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EDITORIAL

Should we do away with case reports?

Medical journals like The Netherlands Journal of Medicine attract quite a number of manuscripts concerning case reports. Many biomedical investigators, basic scientists as well as clinical investigators, tend to view these papers as not very relevant with little contribution to the progress in medicine. Often, case reports are considered at best as a training method, the resident's first step in the area of publishing clinical observations. Papers reporting a randomised controlled clinical trial, a case-control study or a pathophysiological study can count on a much greater esteem. Indeed, our editorial board welcomes manuscripts describing such studies. But what about case reports? In two recent opinion papers, Professor Jan Vandenbroucke, a prominent clinical epidemiologist, makes a plea for the case report as a tool for further development of medical sciences, as a means of education and as an instrument in quality control in medicine.[1,2] Vandenbroucke distinguishes a series of functions of the case report and the case series. The first function deals with the description of new syndromes and diseases. Careful bedside observation is often the basis for such description, which may lead to new hypotheses and initiate further research. A second area is that of the observations concerning side effects of drugs, which again may lead to new hypotheses and more systematic investigations. An interesting example that Vandenbroucke puts forward is the totally unexpected association between pulmonary embolism and use of psychotropic drugs, which was found in a case series.[3,4] A third function deals with a description of mechanisms of disease. Such descriptions, again based on meticulous clinical observation, may make further study superfluous. Another function may be the detection of a new therapy. Some therapies detected in case reports or in case studies may be so convincing that a randomised controlled clinical trial is no longer necessary. The fifth function Vandenbroucke describes is medical education. The case histories in the Lancet, the 'Lesson of the week' in the British Medical Journal and the clinicopathological conference in the New England Journal of Medicine serve as such and as a kind of audit also have the function of improving quality in medicine. Finally, case reports may lead to further recognition of rare manifestations of disease. To my mind, this is nowadays an important function of the case report that has not yet been emphasised enough, and that is the role of published case reports in daily clinical problem-solving. Rare syndromes or disease associations can often be found in the literature, and such discoveries may greatly benefit our patients with a clinical picture that is not readily clear. In the old days performing a literature search to try and solve such a clinical problem was an enormous amount of work. I remember more than once having spent many hours in the library trying to find the solution to a particular clinical problem (for instance with the help of the indigestible Cumulated Index Medicus). These days, such searches can be done electronically in few minutes, and complicated clinical problems can be tackled and solved right away.

It is interesting that Vandenbroucke values case reports and case series as sources for new ideas and progress in medicine; in his concept they are high in the hierarchy of medical publishing, and he considers the controlled trial as a good tool for confirmation. He allies with Jenicek who considers case reports and case series as a first line of evidence.[5]

Of course, there is a down side to the case report and the case series. Not all that is published is relevant or even true, but that also holds for the 'esteemed' medical articles. For the Journal it means that – in addition to original clinical research papers and reviews – we like to see critical, crisp and carefully written case reports. For each of these submissions we will ask the question (and we hope the authors have asked themselves the same question): What does this report add?

Jos W.M. van der Meer
Editor in chief

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REVIEW

Advantages and limitations of (non-)myeloablative allogeneic stem cell transplantation

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ABSTRACT

Allogeneic stem cell transplantation (allo-SCT) is the treatment of choice for a variety of malignant diseases, not only as a method to regain haematopoiesis after myeloablative therapy, but also for its apparent antitumour effect. However, treatment-related morbidity and mortality are considerable. A potential way to overcome this problem is by decreasing the intensity of chemotherapy and/or radiotherapy given prior to transplantation. This reduced-intensity conditioning regimen is the basis of non-myeloablative allo-SCT (also referred to as mini SCT), a new treatment modality that relies more heavily on the antitumour effect exerted by the donor cells than on the antitumour effect of the conditioning therapy.
The aim of this article is to place the concept of non-myeloablative SCT in a historical context and to discuss the advantages and disadvantages of regular myeloablative SCT compared with non-myeloablative SCT.
Furthermore, human trials regarding non-myeloablative SCT are reviewed, and several experimental techniques are discussed that aim to augment the antitumour effect of an allogeneic graft.

