, lactic dehydrogenase, gamma glutamyl transpeptidase and blood glucose. Urine analysis included protein and glucose measurement. Thick smears were carried out daily until two negatives had been obtained for *P. falciparum*. At the end of the study (ten days after first detection of parasitaemia) blood was drawn for standard clinical laboratory tests and a control thick smear.

Data analysis

All available data were analysed in SPSS 10.0 for Windows. Data of three volunteers were excluded from clinical and laboratory analysis, because of the development of concurrent illnesses (influenza A, flu-like syndrome, and myocardial infarction). Prepatent period was defined as the period of time between the challenge and microscopic detection

of parasites; incubation period was defined as time from the challenge until the first fever episode (>38°C). If fever did not occur, incubation time was regarded as missing. Duration of symptoms was defined as the number of days with malaria or chloroquine-related symptoms. Parasite densities were compared after transformation (ln). Clinical differences between group B, group C and reference group A were tested using the nonparametric Wilcoxon's rank-sum test for continuous variables and the Fisher's exact test for dichotomous variables. Thrombocytopenia was defined as a platelet count below 120 x 10^9/l. Changes in blood cell counts were tested by the nonparametric Friedman's rank test.

RESULTS

Infection by 4-7 mosquitoes

Group A of 15 subjects consisted of five males, 13 Caucasians, one Asian and one Black. All volunteers developed parasitaemia after bites of 4-7 infected mosquitoes and were immediately treated with chloroquine. Results of two volunteers were excluded from the analysis because of concurrent illnesses (influenza A and flu-like syndrome). The median prepatent period was 8.8 days (*table 1*). Geometric mean of maximum parasite density was 39.9 parasites/µl. The median incubation period was eight days while median duration of symptoms was six days (from 4 to 122 days after infection). Of the volunteers, 46% (6/13) developed fever, of which 67% in the prepatent period. Altogether, 93% (12/13) of the volunteers showed signs and symptoms one to four days before detection of parasitaemia.

All volunteers in group A developed a mild, uncomplicated episode of clinical malaria. The most common symptoms were headache, malaise and/or fatigue, and myalgia and/or arthralgia (*table 2*).

White blood cell (WBC) count was decreased on the day the thick smear became positive (day 0, *figure 1A*) with a nadir at day 2 and recovery to baseline levels by day 10. Thrombocytopenia (<120 x 10^9/l) occurred in three of the 13 (23%) volunteers. Platelet counts (*figure 1B*) also showed a pattern of significant decline and recovery with a nadir at days 1 to 3. Lymphocyte counts (*figure 1C*) also decreased, but recovered somewhat sooner. Absolute neutrophil counts did not change significantly (data not shown, Friedman rank test: $\chi^2 = 11.2$; df = 6; p=0.08).

Chloroquine treatment was generally uneventful but two volunteers developed side effects. One female became depressed but recovered completely within five days. A second female suffered from paranoia, depersonalisation, nightmares, and concentration problems. There was no medical or family history of neuro-psychiatric disease. Symptoms started on the second day after the start of chloroquine treatment (a total dose of 1.5 g, 27.3 mg/kg). Most symptoms subsided within five days, but the concentration problems took 122 days to resolve.

Infection by 4-7 mosquitoes with delay of treatment

All five volunteers developed signs and symptoms of mild malaria (*table 1*). Clinical presentation of group B was similar to group A, but there was a tendency towards higher fever frequencies with higher maximum temperatures (*tables 1* and *2*). All volunteers developed thrombocytopenia. One volunteer had to be treated with chloroquine after 41.5 hours because of a platelet count of 15 x 10^9/l, without symptoms of bleeding. This was a single observation in a series of measurements showing a gradual decline from 248 to 127 x 10^9/l in four days, followed by a sudden drop to 15 x 10^9/l and a recovery to 120 x 10^9/l

Table 1
Clinical responses to experimentally induced P. falciparum malaria

	A	B	C
NUMBER OF MOSQUITOES	4-7	4-7	1-2
NUMBER OF VOLUNTEERS	13	5	5
ONSET OF TREATMENT	AFTER FIRST POSITIVE THICK SMEAR	DELAYED 48 HOURS	AFTER FIRST POSITIVE THICK SMEAR
Prepatent period (days)	8.8 (7.3-10.3)		9.0 (8.0-13.0)
Incubation period (days)	8 (7-11)[$]		8 (4-11)
Duration of parasitaemia (days)	2 (1-2)	3 (3-5)[*]	2 (1-3)
Duration of symptoms (days)	6 (2-122)[#]	5 (3-6)	5 (1-7)
Highest parasite density (GM[#], per/µl)	39.9 (23-55)	9.6 (32-124)	38.8 (32-55)
Highest temperature (°C)	37.8 (37.0-39.9)	39.4 (38.0-40.2)[**]	38.0 (37.3-39.8)

All values are median (range), except [#] = geometric mean (range); [$]6/13 volunteers did not develop fever, see table 2; [#]due to chloroquine-induced psychiatric side effects; [*]difference between group A and B, Wilcoxon's rank sum p=0.001; [**]difference between group A and B, Wilcoxon's rank sum p=0.05 (borderline significance).

Table 2

Frequency of signs and symptoms in experimentally induced P. falciparum malaria

	A	B	C
NUMBER OF MOSQUITOES	4-7	4-7	1-2
NUMBER OF VOLUNTEERS	13	5	5
ONSET OF TREATMENT	AFTER FIRST POSITIVE THICK SMEAR	DELAYED 48 HOURS	AFTER FIRST POSITIVE THICK SMEAR
Fever	6 (46.2)*	5 (100)	4 (80)
Headache	12 (92.3)	5 (100)	5 (100)
Malaise and/or fatigue	12 (92.3)	4 (80)	5 (100)
Myalgia and/or arthralgia	9 (69.2)	2 (40)	2 (40)
Nausea with/without vomiting	5 (38.5)	2 (40)	3 (60)
Chills	3 (23.1)	1 (20)	3 (60)
Diarrhoea	2 (15.4)	1 (20)	0
Abdominal pain	2 (15.4)	0	1 (20)
Psychiatric symptoms after onset of chloroquine treatment	2 (15.4)	0	0
Thrombocytopenia#	3 (23.1)	5 (100)§	0

Number of volunteers (%); #<120 x 10^9/l; §thrombocytopenia occurred in the period of treatment delay.

Figure 1

Haematological changes after infection with P. falciparum malaria

The influence of infection on white blood cell (1A), platelet (1B) and absolute lymphocyte count (1C) were visualised by plotting the median of all infected volunteers (n=16-21), after subtracting the values on the day of inclusion (=0 on the y-axis).

Day 0 on the x-axis indicates the first thick smear positive day.

Differences were tested using Friedman's rank test. The error bars indicate the interquartile range (IQR).

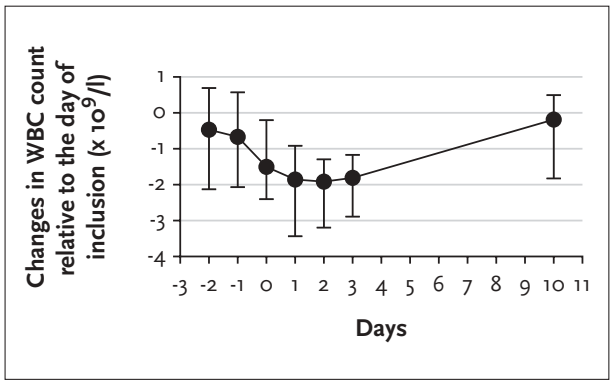

Figure 1A

White blood cell count (WBC)

(Friedman rank: χ^2 = 24.2, df = 6, p<0.001)

Range on the day of inclusion: 4.0- 9.6 x 10^9/l.

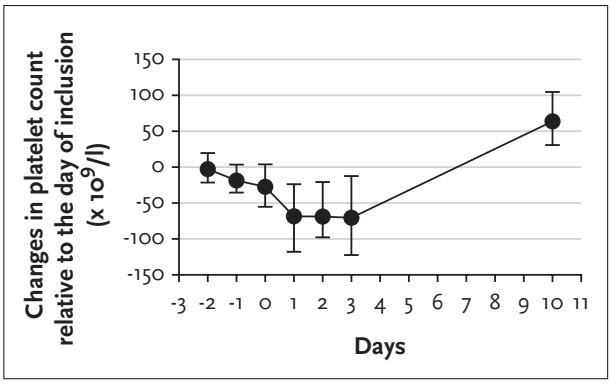

Figure 1B

Platelet count

(Friedman's rank: χ^2 = 67.4; df = 6; p<0.001).

Range on the day of inclusion: 187- 443 x 10^9/l.

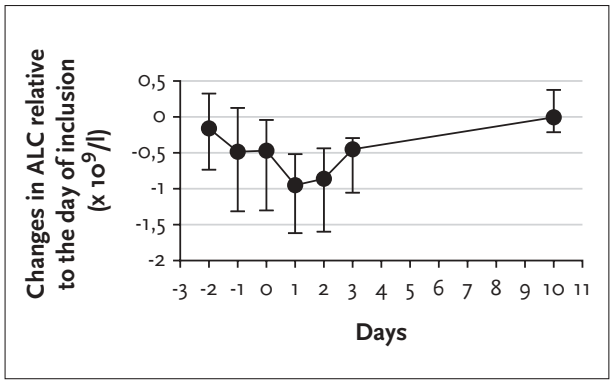

Figure 1C

Absolute lymphocyte count (ALC)

(Friedman's rank: χ^2 = 63.6; df = 6; p<0.001)

Range on the day of inclusion: 1.2- 3.7 x 10^9/l.

within nine hours. An inaccurate reading is most likely in this case. Severity of the thrombocytopenia correlated with parasite density (Pearson's correlation coefficient -0.51, p=0.02). As expected, duration of and maximum parasitaemias were higher than group A (Wilcoxon's rank sum, p=0.001).