INTRODUCTION

For many haematological malignancies, including various forms of leukaemia, non-Hodgkin's lymphoma and multiple myeloma, allogeneic stem cell transplantation (allo-SCT) preceded by a conditioning regimen consisting of high-dose chemotherapy, sometimes combined with radiotherapy, has been a widely used therapy for many years. This article reviews the background of allo-SCT and recently published trials on non-myeloablative SCT. We will also briefly discuss potential future developments in the field of alternative graft-*versus*-host disease (GVHD) prevention,immunotherapy and the selection of partially HLA-mismatched related donors.

STANDARD ALLOGENEIC STEM CELL TRANSPLANTATION

In the past, conditioning therapy prior to allogeneic transplantation was intended to be myeloablative, thus providing an optimal oncolytic effect, 'making space' for the donor stem cells in the recipient bone marrow and suppressing the recipient's immune system sufficiently to ensure engraftment. Therefore, no spontaneous recovery of autologous bone marrow was expected to occur, so subsequent allo-SCT or transplantation of previously harvested autologous stem cells was absolutely necessary to prevent the development of lethal bone marrow aplasia.
New insights into the treatment of haematological tumours developed when it became clear that there was a striking difference between the risk of relapse in patients who underwent an autologous SCT, compared with patients receiving allogeneic stem cells after an identical conditioning regimen.[1-3] This suggested an additional immunologically mediated relapse preventing mechanism of action, exerted by allogeneic but not by autologous stem cell grafts. The existence of this graft-*versus*-tumour (GVT) effect was further supported by the observation of a higher relapse rate in leukaemia patients receiving identical twin

transplants or a T-cell depleted graft, compared with patients receiving stem cells from a non-twin donor or an unmodified allogeneic graft.[4] The GVT effect turned out to be not only directed against haematological diseases, also patients with solid tumours such as metastatic breast cancer[5-7] and refractory metastatic renal cell carcinoma[8] may to some extent benefit from an allo-SCT. The effectiveness of sole donor lymphocyte infusions (DLI) in patients relapsing after an allo-SCT also supports the assumption that donor immune cells are responsible for an anti-tumour effect.[9-11]

Unfortunately, apart from GVT effects, allo-SCTs are also associated with numerous side effects that are also partly immunologically mediated, causing considerable treatment-related mortality (TRM). A summary of these adverse effects follows.

Graft-*versus*-host disease

By a mechanism comparable with that causing the GVT effect, normal host cells are also prone to be affected by donor immune cells, causing a condition called graft-*versus*-host disease (GVHD). For an excellent review of the pathophysiology and management of GVHD, see Ferrara *et al*.[12] or Goker *et al*.[13] Since in non-twin allo-SCTs donor and host cells are not genetically identical, donor leucocytes will recognise recipient iso-antigens as being foreign and will thus attack host cells presenting these antigens. Activated host dendritic cells, being highly immunogenic and specialised in presenting antigens, are potent mediators in the pathophysiology of GVHD.[14] Tissues containing a relatively high number of antigen presenting cells (APC) might therefore be particularly responsible for the symptoms of GVHD. Furthermore, due to a so-called 'cytokine storm' appearing in inflamed tissues, organs that are most severely affected by the chemotherapy and radiotherapy used for conditioning will be more prone to develop GVHD.[12] Consequently, GVHD is characterised by different grades of:

- skin manifestations, leading to mucositis, a pruritic maculopapular rash and, in severe cases, generalised erythroderma, eventually with bullous formation;
- liver function impairment, typically causing conjugated hyperbilirubinaemia and elevated alkaline phosphatase;
- gastrointestinal symptoms, mostly moderate to very severe diarrhoea and abdominal cramping.

Many different protocols have been developed to prevent GVHD, using different combinations of immunosuppressants such as cyclosporin-A, methotrexate, corticosteroids and tacrolimus (FK506). Another method of GVHD prophylaxis is the use of T-cell depleted stem cell grafts: since donor T cells are important mediators in GVHD, especially chronic GVHD, T-cell depletion markedly reduces the intensity of its symptoms. However, the use of T-cell depleted grafts is associated with higher risk of graft rejection, relapse, viral infections and secondary malignancies. A final judgement on the use of T-cell depleted stem cell grafts therefore still remains to be made.[15]

Infections

Due to leucopenia caused by the pre-transplant conditioning regimen, patients undergoing an SCT are particularly susceptible to bacterial, fungal and viral infections in the period after transplantation. According to Meyers and Thomas,[16] the period after SCT can be divided into the following three phases.