Infection by 1-2 mosquitoes

A total of ten volunteers (group C) were exposed to bites of 1-2 infected mosquitoes; five of these ten subjects developed parasitaemia. Parasitaemia and symptoms were similar to volunteers infected with 4-7 mosquitoes (*tables 1* and *2*). None of the volunteers developed thrombocytopenia. No significant differences in laboratory results were observed (data not shown). Volunteers recovered uneventfully from the malaria episode after chloroquine treatment. The five volunteers who remained negative were excluded from data analysis. One of the negative volunteers had an unexpected event during the study. One day after chloroquine treatment, he developed signs of a myocardial infarction. An infero-posterior infarction with a significant stenosis in the circumflex coronary artery was diagnosed. He was transferred to the Cardiology Department and recovered.

Volunteer perception

On the last follow-up visit, we retrospectively evaluated the volunteers' own perceptions on inconveniences and severity of disease through questionnaires. In group A, burden of disease was considered to be severe by 54%, and mild by 46% of the volunteers. Of the volunteers of group B 20% experienced the disease episode as severe, and 80% as mild. Disease perception was comparable in group C: 20% experienced severe disease and 80% mild. Median duration of perceived illness was longer in group A and B, compared with group C (3.0 *vs* 2.0 days). Volunteers perceived headache (37%), malaise/fatigue (19%), and fever/flu-like feeling (15%) as the most unpleasant symptoms. Of the volunteers, 35% considered disease severity higher than anticipated, but 78% of volunteers would participate again.

DISCUSSION

A 100 and 50% infection rate was obtained in 20 and 10 volunteers, respectively, who were experimentally infected by 4-7 and 1-2 *Anopheles stephensi* mosquitoes carrying *P. falciparum* parasites. It has been reported that at least five infected mosquitoes should be used to ensure 100% infection rate, because lower numbers of mosquitoes result in inconsistent infection rates.[6-8] Exposure to 1-2 infected mosquitoes induced parasitaemia in only five out of ten volunteers, which corroborates previous findings.[11]
There are ethical aspects to an infection-inducing challenge experiment, which should be evaluated. Such experiments should not pose risks of irreversible harm if they are confined to self-limiting and completely curable diseases. The expected risks and discomforts for volunteers should be taken into account before the assessment of the study's scientific rationale.[12] In a prospective study, ambulatory management of imported malaria is safe.[13] Follow-up of our volunteers was in principle on an outpatient basis with intensive monitoring, which proved to be satisfactory. Volunteers with an uncomplicated course were only admitted for observation if chloroquine treatment was delayed for 48 hours (group B). The risk of complications was considered to be minimal because of close monitoring and a low threshold for intervention at this low density of parasitaemia. Volunteers participating in other studies had been allowed to develop parasitaemias >10^5 parasites/μl before treatment was initiated.[5,14]

Our protocol with delayed treatment was used for a more precise measure of parasite multiplication. A statistical model was developed that can provide detailed estimates of parasite growth rates and may substantially improve the capacity to evaluate asexual vaccines.[15] In addition, treatment delay provides a possibility to collect data on the initial immune responses during a malaria episode with possibilities to study immune correlates of protection and susceptibility to malaria. All five volunteers in group B developed uncomplicated malaria with a mild increase in severity of symptoms compared with the group that was immediately treated when the thick smear was positive.

Thrombocytopenia was present in all volunteers of group B, but one single platelet count of 15 x 10^9/l was obtained in one individual. This measurement, however, is likely to be incorrect because values directly before and after were similar within a nine-hour period of time. In group A (immediate treatment) 23% (3/13) of the volunteers developed thrombocytopenia (<120 x 10^9/l), while 100% (5/5) developed a low platelet count in group B (delayed treatment). Church *et al.* found thrombocytopenia (<100 x 10^9/l) in ten of 83 (12%) volunteers compared with four of 27 (15%) in our entire study group.[4] A correlation between severity of thrombocytopenia and parasite density has been reported in 89 cases of acute and imported malaria.[15] Nonetheless, this event stresses the need to stay alert and perform frequent tests. The significant decrease in WBC, in particular lymphocytes, is consistent with previous studies.[4,16] It has been speculated that redistribution of lymphocytes into body compartments and apoptosis of T cells occur in parallel during malaria attacks.[17-19]

The clinical response to all challenges, i.e. duration of parasitaemia, and geometric mean parasitaemia corroborates previous studies.[4-8,14] However, our studies show shorter

prepatent periods, which may be due to differences in parasite strain (as has been shown before) or different protocols of laboratory diagnosis.[4,11] The number of sporozoites released from the mosquitoes may vary, or a higher efficiency of liver stage development may be obtained with some strains. A weak inverse relationship was found between prepatent period and number of mosquitoes (data not shown, Pearson's correlation coefficient: -0.397, p=0.04). Previous studies have shown inverse correlations between the estimated inoculum dose and prepatent period.[8]
Our study shows that incubation time is often shorter than the prepatent period, which is in contrast to other challenge studies.[6-8,11] Incubation period has been previously reported from six to 32 days. It is, however, difficult to compare results from various studies, due to different monitoring of volunteers and definitions of incubation time.

Clinical symptoms are comparable with previous studies, but headache was more frequently reported by our volunteers.[4] A relation between parasite inoculum and severity of disease has been suggested.[20] Challenging with 1-2 mosquitoes does not decrease the symptoms, although disease perception is less severe.

Unexpected side effects occurred in three volunteers after the onset of chloroquine treatment. Two volunteers developed reversible psychiatric symptoms following treatment (Telgt, *et al.* in press). Psychiatric side effects following therapeutic doses of chloroquine have been reported but are relatively rare.[21,22] Symptoms usually occur when 2 to 6 g of chloroquine is administered, but both our volunteers received a chloroquine dose below 2 g. For future studies, chloroquine will be replaced by another antimalarial agent, such as co-arthemeter.
One male volunteer who did not develop parasitaemia had a myocardial infarction two days after chloroquine administration. Cardiac complications during and after adequate treatment of malaria are extremely rare (0.6%).[23,24] Retrospectively, this volunteer appeared to have a moderate risk of a coronary event within ten years. Volunteers with a risk of a coronary event greater than 10% will be excluded in future challenge studies.

In conclusion, *P. falciparum* (NF54) experimental human malaria infections with *Anopheles stephensi* mosquitoes induced a 100% infection rate after bites of 4-7 infected mosquitoes and 50% after 1-2 mosquitoes. Using stringent criteria, including risks for cardiac events, and close monitoring, with immediate administration of antimalarial treatment on first detection of parasitaemia, experimental human malaria challenges can be considered to be safe and generally well tolerated. In this way, phase IIa challenge trials can be a powerful tool in the difficult decision-making process of malaria vaccine development and testing.

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PR and QTc interval prolongation on the electrocardiogram after binge drinking in healthy individuals

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ABSTRACT

Background: Acute, excessive alcohol intake has been associated with an increased cardiovascular mortality in otherwise healthy individuals. It predisposes to accelerated atherosclerosis resulting in acute coronary events but also arrhythmias have been described, such as atrial fibrillation and life-threatening re-entrant ventricular arrhythmias. QTc prolongation is associated with an increased risk of ventricular tachyarrhythmias and an independent risk factor for sudden cardiac death. The aim of the study is to investigate the effect of binge drinking on the conduction intervals in healthy individuals.

Methods: Ten of the volunteers drank red wine while the other ten volunteers drank a sweet designer drink. A follow-up of blood pressure, heart rate, ECG and laboratory findings was performed at an ethanol level of 0, 0.4 and 0.8%, respectively.

Results: Fifteen volunteers showed a prolongation of the PR interval, 13 of the QRS complex, 9 of the QT interval and 13 of the QTc interval. PR interval increased from 149 ± 16 ms to 163 ± 11 ms ($p<0.05$). The heart rate-adjusted QT interval increased from 400 ± 24 ms to 426 ± 52 ms ($p<0.05$). Heart rate and systolic blood pressure did not significantly change due to the ingestion.

Conclusion: Acute ingestion of alcohol in a healthy population can induce prolongation of PR and QTc interval.

INTRODUCTION

Alcohol is widely used and in contrast to moderate intake, which can reduce the risk of coronary heart disease, chronic and excessive use is associated with increased cardiac morbidity and mortality.[1,2] In chronic alcoholism, patients first develop diastolic dysfunction and later a systolic dysfunction with hypertrophy and dilatation of the ventricular chambers. This results in a decrease in ventricular ejection fraction and possible symptoms of heart failure.[3,4] Electrocardiographic changes may develop after long-term alcohol consumption, such as prolonged heart rate-adjusted QT interval, conduction disturbances, nonspecific T-wave changes and shortening of the action potential. These changes can predispose to the development of atrial fibrillation.[5-7] Binge drinking is also associated with an increased cardiovascular mortality in otherwise healthy individuals. The exact definition of binge drinking is not provided in the literature but it is considered as an acute and excessive alcohol intake. This drinking pattern causes an acute inhibition of fibrinolysis and may predispose to accelerated atherosclerosis resulting in acute coronary events.[8] Atrial fibrillation but also life-threatening re-entrant ventricular arrhythmias have been described after a binge.[9-12] Because QTc prolongation is associated with ventricular tachyarrhythmias and sudden cardiac death we invested the effect of binge drinking on the conduction intervals in individuals without any signs of cardiac heart disease. We chose for alcohol ingestion instead of infusion in order to imitate oral intake and compared red wine with a sweet designer drink to differentiate between a possible alcohol effect as compared with the effect of polyphenols in wine.

METHODS

Study design

A prospective study was performed among 20 healthy individuals, 24 to 56 years of age, without a history of atherosclerosis, hyperlipidaemia, diabetes mellitus, hyperhomocystenaemia or cerebrovascular disease. Exclusion criterion was the use of prescribed medication, with an exception of oral contraceptives. The average alcohol consumption before entering the study was 1.5 drinks daily. Before entering the study a medical check was performed involving history taking, ECG and general laboratory assessment (*table 1*). The study was designed to achieve an ethanol level of 0.4 and 0.8% after ingestion of 40 and 60 g of alcohol, respectively. Ten individuals ingested a sweet designer drink (Bacardi breezer, 275 ml with 5.0 vol% alcohol, adding up to 11.0 g of alcohol). The other ten volunteers drank red wine (Rioja, 110 ml 13.0 vol% of alcohol, adding up to 11.4 g of alcohol per glass). As one glass of wine contains 11.4 g ethanol and a sweet designer drink contains 13.75 g ethanol, volunteers had to drink four to six glasses of wine and three to four designer drinks, respectively, to reach an ethanol level of 0.8%. Three glasses of wine and two designer drinks were consumed in 45 minutes and after the last drink, 45 minutes were allowed for alcohol uptake in the circulation. After these 90 minutes, ECGs were obtained and alcohol level was measured by blood sampling using an enzymatic method (t = 0.5). If the alcohol level did not reach the 0.45%, the amounts of alcohol were adjusted. Hereafter, the cycle was repeated and 180 minutes after starting ECGs and blood samples were again collected (t = 1). Pasters were used for the precordial location determination in serial electrocardiography. A Siemens ambulant electrocardiography machine was used to obtain all the electrocardiograms. All subjects had not eaten or smoked for four hours before entering the study and participants gave informed consent. The medical ethical committee of the Meander Medical Centre approved the study protocol.

Table 1
Baseline subject characteristics (mean and range)

	N=20	(RANGE)
Sex (male/female)	14/6	
Age (years)	35.5	(21-56)
Systolic blood pressure (mmHg)	116	(105-140)
Diastolic blood pressure (mmHg)	62	(55-80)
Ethanol intake (g/week)	175	(0-532)
Daily ethanol intake (consumption)	1.5	(0-4)
CDT%	2.2	(1.6-5.8)
Smokers	7	
Cholesterol (mmol/l)	4.65	(3.3-6.2)
LDL cholesterol (mmol/l)	2.85	(1.7-4.0)
Triglyceride (mmol/l)	1.2	(0.5-2.8)
HDL cholesterol (mmol/l)	1.3	(0.7-2.0)
Cholesterol/HDL	4	(2-6)

CDT% = carboxyl deficient transferring; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

ECG analysis

The ECGs were analysed for heart rhythm, heart rate, PR interval, QRS interval, QT interval and the heart rate-adjusted QT interval. The intervals were hand-measured. The QTc interval was calculated by using Bazett's correction formula $QTc = QT/\sqrt{RR}$. Furthermore, bundle branch block, ST segment elevation (≥0.1 mV) and/or depression (≥0.05 mV), T-wave morphology and ECG criteria for LVH and U-wave presence were analysed. Left ventricular hypertrophy is considered to be present when the S wave in lead V1 and V2 plus the R wave in lead V4 to 6 is more than 3.5 mV (5.3 mV in patients younger than 25 years), R + S >4.0 mV in the precordial leads, R in lead I >1.5 mV, R in lead aVL >1.3 mV (and no signs of left anterior hemiblock), R in lead aVF >2.0 mV (and no signs of left posterior hemiblock) and R in lead I and S in lead III >2.5 mV (and no signs of left anterior hemiblock).

Statistical analysis

Data were analysed using the Student's paired t-test. The comparisons of the intervals between the two study groups were made by the χ^2 test. These results are expressed as relative risks. A p value <0.05 was regarded as statistically significant.

RESULTS

In 18 volunteers, an ethanol level of ≥0.45% (t = 0.5) was measured after ingestion of 20 to 30 g of ethanol. In 19 persons a level of ≥0.8% (t = 1.0) was reached after ingestion of 40 to 60 g of ethanol. At a level of 0.4% 12 individuals had a prolongation of the PR interval, 8 of the QRS complex, 6 of the QT interval and 12 of the QTc interval. At a level of 0.8%, 15 persons showed a prolongation of the PR interval, 13 of the QRS complex, 9 of the QT interval and 13 of the QTc interval (*table 2*). PR interval increased from 149 ± 16 ms to 163 ± 11 ms (p<0.05). The QRS complex increased from 90 ± 4 ms at baseline to 95 ± 1 ms at t = 1 (NS). QT interval was 383 ± 25 ms at t = 0 and rose to 393 ± 29 ms (p<0.05) at t = 0.5. However, at t = 1, the QT interval was 385 ± 33 ms (NS). The heart rate-adjusted QT interval increased from 400 ± 24 ms to 426 ± 52 ms (p<0.05). The systolic blood pressure was 116 ± 3 mmHg before ingestion and 110 ± 2 mmHg after consumption of alcohol (NS). The diastolic blood pressure did not change during intake. The heart rate at baseline was 67 ± 1 beats/

min and after ingestion 67 ± 8 beats/min (NS) (*table 3*). There was no significant difference in conduction intervals between the red wine and sweet designer drink group after ingestion of alcohol. Sixteen volunteers showed nonspecific T wave changes. ECG changes like ST-segment depression and first-degree atrioventricular block occurred in one volunteer. A U wave developed in three persons during the ingestion of ethanol. Arrhythmias did not occur in any of the subjects.

DISCUSSION

The current study shows that binge drinking can cause a prolongation of the PR and QTc interval in a healthy study population. These intervals show a statistically significant prolongation at an ethanol level of 0.8%. However, prolongation of the PR interval (>200 ms) and QTc interval (>450) ms occurred in one and in three individuals, respectively. Prolongation of the heart rate-adjusted QT interval has been described before but after intravenous infusion and in patients with stable coronary heart disease. Rossinen *et al.* studied whether acute alcohol after intravenous infusion prolonged the ventricular repolarisation in patients with stable heart disease. At an ethanol level of 1.2 ± 0.2% the QTc interval increased on average by 12 to 23 ms (p<0.005) over a 12-lead ECG in the study group as well as in a healthy control group. These authors concluded that alcohol indeed prolongs the QTc interval which reflects abnormal repolarisation and may increase the risk of life-threatening arrhythmias.[7]

The question remains whether the prolongation is due to depolarisation or repolarisation. The PR interval reflects the time needed to activate the atria, to conduct the impulse to the AV node and His bundle and start the ventricular depolarisation. The QTc interval reflects ventricular depolarisation and repolarisation. Even if the delays are mainly due to repolarisation, considering the fact that the QRS intervals did not significantly increase during alcohol intake, Cardy *et al.* demonstrate P wave and QRS complex length-

Table 2
ECG characteristics after 40-60 g ethanol ingestion

	SWEET DRINK (N)	RED WINE (N)	RR (CI)	P
PR interval prolongation	8	7	1.14 (0.69-1.9)	NS
QRS complex prolongation	6	7	0.86 (45-1.64)	NS
QT interval prolongation	4	5	0.8 (0.3-2.13)	NS
QTc interval prolongation	5	7	0.63 (0.31-1.25)	NS
ST-segment elevation	0	1	-	-
ST-segment depression	1	0	-	-
Aspecific T wave change	9	5	1.8 (0.94-3.46)	NS
U wave presence	3	0	-	-
Bundle branch block	0	0	-	-
Nonspecific conduction disturbance	0	2	-	-

RR = relative risk; CI = confidence interval; NS = nonsignificant.

Table 3
Conduction intervals, blood pressure and heart rate after ingestion of 20-40 and 40-60 g of alcohol

	BASELINE T = 0	20-40 G T = 0.5	P	40-60 G T = 1	P
PR	149 ± 16	170 ± 11	0.001	163 ± 11	0.01
QRS	90 ± 4	95 ± 7	NS	95 ± 1	NS
QT	383 ± 25	393 ± 29	0.002	385 ± 33	NS
QTc	400 ± 24	411 ± 28	0.016	426 ± 52	0.036
SBP	116 ± 3	114 ± 2	NS	110 ± 2	NS
DBP	62 ± 6	68 ± 2	NS	62 ± 0	NS
MBP	80 ± 3	83 ± 4	NS	90 ± 8	NS
Heart rate	67 ± 0.7	67 ± 5	NS	67 ± 8	NS

Conduction intervals in milliseconds, blood pressure in mmHg, heart rate in beats per minute, NS = not significant; SBP = systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure.

ening after ingestion of ethanol and might explain in some part the purported changes.[13] They investigated whether atrial and ventricular signal-averaged electrocardiograms change after acute ingestion of ethanol in ten healthy volunteers. They reported P wave and QRS complex prolongation in nine of ten and ten of ten subjects, respectively, after acute alcohol intake with peak alcohol levels of 0.75 ± 0.05%. In their control group, who only drank fruit punch, prolongation of the P wave and QRS complex was also shown. The difference between the experimental and control group was significant. These studies elicit the question of what would have happened in the present study if the volunteers had drunk a nonalcoholic drink on another occasion.