1. A granulocytopenic period, lasting from a few days after transplantation until the recovery of neutrophils, usually defined as the first of three consecutive days on which the absolute neutrophil count (ANC) rises above 0.5 x 10^9/l (day 12-30; longer in case of graft rejection or failure after a myeloablative regimen). During this phase, patients are particularly susceptible to bacterial and fungal infections.
2. A phase lasting from the recovery of granulocytes until approximately day 100 in which patients have an increased risk of developing various viral and protozoal infections, due to delayed recovery of immunity. However, especially helper T-cell recovery may take several years after transplantation, regardless of the occurrence of GVHD. As a result of this, for instance cytomegalovirus (CMV) infection and Ebstein Barr Virus (EBV) reactivation and subsequent EBV-related lymphoproliferative disease are complications frequently observed following allo-SCT, especially when a T-cell depleted graft is used.[17]
3. Infections occurring more than 100 days post-transplant are partly caused by chronic GVHD and partly by prolonged immunosuppressive treatment against chronic GVHD.

Additional treatment-related toxicity

Pre- and post-transplant medication is responsible for many symptoms associated with SCT.[18,19] Possible side effects include nausea and vomiting, diarrhoea, nephrotoxicity, hepatotoxicity including veno-occlusive disease, CNS toxicity, haemorrhagic cystitis, cardiotoxicity, electrolyte disturbances, mucositis and cutaneous toxicity.

Given the advantages and disadvantages of allo-SCT, it is considered the treatment of choice for several diseases, including acute and chronic myeloid leukaemia (AML/CML), acute lymphoid leukaemia (ALL) and aplastic anaemia (*table 1*). A cure rate of 60 to 70% can be realised in CML and approximately 55% in AML. For ALL and aplastic anaemia, cure rates are highly dependent on disease status and age. In follicular non-Hodgkin's lymphoma, allo-SCT can be considered when poor prognostic factors are present

Table 2
Trials with non-myeloablative conditioning regimens; protocols and results

STUDY (REFERENCE)	INDICATION (NUMBER OF PATIENTS)	PRE-TRANSPLANT CONDITIONING (NUMBER OF PATIENTS)	ENGRAFTMENT	CHIMERISM: %DONOR CELLS IN NUMBER OF PATIENTS (NUMBER ASSESSED)	GVHD >GRADE 1 (NUMBER OF ASSESSABLE PATIENTS)	TRM	TUMOUR RESPONSE (PR OR CR)
Giralt *et al.*(27)	AML (13) MDS (2)	(a) Flu/ara-c/ idarubicin (7) (b) Ara-c/2-CDA (7) (c) Flu/melphalan (1)	87%	≥85%*: 3/5 ≥30%: 2/5	Acute: 3/14 Chronic: 0/5	13%	53%
Ueno *et al.*(6)	Metastatic breast cancer (10)	CTX/ carmustine/ thiotepa	100%	100%**: 8	Acute: 3/10 Chronic: 4/9	20%	50%
Khouri *et al.*(42)	CLL (6) Richter's trans-formation (2) Lymphoma (7)	(a) CTX/flu (10) (b) Cisplatin/flu/ cytarabine (5)	73%	≥80%†: 5/9 0%: 4/9	Acute: 1/15 Chronic‡: - limited: 2/8 - extensive: 2/8	33%	73%
Giralt *et al.*(25)	AML/ MDS (43) CML (27) ALL (3) Hodgkin's (4) Non-Hodgkin's (9)	(a) Flu/ melphalan (78) (b) Cladribine/ melphalan (8)	88%	>90%†: 40/42	Acute: 34 Chronic: 21/46	(a) 37%§ (b) 88%	57%
Michallet *et al.*(43)	ALL (13) AML (18) MDS (10) Non-Hodgkin's (16) MM (14) Hodgkin's (3) CLL (3) CML (12) Solid tumours (3)	(a) Flu/ busulphan/ ATG (53) (b) Idarubicin/ flu/cytarabine (19) (c) Other combinations including ATG or ALG (17)	86%	100%**: 27/48 Mixed: 11/48 0%: 10/48	Acute: 17/79 Chronic: - limited: 13/36 - extensive: 3/36	37%††	71%
Slavin *et al.*(24)	CML (8) AML (8) ALL (2) NHL (2) MDS (1) MM (1) Non-malignant (4)	Flu/ busulphan/ ATG	100%	Mixed**: 9/26 (transient)	Acute: 10/26 Chronic: - limited: 9/21 - extensive: 0	15%	82%
Nagler *et al.*(44)	Non-Hodgkin's (19) Hodgkin's (4)	Flu/ busulphan/ ATG	100%	100%**: 16/23 Mixed: 7/23	Acute: 8/23 Chronic: - limited: 3/16 - extensive: 1/16	30%	NA
Childs *et al.*(8)	Metastatic renal cell carcinoma (19)	CTX/flu	100%	Sustained in all patients; mixed or complete	Acute: 10/19 Chronic: - limited: 3/17 - extensive: 1/17	11%	53%
Mc-Sweeney *et al.*(26)	AML (11) CLL (8) CML (9) Hodgkin's (4) MM (8) Other (5)	TBI 200 cGy	100%	>95%‡‡: 6/44 → 9/41 1-95%: 38/44 → 32/41 9/44: graft rejection at 2-4 months	Acute: 21/36§§ Chronic: - limited: 7/31 - extensive: 16/31	7%	56%
Carella *et al.*(45)	Hodgkin's (4) NHL (2) CML (2) MDS (1)	CTX/flu	100%	≥88%†: 3/4 0%: 1/4	Acute: 2/9 Chronic: 1/4	0	78%
Grigg *et al.*(46)	(a) A/CML (5) (b) NHL (4) (c) MM (2)	(a) Flu/ara-c/G-CSF (b) Flu/ara-c/cisplatin (c) Flu/melphalan	73%	≥95%†: 5/8 Mixed: 1/8 0%: 2/8	Acute: 7/8 Chronic: - limited: 2/4 - extensive: 2/4	56%	45%

STUDY (REFERENCE)	INDICATION (NUMBER OF PATIENTS)	PRE-TRANSPLANT CONDITIONING (NUMBER OF PATIENTS)	ENGRAFT-MENT	CHIMERISM: %DONOR CELLS IN NUMBER OF PATIENTS (NUMBER ASSESSED)	GVHD >GRADE 1 (NUMBER OF ASSESSABLE PATIENTS)	TRM	TUMOUR RESPONSE (PR OR CR)
Spitzer *et al.*(47)	NHL (11) Hodgkin's (4) AML (3) ALL (1) CLL (2)	CTX/thymic irradiation/ ATG	86%***	≥99%†††: 6/10 30-70%: 3/10 <1%: 1/10	Acute: 6/21	5%	67%
Wäsch *et al.*(48)	Lymphoma (11) AML/MDS (4) CML/MPS (5) Metastatic melanoma (1)	Flu/ carmustine/ melphalan	100%‡‡‡	100%**: 16/21 Mixed: 5/21	Acute: 13/21 Chronic: - limited: 5/17 - extensive: 9/17	19%	90%
Garban *et al.*(49)	MM (12)	Flu/ ATG/ busulphan	100%	100%**: 7/12 Mixed: 2/12 Not determined: 3	Acute: 6/12 Chronic: - limited: 5/7 - extensive: 2/7	NA	92%

ALG = anti-lymphocyte globulin, ALL = acute lymphoid leukaemia, AML = acute myeloid leukaemia, Ara-c = cytarabine, ATG = antithymocyte globulin, 2-CDA = 2-chlorodeoxyadenosine, CLL = chronic lymphoid leukaemia, CML = chronic myeloid leukaemia, CTX = cyclophosphamide, DRM = disease-related mortality; mortality due to relapse or progressive disease, Flu = fludarabine, G-CSF = granulocyte-colony stimulation factor, NA = not available, NHL = non-Hodgkin's lymphoma,
MDS = myelodysplastic syndrome, MM = multiple myeloma, TBI = total body irradiation, TRM = treatment-related mortality.

** at day 60-90, † at three months, ‡ only patients in CR are evaluated, ∫ before day 100 post-transplantation, ** time of last follow-up unknown, †† only engrafted patients were included, ‡‡ T-cell engraftment on day 28 → day 56, ∫∫ only patients without rejection were assessed, *** defined by >1% donor cells in peripheral blood until at least day 35, ††† 2-8 weeks post-DLI (prophylactic), ‡‡‡ defined as neutrophil recovery of >1.0x10⁹/l and platelet recovery of >20 x 10⁹/l.*

and in myelodysplastic syndrome if there are high-risk factors. Due to high TRM and a low cure rate, if any, the use of allo-SCT in multiple myeloma is questionable.