The exact mechanism causing alcohol-induced arrhythmias remains unclear. Alcohol and its metabolite acetaldehyde can indirectly stimulate the release of catecholamines, which are capable of increasing P wave duration.[10] An exaggerated sympathetic reaction on alcohol can predispose to atrial fibrillation.[12] Furthermore alcohol is capable of inhibiting Na-K-ATPase. Decreases in the activity of this pump could eventually alter the resting membrane potential across the sarcolemma, as well as the intracellular and extracellular ionic homeostasis. Also the calcium binding and transport by the cardiac sarcoplasmatic reticulum may be delayed by alcohol. Alcohol consumption may affect the number of calcium ions entering the cardiac cell through voltage-dependent calcium channels during the plateau of the action potential and the amount of activity of these channels located on the sarcolemma.[6] Therefore, the ventricular repolarisation, which depends on the reduction in L-type Ca current and an increased outward K current, may be prolonged by the effect of alcohol.[6,7] Recently O'Leary reported the results of inhibition of the cloned DNA HERG potassium channel by alcohol, cocaine and cocaethylene (a metabolite of cocaine and alcohol).[14] The HERG channel is responsible for the rapidly activating component (I_{Kr}) of the delayed rectifier potassium current which plays a major role in myocardial repolarisation and is the important determinant of action potential duration. The cloned HERG channel resembles the I_{Kr} of the delayed rectifier current. Inhibition of the cDNA HERG channel by ethanol prolongs the repolarisation time and increases the QT interval.[14] This may be an explanation for the significant prolongation of the QTc interval in our study population.

There was no significant difference in conduction interval between the red wine and sweet designer drink group. Therefore the prolongation of the intervals should be attributed to alcohol rather than other compounds in wine. Wine contains more than 500 compounds. These include water, alcohols, organic acids, sugars and glycerol and polyphenols, also known as flavonoids. Polyphenol-rich beverages are tea, cocoa, fruit juices and wine. Wine contains 500 mg/l of flavonoids in contrast to beer which contains no more than 60 mg/l. Polyphenols exhibit a wide range of biological effects as antioxidants, inhibitors of platelet aggregation, and modulators of prostaglandin and nitric oxide metabolism and might have a potential role in atherosclerotic disorders.[15] The positive effect of flavonoids on the cardiovascular morbidity and mortality seems to be related to long-term low intake of alcohol. It is not to be expected that acute ingestion of red wine will result in a positive effect of the flavonoids.

In the present study systolic blood pressure decreased, although not significantly, after ingestion of alcohol. Heart rate did not show any change. This phenomenon has been described earlier.[7] Alcohol primarily causes vasodilatation resulting in a decline in blood pressure. Heart rate will increase as a reflex mediated by baroreceptors. On the contrary, by increasing the total circulating volume by the alcohol intake, this effect of vasodilatation is overruled and heart rate will not rise. The total amount of fluid ingested in the two groups was different. In the sweet designer drink group a total amount of 1650 ml was ingested as compared with 660 ml in the red wine group. Despite this difference, there was no significant difference in blood pressure, heart rate or conduction intervals between the two groups.

CONCLUSION

In conclusion, this study shows that acute, excessive ingestion of alcohol in a healthy study population can result in a significant increase in the PR and QTc intervals. However, there was no comparison with a control group. A larger, randomised and controlled study is mandatory to investigate the effect in individuals subjected to the same volume challenge, without alcohol.

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Caribbean female patients with type 2 diabetes mellitus have lower serum levels of adiponectin than nondiabetic subjects

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ABSTRACT

Background: Previous studies in other populations suggest that low levels of serum adiponectin may be a cardiovascular risk factor. We aimed to determine the baseline concentration of serum adiponectin and its relationship with selected biochemical risk factors for coronary artery disease (CAD) in a cross-section of Caribbean patients with type 2 diabetes.

Methods: Anthropometric indices and fasting plasma concentrations of glucose, insulin, adiponectin, triglyceride, and total and HDL cholesterol were measured in 56 type 2 diabetic patients and 33 nondiabetic subjects. Insulin resistance (IR) was determined using the homeostatic model assessment (HOMA) method.

Results: Consistent with previous reports, Caribbean type 2 diabetic patients had significantly lower fasting serum adiponectin levels and higher mean levels of glucose, triglyceride and IR than the nondiabetic subjects (all, $p<0.01$). The nondiabetic female subjects had significantly higher serum adiponectin levels than did the female diabetics or nondiabetic males ($p<0.01$). Serum adiponectin level was negatively correlated with triglyceride or LDL cholesterol and positively related with HDL cholesterol among nondiabetic subjects, and the latter relationship persisted after adjusting for the effects of age, sex and BMI ($r=0.70$, $p<0.01$).

Conclusion: Similar to reports from other populations, Caribbean patients with type 2 diabetes, particularly the females, have lower levels of serum adiponectin than their nondiabetic counterparts and this is an additional CVD risk factor for the patients.

INTRODUCTION

Trinidad and Tobago is a multiethnic population compromising mainly peoples of African (40.8%) and East Indian (40.7%) origin.[1] While the prevalence of type 2 diabetes mellitus is higher in people of East Indian descent, the people of African origin had a higher prevalence rate of hypertension.[2,3] Recent reports from Trinidad and Tobago have shown increased risk of cardiovascular disease (CVD) among type 2 diabetic patients at the primary care setting;[4-6] this was thought to be related to poor postprandial hyperglycaemic control especially after consuming some ethnic carbohydrate foods.[7,8] The high CVD risk in this population has also been reported in other developing countries undergoing socioeconomic transformations such as Taiwan,[9] Mexico,[10] and countries in the Arabian Gulf.[11,12]

Thus, studies are warranted to identify modifiable and nonmodifiable CVD risk factors that may have accounted for its recent increase in developing countries. For example, although nonmodifiable, low serum concentration of the newly identified adipose tissue derived cytokine, adiponectin, has been shown to increase the risk of developing diabetes in both Japanese and Pima Indian populations.[13,14] Similarly, other studies in Caucasian and Pima Indian populations have shown that patients with type 2 diabetes and/or obesity have low serum concentrations of adiponectin.[15] Thus, given that low serum adiponectin level is now considered a CVD risk factor,[16-18] type 2 diabetic patients with low concentrations of this protein would have increased risk of developing premature arteriosclerosis. We therefore considered it important to determine the baseline concentration of adiponectin in this population where a cross-section of diabetic patients has previously been shown to

have increased risk of CVD.[4-6] Furthermore, this study is warranted considering a recent report from this population, which showed significant relationships between serum adiponectin levels and selected biochemical risk factors for developing diabetes in the offspring of patients with type 2 diabetes.[19] It is believed that the determination of the baseline value of this important adipocytokine in patients at increased risk for CVD would assist in early identification and management of the patients with high propensity of developing heart disease.

SUBJECTS AND METHODS

The recruitment strategies for diabetic and nondiabetic subjects were the same as has recently been published.[7,8] Briefly, type 2 diabetic patients were recruited from a database of 244 type 2 diabetic patients. The patients were randomly contacted by telephone and the study protocol and objectives of the study were thoroughly explained to them. Patients who expressed interest in participating in the study were required to visit our laboratory for registration and signing of the consent forms. The nondiabetic subjects were recruited through posters and flyers. Interested persons were required to contact our laboratory for thorough explanations of the study protocol and objectives and to perform a standard oral glucose tolerance test (OGTT), which was compulsory to exclude healthy subjects who might have undiagnosed diabetes. Thus, after collecting a fasting blood sample each nondiabetic subject consumed 75 g of anhydrous glucose (Cow & Gate Glucose, Nutricia, Rokkeveenseweg 49, Zoetermeer, the Netherlands) dissolved in 250 ml of water and a blood sample was collected at 120 minutes. Subjects with fasting and two-hour postprandial plasma glucose >7.0 and 11.1 mmol/l respectively were excluded from the study.[20]

Study protocol

The study protocol was reviewed and approved by our institutional Ethics Review Committee. All subjects were studied in our laboratory the morning after an overnight (12 to 14 hour) fast. During the visit, details of ethnic origin and age were directly ascertained from the subjects and recorded. Then, waist (cm), at the level of the umbilicus with the patient standing and breathing normally, and hip circumferences (cm), at the level of the largest projection of the buttocks, were obtained by tape measure while weight (kg), with standard hospital scales, and height (m), with a metal rule, were measured (in light clothing, without shoes). Then a fasting blood sample was collected from each subject. The blood samples were preserved in fluoride oxalate and plain tubes and plasma and serum specimens, respectively, were removed after centrifugation within 30 minutes of collection and stored at -20°C.

Biochemical analysis

Plasma glucose and serum triglyceride (TG), total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol were measured in multichannel auto-analysers using dry slide kits (Johnson & Johnson Vitros 250, Ortho-Clinical Diagnostics Inc., Rochester NY 14626, USA) while low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation.[21] HbA_{1c} was determined using a nonenzymatic reaction kit for DCA 2000 (Bayer Corp., Elkhart, IN 46515, USA). The serum insulin (Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden) and adiponectin (B-Bridge International, Inc, BioCat GmbH, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany) levels were determined by enzyme linked immunoabsorbent assay (ELISA). The intra- and inter-assay coefficients of variation for insulin were 3.7 and 6.4%, respectively.

Statistics and calculations

The results are expressed as mean ± SE. The Statistical Package for the Social Sciences (SPSS Inc., 233 South Wacker Drive, Chicago IL 60606-6307, USA) software was used in all analyses. Insulin resistance (IR), defined as the product of fasting serum insulin and plasma glucose divided by 22.5, was assessed using fasting serum insulin and plasma glucose concentrations in homeostasis model assessment (HOMA).[22] Comparisons of the mean differences in biochemical parameters between diabetic and nondiabetic subjects were performed using Students' t-tests while χ^2 was used for nonparametric tests. The relationships between adiponectin and selected biochemical parameters were explored using Pearson's correlation technique. Multiple linear regression analysis was employed to determine the influence of age, sex, BMI, waist circumference, ethnicity and diabetes status on serum levels of adiponectin. A p value <0.05 was considered statistically significant on two-tailed testing for all analysis.