In summary, allo-SCT is used with the intention to cure in some highly malignant diseases. However, its high TRM (20-30% after one year) makes it a high-risk procedure that is therefore usually limited to patients less than 60 years of age. For this reason, efforts are being made to decrease TRM without losing the beneficial GVT effect. Non-myeloablative SCT, i.e. an SCT following a non-lethal conditioning regimen, is one of the potential solutions currently under investigation.

Table 1
Standard indications for allogeneic stem cell transplantation

Chronic myeloid leukaemia
Acute myeloid leukaemia
Acute lymphoid leukaemia
Follicular non-Hodgkin's lymphoma*
Myelodysplastic syndrome*
Severe aplastic anaemia*
Multiple myeloma†

** depending on risk group, † this indication is questionable due to high TRM.*

RESULTS OF NON-MYELOABLATIVE SCT

The assumption of donor stem cells needing space in the bone marrow to engraft successfully and that this space must be provided by myeloablative host conditioning has recently been questioned.[20] By establishing a stable presence of both donor and recipient blood cells (mixed chimerism) in dogs after a conditioning regimen consisting of lymph node irradiation only and therefore not affecting recipient bone marrow, Storb *et al.*[20] proved that donor stem cells are able to create their own niche in unconditioned bone marrow. They also discovered that the immunosuppressive effect of total body irradiation and chemotherapy probably plays a more important role in achieving engraftment of donor stem cells. In mice, engraftment of fully mismatched donor bone marrow is demonstrated after pre-transplant conditioning with anti-CD4 and anti-CD8 antibodies, cyclophosphamide and irradiation of the thymus,[21] or after treatment with one dose (200 cGy) of total lymphoid irradiation and cyclophosphamide,[22] indicating that not myeloablation, but immunosuppression is the cornerstone of graft acceptance. Ramshaw *et al.*[23] demonstrated that even without conditioning, engraftment of syngeneic male stem cells could be obtained by transplanting a very large graft.
In the past years, there have been numerous human trials investigating less toxic conditioning regimens that do not rely on myeloablation but on immunosuppression. There

are several reasons for the apparent interest in non-myeloablative SCT, also called as mini SCT, for reducing TRM. The most important one is that severity of the conditioning regimen might be positively correlated to the incidence and severity of GVHD:[12,13] less intensive pre-transplant conditioning would result in less tissue damage and inflammation, leading to a less severe 'cytokine storm' and therefore to a decreased target organ susceptibility to GVHD. The reverse correlation between the degree of immunosuppression and GVHD has been elegantly demonstrated by Prigozhina *et al.* in a mouse model.[22] Furthermore, non-myeloablative conditioning will require lower doses of medication and radiotherapy and will therefore lead to a possible reduction in treatment-related toxicity. Also, less severe conditioning might lead to a less profound thrombopenia and granulocytopenia in the period directly post-transplantation, resulting in a decreased risk of haemorrhage and infection. In conclusion, allo-SCT following non-myeloablative conditioning might very well lead to a decrease in TRM compared with myeloablative allo-SCT.

Table 2 summarises the preparative protocols and results of recent trials on allo-SCT following non-myeloablative conditioning. Most of the studies included only patients with haematological malignancies, although some groups also investigated patients with (metastatic) solid tumours. Slavin *et al.* included four patients with non-malignant genetic diseases (β-thalassaemia major, Fanconi's anaemia, Blackfan Diamond anaemia and Gaucher's disease, respectively), all alive with a Karnofsky score of 100% at the time of last follow-up.[24] As pre-transplant conditioning therapy, cyclophosphamide (CTX), melphalan and fludarabine are the most widely used. Giralt *et al.* investigated a combination of cladribine and melphalan, but had to discontinue this arm prematurely because of excessive toxicity.[25] Remarkable is that only McSweeney *et al.* used a sublethal dose of total body irradiation (TBI) in their conditioning therapy.[26]

The vast majority of donors were HLA-matched siblings; Giralt *et al.* however also included patients with one antigen-mismatched sibling donors and matched unrelated donors.[25,27] $CD34^+$ stem cells were obtained by leukapheresis of peripheral blood after stimulation with G-CSF, or harvested by bone marrow aspiration. T-cell depleted grafts were not used in any of the trials. GVHD prophylaxis consisted mainly of cyclosporin A (CsA); this was sometimes combined with methotrexate (MTX), steroids or other immunosuppressants. One study used mycophenolate mofetil (MMF), a lymphocyte-specific immunosuppressant, in combination with CsA.[26] Generally, patients were considered assessable for chronic GVHD if they were alive 100 days post-transplantation.