RESULTS

Table 1 shows the background characteristics of the diabetic and nondiabetic subjects studied. The diabetic patients were older than nondiabetic control subjects. Although both groups had similar body mass indexes, the diabetic patients had significantly higher waist circumference ($p<0.05$, *table 1*). The diabetic patients had significantly lower fasting serum adiponectin levels and higher mean levels of HbA_{1c}, glucose, triglyceride and HOMA-derived insulin resistance (IR) than the nondiabetic subjects ($p<0.01$, *table 2*). The nondiabetic female subjects had significantly higher mean serum adiponectin levels than did diabetic females or nondiabetic males ($p<0.01$, *table 2*). However, serum adiponectin levels did not differ between subjects of African and East Indian origin irrespective of gender (data not

Table 1
Clinical characteristics and anthropometric indices of the diabetic and nondiabetic subjects studied

CHARACTERISTICS	DIABETIC PATIENTS N=56	NONDIABETIC SUBJECTS N=33
Male/female ratio	23/33	11/22
Drinkers of alcoholic beverages‡ (%)	30 (53.6)	15 (46.9)
Cigarette smokers (%)	6 (10.7)	2 (6.3)
Ethnicity		
African origin (%)	23 (41.1)	15 (45.5)
East-Indian origin (%)	33 (58.9)	18 (54.5)
Diabetes management		
Diet/exercise (%)	2 (3.6)	-
Tablets/insulin‡ (%)	14 (25.9)	-
Tablets (metformin, sulphonylurea or combination therapy) (%)	40 (71.4)	-
Age (years)	55.5 ± 1.1*	50.1 ± 1.9
Weight (kg)	77.6 ± 2.3	73.8 ± 2.2
Body mass index (kg/m²)	29.5 ± 0.8	27.4 ± 0.8
Waist circumference (cm)	100.1 ± 1.8*	93.8 ± 1.8

**p<0.05 for comparisons between the healthy control subjects and the patients; ‡units of alcohol or insulin was not ascertained.*

Table 2
Baseline serum adiponectin levels and some selected biochemical parameters in all subjects, and in male and female diabetic and nondiabetic subjects

	DIABETIC SUBJECTS			NONDIABETIC SUBJECTS		
	ALL	MALES	FEMALES	ALL	MALES	FEMALES
Age (years)	55.5 ± 1.1	58.3 ± 1.7‡	53.5 ± 1.4	50.1 ± 1.9‡	52.2 ± 3.3	49.1 ± 2.4
Body mass index (kg/m²)	29.5 ± 0.8	26.4 ± 0.7‡	31.6 ± 1.1*	27.4 ± 0.8	26.1 ± 0.9	28.1 ± 1.1
Adiponectin (µg/ml)	5.2 ± 0.5	4.9 ± 0.9	5.3 ± 0.6**	10.4 ± 1.4‡‡	4.5 ± 0.7	12.8 ± 1.7‡‡
Insulin (mU/l)	13.3 ± 2.4	16.3 ± 5.3	11.1 ± 1.8	8.5 ± 1.3	7.1 ± 1.5	9.2 ± 1.8
Glucose (mmol/l)	8.7 ± 0.5	7.9 ± 0.7	9.3 ± 0.6	5.5 ± 0.1‡‡	5.7 ± 0.2	5.5 ± 0.1
Glycated haemoglobin (%)	8.7 ± 0.5	8.1 ± 0.3‡	9.1 ± 0.4	5.6 ± 0.1‡‡	5.5 ± 0.2	5.6 ± 0.1
Triglyceride (mmol/l)	1.8 ± 0.1	2.0 ± 0.3	1.7 ± 0.2**	1.3 ± 0.1‡	1.9 ± 0.4	1.1 ± 0.01‡
HDL cholesterol (mmol/l)	1.5 ± 0.01	1.4 ± 0.1	1.6 ± 0.1	1.6 ± 0.01	1.3 ± 0.1	1.7 ± 0.1
LDL cholesterol (mmol/l)	3.3 ± 0.2	3.3 ± 0.3	3.4 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	2.8 ± 0.2‡
Total cholesterol (mmol/l)	5.2 ± 0.2	5.1 ± 0.3	5.3 ± 0.2	4.9 ± 0.2	5.3 ± 0.2	4.7 ± 0.2
Insulin resistance (pmol/mmol/l)	37.8 ± 6.9	41.3 ± 15.5	35.4 ± 5.0**	15.5 ± 2.6‡‡	13.3 ± 3.3	16.7 ± 3.6

**p<0.05 and **p<0.01 for within gender (diabetic females vs nondiabetic females) comparisons; ‡p<0.05 and ‡‡p<0.01 for all, and between gender (male vs female) comparisons in both diabetic and nondiabetic groups.*

shown). There was a significant inverse relationship between adiponectin and triglyceride or LDL cholesterol in nondiabetic subjects but not in diabetic patients. Again, a significant positive correlation was observed between adiponectin levels and HDL cholesterol in nondiabetic subjects, but not in diabetic patients, and the relationship persisted after adjusting for the effects of age, sex and BMI (r = 0.70, p<0.01, *table 3*). However, multiple linear regression analysis suggests that sex, BMI and diabetes status are the major determinants of serum adiponectin levels in the subjects studied (*table 4*).

DISCUSSION

The present study has shown that in a Caribbean population (i) serum adiponectin levels are lower in type 2 diabetic patients than in nondiabetic subjects, (ii) serum adiponectin

Table 3

Relationship between adiponectin and selected biochemical variables before and after controlling (partial correlation) for age, sex and BMI in diabetic and nondiabetic subjects

	DIABETIC PATIENTS N=56		NONDIABETIC SUBJECTS N=33	
ADIPONECTIN (MG/ML) VS	BIVARIATE CORRELATION	PARTIAL CORRELATION	BIVARIATE CORRELATION	PARTIAL CORRELATION
Insulin (mU/ml)	-0.23	-0.17	-0.16	-0.25
Glucose (mmol/l)	-0.11	-0.03	0.02	0.14
Triglyceride (mmol/l)	-0.26	-0.23	-0.39*	-0.20
HDL cholesterol (mmol/l)	0.07	-0.03	0.67**	0.70**
LDL cholesterol (mmol/l)	-0.08	-0.04	-0.47**	-0.37
Insulin resistance (pmol/mmol/l)	-0.26	-0.21	-0.16	-0.23
Body mass index (kg/m²)	-0.21	-	0.05	-

**p<0.05 and **p<0.01 for levels of significance of correlation coefficient at two-tailed testing.*

Table 4

Multiple linear regression analysis showing the influence of independent variables (age, sex, ethnicity, BMI, waist circumference and diabetes status) on serum adiponectin levels in all subjects, and diabetic and nondiabetic subjects studied

	ALL SUBJECTS N=89		NONDIABETIC SUBJECTS N=33		DIABETIC PATIENTS N=56	
	B-COEFFICIENT	SE	B-COEFFICIENT	SE	B-COEFFICIENT	SE
Age	0.1	0.07	0.17	0.13	0.14	0.07
Sex	0.33**	1.41	0.48*	3.29	0.29	1.18
Ethnic group	-0.14	1.2	-0.13	2.75	-0.15	1.06
Body mass index	-0.29	0.24	0.05	0.53	-0.78*	0.20
Waist circumference	0.11	0.1	-0.13	0.23	0.50	0.08
Diabetes status	0.38**	1.28	_	_	_	_

**p<0.05 and **p<0.01 for levels of significance of β-coefficient at 2-tailed testing. SE = standard error.*

level has a significant positive relationship with HDL cholesterol in nondiabetic subjects but not in diabetic patients, (iii) nondiabetic females had higher levels of serum adiponectin than did diabetic females or nondiabetic males. The implications of these findings in diabetes management in this population are further discussed.

The finding of lower serum adiponectin levels in type 2 diabetic patients (particularly female subjects) in comparison with nondiabetic subjects is absolutely consistent with previous reports from other populations.[15,23] A possible explanation for the observed differences in serum adiponectin levels might be related to the levels of fasting insulin and insulin resistance. Indeed, the diabetic patients have higher basal insulin and HOMA-derived insulin resistance levels, and a previous report has shown that adiponectin levels were suppressed below basal levels in both diabetic and nondiabetic subjects during hyperinsulinaemic euglycaemic clamp study.[24] Again, the comparatively higher waist circumference, an index of abdominal obesity and intra-abdominal fat deposition,[25,26] among the type 2 diabetic patients might have contributed to the lower serum adiponectin levels given that plasma adiponectin concentration decreases with increasing adiposity.[27] Interestingly, multiple linear regression analysis of the current data suggested that body mass index, but not waist circumference, is a significant determinant of serum adiponectin levels in the diabetic patients. Indeed, the female diabetic patients that constituted the majority of patients (59%) had higher BMIs than female nondiabetic subjects. It should be noted that in this population, patients of East Indian origin have higher prevalence of diabetes[2,3] and are at greater risk of CVD than patients of African descent.[4-6] However, univariate analysis of the current data (not shown) did not indicate any differences in the serum adiponectin levels of the patients of the two ethnic groups irrespective of gender. Indeed, multiple linear regression analysis confirmed that ethnicity is not a cofounder in serum adiponectin levels in this study (*table 4*).

Several previous studies have shown that plasma adiponectin is positively related to HDL-cholesterol levels and maybe protective against CVD.[16-18] Thus, the findings of the present study agreed with the previous observation at least among the nondiabetic subjects where the observed relationship between serum adiponectin levels and HDL cholesterol was not influenced by age, sex or BMI on partial correlation. Interestingly, the present finding in the nondiabetic subjects is consistent with a recent report in Caribbean subjects with and without positive family history of diabetes.[19] However, the finding that the relationship between serum adiponectin level and HDL cholesterol in type 2 diabetic patients was not significant in this study is not completely clear and is in contrast to previous reports where strong relationships between plasma adiponectin and HDL-cholesterol levels were documented.[16-18] Perhaps it is important to note that diabetes is a disorder of metabolic function, meaning that the anti-inflammatory and antiatherogenic activities of adiponectin are not restricted to its relationship with HDL cholesterol, hence certain activities of adiponectin are independent of HDL-cholesterol levels.[28,29] Indeed, experimental cell studies, for example, have shown that adiponectin is involved in modulating nuclear factor-kB signalling through a CAMP-dependent pathway and also act as an endogenous regulator of endothelial cells in response to inflammatory stimuli.[30,31] The finding of gender-related differences in plasma adiponectin concentration has previously been reported in Japanese diabetic patients without coronary artery disease.[23] Similarly, other studies in nondiabetic subjects in Japanese, Caribbean, North American and Canadian populations have shown that women have higher adiponectin levels than men.[19,32-34] Other workers have speculated that sex hormones such as oestrogen, progesterone and androgen might have an affect on plasma adiponectin levels.[23] It should, however, be noted that previous studies where sexual dimorphism in adiponectin concentration have been reported included postmenopausal[23,34] and premenopausal[19] women indicating that the reported differences may not be entirely related to the possible effect of sex hormones. It is therefore suggested that further studies aimed at addressing sexual dimorphism in plasma adiponectin levels and its role in insulin and glucose metabolism are warranted.

We acknowledge that the type 2 diabetic patients studied here might have included patients with latent atherosclerotic vascular diseases considering that diabetic patients with coronary artery disease are often asymptomatic.[23] Although none of the patients reported or admitted a case of CAD, the patients were not clinically examined for CAD and the presence of latent atherosclerotic disorder would worsen the plasma adiponectin levels among the diabetic patients, especially the males.[34] This limitation notwithstanding, our findings are consistent with previous reports from other populations. Thus, we conclude that Caribbean patients with type 2 diabetes, particularly the females, have lower levels of serum adiponectin than their nondiabetic counterparts or male subjects, and this is an additional CVD risk factor for the patients.

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CASE REPORT

Sarcoidosis mimicking ischaemic ventricular arrhythmia and pulmonary embolism

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ABSTRACT

Sarcoidosis is a multisystem granulomatous disorder characterised pathologically by the presence of noncaseating granulomas in the organs involved. Cardiac involvement, although well known, is rare.
We describe a 72-year-old patient who was admitted to the intensive care unit after coronary artery bypass grafting. She developed refractory right and left ventricular failure complicated by multiple organ failure and died three days later. Postmortem examination revealed extensive sarcoidosis. On hindsight, preoperative ventricular tachycardia and an abnormal perfusion-ventilation scintigraphy of the lungs were manifestations of an underlying sarcoidosis.

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown aetiology characterised pathologically by the presence of noncaseating granulomas in the organs involved.[1] Most frequently sarcoidosis involves the lung. Extrapulmonary sarcoidosis can affect all organ systems. Granulomatous involvement of the heart may lead to various cardiac problems especially arrhythmias. Nevertheless cardiac involvement only gives rise to clinical manifestations in 5% of the patients.[2,3] Initial cardiac presentation of sarcoidosis is rare.[4] We report a patient who underwent coronary artery bypass grafting (CABG) because of symptomatic single-vessel disease and recurrent ventricular tachyarrhythmia who was found to have extensive sarcoidosis with involvement of the heart.

CASE REPORT

A 72-year-old woman was admitted to our intensive care unit after coronary artery bypass grafting of the obtuse marginal branch. Four months before admission a diagnosis of pulmonary embolism was made after she had presented with dyspnoea and pleuritic pain. The diagnosis of pulmonary embolism was based on a positive D-dimer and a high probability mismatch on nuclear perfusion ventilation scanning. Her previous medical history was unremarkable apart from an asymptomatic left-sided carotid stenosis.
Three months before admission she presented with chest pain and ventricular tachycardia that converted to sinus rhythm after amiodarone therapy. Subsequent cardiological evaluation showed normal left and right ventricular function on echocardiography. ECG showed sinus rhythm with right bundle branch block.
Coronary angiography revealed an occluded obtuse marginal branch while the other coronary arteries were normal. Radionuclide imaging (myocardial scintigraphy, TC-99M MIBI) showed an irreversible posterolateral defect. There were no segmental areas of decreased uptake of the ventricular myocardium corresponding to areas of fibrogranulomatous replacement. Initially percutaneous coronary intervention was intended. This turned out to be technically impossible and subsequently the patient was scheduled for an off-pump CABG. During cutdown of the left internal thoracic artery (LITA), the patient developed a refractory cardiogenic shock, needing extracorporeal circulation. The LITA was very small with an almost absent flow and considered unsuitable. An aorto-coronary venous bypass graft was constructed to the marginal branch. The patient could be easily weaned from bypass without signs of ischaemia.

Postoperatively the patient was on mechanical ventilation with normal bilateral breathing sounds. Haemodynamically she was stable. Swan-Ganz pressure tracings recorded elevated pulmonary artery pressures and a low cardiac index (*table 1*). There were no enlarged lymph nodes and no pathological findings on abdominal examination. There was slight peripheral oedema at the extremities. Laboratory findings directly postoperatively were a haemaglobin of 6.5 mmol/l, thrombocyte count 125 x 10.9/l, leucocyte count 14.4 x 10.9/l, partial thromboplastin time (PTT) 1.82 and activated PTT 67. Electrolytes, liver and renal function tests were normal. Arterial blood gas analysis was unremarkable. Despite the low cardiac index the patient was extubated after initial postoperative care and on the first postoperative day she was discharged to the regular ward. In the next three days the patient complained of increasing dyspnoea and chest pain. Transthoracic echography showed pericardial effusion and a decreased right ventricular function. Both right atrium and ventricle were enlarged. A diagnosis of pericardial tamponade was made and rethoracotomy followed. During this procedure pericardial fluid was removed. Postoperatively the patient was re-admitted to the ICU.

In the postoperative phase the patient developed cardiac shock. Swan-Ganz measurements showed high pulmonary artery and central venous pressures and a low cardiac index (*table 1*). Despite therapy the patient developed multiple organ failure and died the following day.

The postmortem examination showed a hypertrophic heart with dilatation of the right ventricle (*figure 1*), and an old infarction and fibrosis of the left ventricle. The graft was open. Surprisingly, microscopic examination revealed myocardial sarcoidosis (*figure 2*), and extensive granulomas in the mediastinum, liver and lungs. Tuberculosis as well as fungal infection was excluded using polymerase chain reaction. There was no evidence of pulmonary embolism.

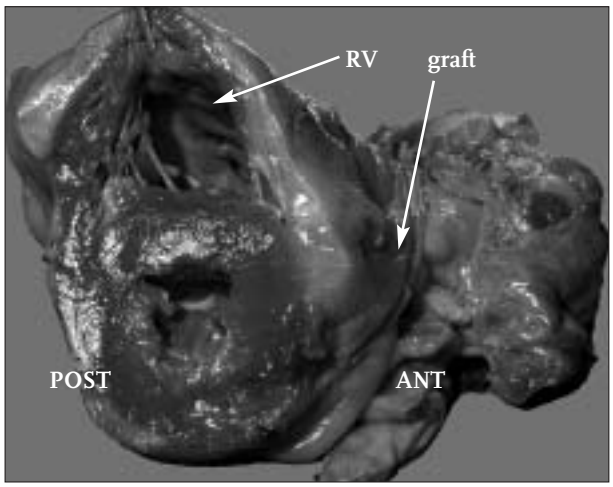

Figure 1
Cardiac hypertrophy (weight 430 g) and dilatation right ventricle
RV = right ventricle; post = posterior; ant = anterior.

DISCUSSION

Sarcoidosis is a systemic disorder of unknown cause which is characterised by noncaseating granulomas in the organs involved.[1] Although the lung is usually involved, the disease is known for its extrapulmonary manifestations.[5]

Table 1
Preoperative and postoperative haemodynamic parameters (after CABG and re-thoracotomy)

PARAMETERS (REFERENCE VALUE)	PREOPERATIVE	POST-CABG	POST-RETHORACOTOMY
Heart rate	90	63	112
Systolic BP (mmHg)	145	118	108
Diastolic BP	57	50	65
Mean BP	88	74	83
CVP (1-6 mmHg)	5	4	10
PAP systolic (15-28 mmHg)	40	41	57
PAP diastolic (5-15 mmHg)	20	17	30
PAOP (6-12 mmHg)	11	10	15
CI (2.4-4.0 l/min/m^2)	-	1.7	1.5
SVRI (1600-2400 dynes.sec. m^2/cm^5)	-	3388	3972
PVRI (200-400 dynes.sec. m^2/m^5)	-	774	1453
LVSWI (40-60 g.m/m^2)	-	26	15
RVSWI (4-8 g.m/m^2)	-	9	7
SV (60-70 ml/stroke)	-	41.3	13

BP = blood pressure; CVP = central venous pressure; PAOP = pulmonary artery occlusion pressure; PAP = pulmonary artery pressure; CI = cardiac index; SVRI = systemic vascular resistance index; LVSWI = left ventricular stroke work index; RVSWI = right ventricular stroke work index; SV = stroke volume.

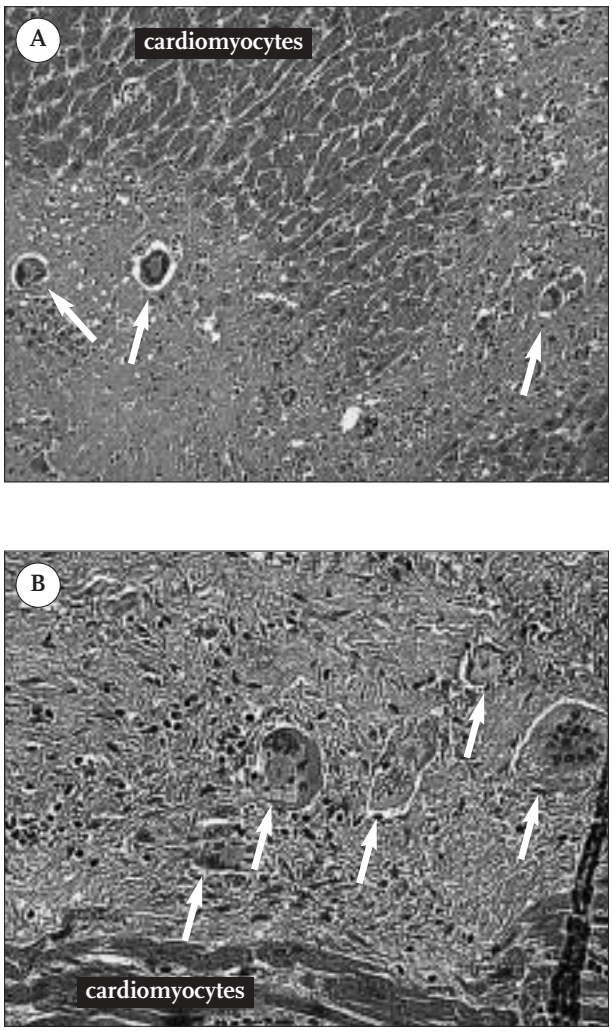

Figure 2
Myocardial sarcoidosis (A: lateral wall, B: posterior wall, cardiomyocytes and area with noncaseating granulomas, giant cells (arrow) and fibrosis

Cardiac manifestations of the disease are rare and reported in up to 5% of the patients with sarcoidosis.[2,3] It is unusual for sarcoidosis to present with isolated cardiac involvement.[4] Autopsy studies show a higher percentage of myocardial sarcoidosis. In an autopsy study, cardiac involvement proved to be the cause of death in 37% of the patients with sarcoidosis. In only 45% the diagnosis of sarcoidosis was suspected or established antemortem.[6] Cardiac sarcoidosis may cause interstitial inflammation which initially impairs diastolic function, whereas systolic function remains normal or nearly normal.[7] Subsequent inflammation and fibrosis result in impaired systolic function. Diffuse hypokinesia can occur, as well as focal abnormalities of regional wall motion that especially affect the basal septum but spare the apex.[8] The course of the disease is variable; in some patients it progresses rapidly to death with no preexisting symptoms.[9-11]

Half of the patients with cardiac sarcoidosis have electrocardiographic abnormalities of rhythm conduction and repolarisation. Clinical manifestations depend upon the location and extent of inflammation and should especially be suspected in young patients with known sarcoidosis presenting with arrhythmias. Involvement of the ventricular septum and conduction system may lead to a variety of arrhythmias and sudden death. Sudden death due to ventricular tachyarrhythmias or conduction block accounts for 25 to 65% of the deaths due to cardiac sarcoidosis.[12-14] Chronic pulmonary hypertension and cor pulmonale result from inflammation and subsequent severe scarring of the pulmonary parenchyma and vascular obliteration. In this setting death from sarcoidosis commonly results from right ventricular failure. The various clinical manifestations due to cardiac involvement are presented in *table 2*.

Table 2
Manifestations of cardiac sarcoidosis

Conduction abnormalities	First-degree heart block Intraventricular conduction defects Complete heart block
Ventricular arrhythmias	Abnormal automaticity Disrupted ventricular activation and recovery Sustained or nonsustained ventricular tachycardia
Supraventricular arrhythmias	Ectopic atrial activity Paroxysmal atrial tachycardia Atrial flutter/fibrillation
Heart	Systolic dysfunction Diastolic dysfunction Ventricular aneurysm
Valvular dysfunction	Mitral incompetence due to papillary muscle involvement
Simulated infarction	Transmural, non-Q-wave
Pericarditis	Rare, detected by echocardiography
Cor pulmonale, right-sided heart failure	Due to advanced pulmonary sarcoidosis

Sarcoidosis may mimic pulmonary embolism. Nuclear imaging (V/Q scan) can be falsely interpreted as pulmonary embolism.[15-18] Moreover D-dimer concentrations may be elevated in pulmonary sarcoidosis.[19-21]
Cardiac sarcoidosis may cause cardiomegaly and heart failure but may be difficult to establish. Firm diagnostic tests are not available and the diagnosis can only be established on the combined diagnostic modalities available and the exclusion of (other) structural heart disease. Although the prognosis of symptomatic cardiac sarcoidosis is not well defined, treatment with corticosteroids seems to delay the progression of inflammation and fibrosis.[1,12,14] Pacemakers are indicated when evidence of high-grade conduction disease is present. Automatic implantable cardioverter-defibrillators (AICD) are recommended in survivors of sudden death or patients with refractory ventricular tachyarrhythmias.[22-24]

CONCLUSION

Retrospectively, a high index of suspicion for a noncoronary explanation of the chest pain and the arrhythmia could have placed the ventricular tachycardia and the abnormal V/P scan in a different perspective: both fit the diagnosis of sarcoidosis, especially while there was single-vessel coronary stenosis. Even in retrospect there was little evidence of sarcoidosis in our patient preoperatively.
In conclusion, sarcoidosis is often not diagnosed nor suspected antemortem. The combination of ventricular arrhythmia, pulmonary hypertension, abnormal V/P scan and a positive D-dimer may be a clue to the right diagnosis

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CASE REPORT

Staging for CLL-type non-Hodgkin's lymphoma reveals a gastrointestinal stromal tumour

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ABSTRACT

We report a 73-year-old man presenting with fatigue, lymphadenopathy and weight loss. He had no abdominal pain, fever or night sweats. Physical examination revealed a palpable 1.4-cm hard nontender lymph node behind the left sternocleidomastoid muscle and a palpable 2-cm lymph node in the left axilla. Bone marrow examination and excisional biopsy of the lymph node behind the left sternocleidomastoid muscle showed a CLL-type non-Hodgkin's lymphoma (CLL-type NHL). Staging by CT scanning revealed, besides axillary and mediastinal adenopathy, an unexpected mass in the stomach. Gastroscopy and pathological evaluation showed a gastrointestinal stromal tumour (GIST) with immunohistochemical staining for CD 34 and CD 117. The patient was treated with imatinib.
CLL-type NHL and GIST both tend to occur in middle-aged and older patients. A double-tumour consisting of both these tumours is rare: the incidence is estimated to be 3 per 10 billion people.

INTRODUCTION

A CLL-type non-Hodgkin's lymphoma (NHL) is a neoplasm that usually occurs in middle-aged and older patients (aged 40 to 80 years). The incidence in the age category above 60 years is 20 per million people.[1] Gastrointestinal stromal tumours (GISTs), although relatively rare (0.1 to 3% of all gastrointestinal neoplasms), are the most common mesenchymal tumours of the gastrointestinal tract. They usually occur in the stomach (60%), with the remainder in the small intestine (15%) and other sites, including large bowel, oesophagus, rectum, mesentery and omentum. They also tend to occur in middle-aged and older adults, with a slight predilection for men. The approximated incidence in the Netherlands is 16 per million people.[2-5]
We report a male patient with a NHL and a GIST at the same time. This double-tumour is rare and, as far as we know, has never been described before. The approximated coincidence of these tumours is, on the basis of their presumed independent incidences, 3 per 10 billion people.

CASE REPORT

A 73-year-old man presented with fatigue, lymphadenopathy and weight loss. He had no abdominal pain, fever or night sweats. On physical examination he had a palpable firm nontender lymph node with a diameter of 1.4 cm behind the left sternocleidomastoid muscle and a palpable 2-cm lymph node in the left axilla. No other abnormalities were found on physical examination. Laboratory examination revealed an erythrocyte sedimentation rate of 4 mm, haemoglobin 5.7 mmol/l, leucocytes 5.6 x 10^9/l with normal differentiation, thrombocytes 249 x 10^9/l, albumin 33 g/l and ferritin 6 µg/l. Glucose, minerals, liver enzymes, lactic acid dehydrogenase, bilirubin, thyroid stimulating hormone, vitamin B12 and folate were normal and no M-proteins were found.
Bone marrow examination showed a diffuse infiltration of a small lymphocytic NHL. Histological examination of the lymph node behind the left sternocleidomastoid muscle revealed a CLL-type NHL with positive immunohistochemical staining for CD 20 and CD 5; CD 23 was not

clearly positive, which is unusual. Molecular examination showed karyotype 47 XY +12, which is typical for CLL-type NHL.
Staging by CT scanning of the thorax and abdomen revealed, besides axillary and mediastinal adenopathy, an unexpected mass in the stomach (*figure 1*). Gastroscopy showed a tumour in the stomach. Pathological evaluation showed a GIST with immunohistochemical staining for NSE, CD 39, CD 34 and KIT (CD 117) in the cytoplasm of the abnormal cells.
A complementary positron emission tomography (PET) showed high activity in the stomach, characteristic of a malignancy of the stomach and positive axillary and mediastinal regions as can be seen in lymphoma.
The patient was treated with imatinib. Gastric resection was not performed because of the predicted high morbidity due to the coexistent NHL. The follow-up of the NHL consisted of observation. After one month of treatment with imatinib the PET scan showed regression of the activity in the stomach.

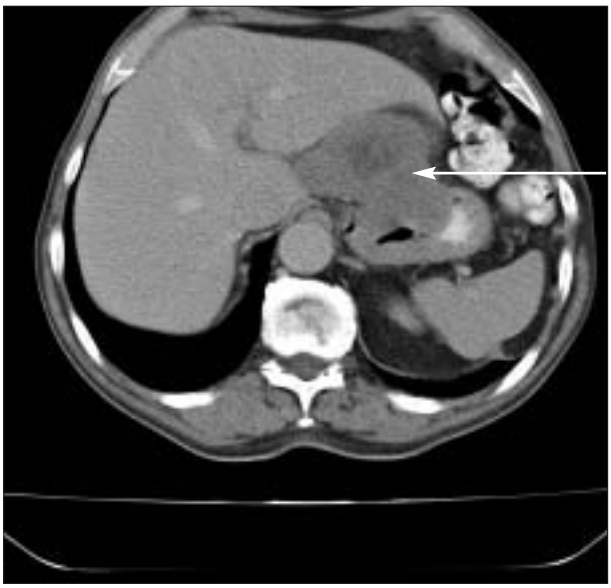

Figure 1
CT of the abdomen showed a gastointestinal stromal tumour

DISCUSSION

Patients with a CLL-type NHL usually present with persistent painless generalised lymphadenopathy. The peripheral blood may be normal or reveal only a mild lymphocytosis. M-proteins are found in 20% of the cases. Patients can often be observed without treatment for three to four years. The median survival is eight to ten years.[1]
Patients with a GIST may present with a variety of symptoms such as vague abdominal pain, gastrointestinal bleeding, fever, night sweats and weight loss.[2-5] GISTs range from small indolent tumours curable with surgery alone to aggressive metastatic cancers. Predicting the clinical behaviour of a newly diagnosed GIST is difficult in the absence of frank neoplastic spread.[6]
GISTs express CD 34 (approximately 70% of cases) and KIT (CD117), a transmembrane tyrosine kinase receptor that is the protein product of c-kit proto-oncogene (up to 100% of cases). Most GISTs have a mutation in the c-kit proto-oncogene that translates into a gain-of-function constitutive activation of KIT. KIT activation seems to play a central role in GIST pathogenesis, seemingly serving as a requisite for neoplastic behaviour in the majority of GISTs.[3,6]
Complete gross surgical resection is the main treatment modality for GISTs. Until the advent of imatinib, there was no effective treatment for unresectable or metastatic GISTs. Imatinib is a well-tolerated agent that can inhibit the disrupted tyrosine kinase signalling pathways in GIST. Therapy with imatinib can induce objective responses and stabilisation of disease and can provide clinical benefit in the majority of GIST patients treated with the drug. It is important that imatinib is continued, because the disease will progress if the drug is stopped. The most common side effects seen in patients continuing on therapy have been periorbital oedema, peripheral oedema, fatigue, skin rash, myelosuppression and nausea/vomiting.[3,6,7] Resistance to imatinib may occur in some patients caused by mutations in the targeted oncogene. Further investigations are necessary to identify drugs that override or reduce this refractoriness to imatinib.

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A remarkable ECG of a patient with swollen legs

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CASE REPORT

A 69-year-old male, without any remarkable medical history, was admitted to the hospital because of predominantly right-sided heart failure. Despite initiation of furosemide two weeks before admission, his physical condition did not improve. Physical examination revealed oedematous legs and presacral oedema. His blood pressure was 100/60 mmHg with a pulse rate of 88 beats/min. The laboratory results were as follows: normal peripheral blood cell count, ureum 7.5 mmol/l, creatinine 80 μmol/l, ASAT 38 U/l, ALAT 40 U/l, γ-glutamyltransferase 55 U/l, alkaline phosphatase 118 U/l and C-reactive protein 3 mg/l.
The ECG (*figure 1*) showed sinus rhythm with microvoltages in the frontal leads and slow R progression in the precordial leads. Echocardiography revealed a concentric hypertrophic heart with moderate left systolic function, based on diffuse hypokinesia. Doppler showed a restrictive diastolic flow pattern. Hypoalbuminaemia (24g/l) was found, while monoclonal gammopathy was absent. Albuminuria of 2 g/day was documented.

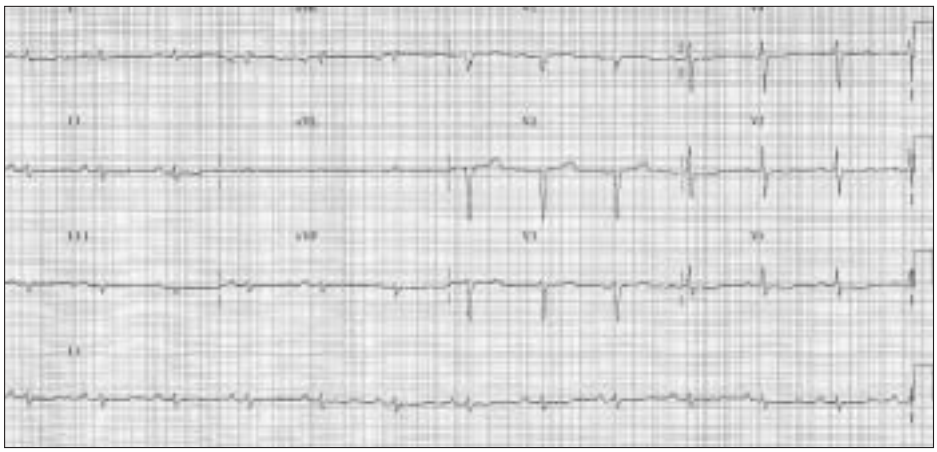

Figure 1
The ECG shows sinus rhythm with microvoltages in the frontal leads and slow progression in the precordial leads

WHAT IS YOUR DIAGNOSIS?

See page 77 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (ON PAGE 76)

A REMARKABLE ECG OF A PATIENT WITH SWOLLEN LEGS

DIAGNOSIS

The combination of a hypertrophic myocardium with diffuse diminished left ventricular function and glomerular involvement (macroalbuminuria) was suspicious for a systemic disease. The ECG pattern was concordant with cardiac amyloidosis. Invasive work-up confirmed restriction with square root sign, elevated pulmonary capillary wedge pressure and left ventricular end-diastolic pressure. Myocardial amyloidosis was histologically confirmed by endomyocardial biopsy. This case demonstrates the two most common and diagnostically useful ECG patterns in primary amyloidosis: pseudoinfarction pattern (sensitivity 63 to 80%) and low QRS voltage (sensitivity 60 to 93%).[1,2]

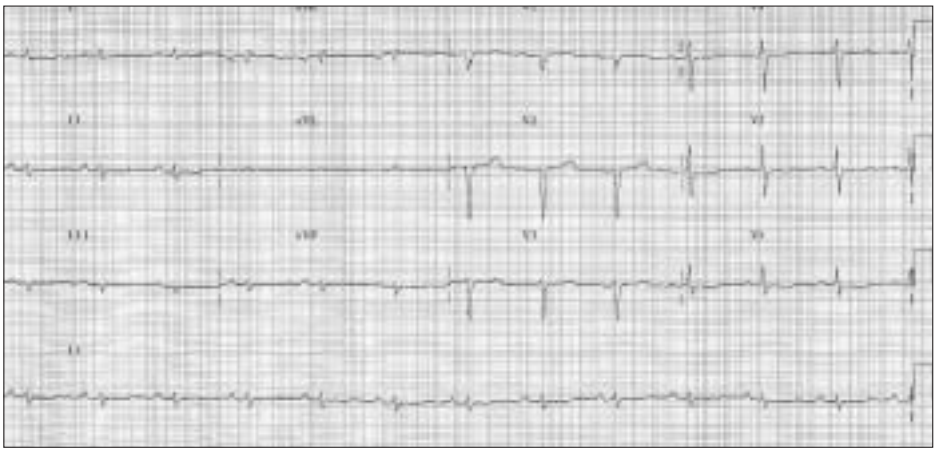

Figure 1
The ECG shows sinus rhythm with microvoltages in the frontal leads and slow progression in the precordial leads

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INFORMATION FOR AUTHORS

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

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

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

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

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

Subheadings should not exceed 55 characters, including spaces.

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

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

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

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

The *Results* should be presented precisely without discussion.

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

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

References should be numbered consecutively as they appear in the text (in square brackets after the punctuation). Type the reference list with double spacing on a separate sheet. References should be in the language they are published in, conform the 'Vancouver' style for biomedical journals (N Engl J Med 1991;324:424-8). Journal abbreviations should conform to the style used in the Cumulated Index Medicus. Examples:

1. Smilde TJ, van Wissen S, Wollersheim H, Kastelein JJP, Stalenhoef AFH. Genetic and metabolic factors predicting risk of cardiovascular disease in familial hypercholesterolemia. Neth J Med 2001;59:184-95.
2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
3. Powell LW, Isselbacher KJ. Hemochromatosis. In: Braunwald E, Fauci AS, Kasper DL, et al., editors. Harrison's Principles of Internal Medicine. 15th edition. New York: McGraw-Hill; 2001. p. 2257-61.

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

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

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

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

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

Books for reviewing
Books, which are to be considered for review, should be sent to the Editor in chief.

Submission
Manuscripts should be sent to the Editor in chief, Prof. A.F.H. Stalenhoef, Radboud University Nijmegen Medical Centre, Department of General Internal Medicine (541), PO Box 9101, 6500 HB Nijmegen, the Netherlands, tel.: +31 (0)24-361 04 59, e-mail: g.derksen@aig.umcn.nl. They should be submitted in four complete copies, which include four sets of the figures; authors should retain one copy of the manuscript. Rejected manuscripts will not be returned to the author unless specially requested at the time of submission.

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

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

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