In most trials, engraftment was defined by neutrophil or full haematopoietic recovery, although since pre-transplant conditioning was intended to be non-myeloablative, leucocytes could not only be donor-derived, but also originating from recovering recipient stem cells. Therefore in non-myeloablative allo-SCT the existence of donor chimerism (donor cells detectable in the recipient peripheral blood) is a more informative parameter. In *table 2*, chimerism data at three months post-transplantation is provided whenever available. This period exceeds the normal lifespan of most peripheral blood cells, except long living T cells. Consequently, the donor leucocytes detected at that time cannot be cells that were already present in the graft, but must be derived from stem cells that have started to proliferate in the recipient and thus have engrafted. As can be seen in *table 2*, engraftment is realised in most of the studies published thus far. However, data on long-term chimerism are virtually absent.

In the trials summarised in *table 2*, mean TRM was 27% (range 0-88%); of 61 patients with CML, 13 (21%) died from their treatment. These percentages are comparable with mortality rates observed in SCT following myeloablative conditioning. This might seem disappointing, but since most studies only included poor-risk patients that were heavily pre-treated and/or excluded from standard allografting, patient selection might be an explanation for this outcome. Anyway, thus far the mini transplant procedure does not meet its expectations with respect to TRM.

Mean tumour response reported in *table 2* is 66% (range 45-92%); of a total of 61 patients with CML, the disease entity known to be most sensitive to an allo-immune response, 44 (72%) had a partial or complete response. However, relapse was common in most studies, and/or follow-up was too short to draw conclusions on long-term outcome.

In our own hospital, up until now 15 patients with haematological malignancies or metastatic breast cancer have received a mini SCT. The results are reported in a separate paper in this issue. Our own results as well as the results in *table 2* show that non-myeloablative conditioning followed by allo-SCT can result in engraftment and chimerism in patients with various haematological malignancies and solid tumours. It can also lead to a more or less sustained complete or partial remission in a subset of patients. However, TRM may still be disappointing and thus far in conflict with the hypothesis that non-myeloablative conditioning would lead to less complications and mortality, although this might be due to unfavourable patient selection. In the majority of publications graft rejection and disease recurrence are common, and/or follow-up is too limited to draw conclusions on long-term chimerism, tumour response, TRM and disease-free survival. Moreover, no prospective randomised trials have been carried out to compare myeloablative and non-myeloablative conditioning with respect to all these parameters. Therefore, at this moment non-myeloablative allo-SCT must still be regarded

to as an experimental treatment procedure.
A disadvantageous 'side effect' of the decreased toxicity of mini SCTs is that the oncolytic potential of the conditioning regimens used is also less compared with myeloablative regimens or regular chemotherapy/radiotherapy. Consequently, the concept of allo-SCT preceded by non-myeloablative conditioning relies heavily on the GVT effect as the most important factor in the treatment of malignancies. It is not yet clear whether the GVT effect after mini SCTs is comparable with the effect observed after SCT following myeloablative conditioning. In any case, in order not to lose the oncolytic effect of chemotherapy and radiotherapy prior to SCT altogether, attempts should be made to design a conditioning regimen in which the toxicity is as low as possible to ensure an optimal balance between antitumour effect (exerted by the conditioning regimen as well as the allograft) and TRM.

FUTURE PERSPECTIVES

Most of the current protocols for allo-SCT require the availability of an HLA-identical donor, imposing an important restriction on the application of this therapy. It is estimated that in the overall population, in less than 30% of all patients a genotypically HLA-identical related donor can be found.[28] An HLA-matched unrelated donor can be found for approximately 50% of Caucasians; for non-Caucasians this chance is thought to be less than 10%. Therefore, efforts are being made to make allo-SCT available to more patients, for instance by accepting HLA-haploidentical relatives to serve as a source for stem cells. The overall chance of identifying such a donor within the family of the patient exceeds 90%.[28] Mismatched allo-SCT is, however, associated with higher incidence of ≥grade II acute GVHD and graft failure.[29] Although in several studies, after a myeloablative conditioning regimen and using T-cell depleted 0-3 antigen-mismatched grafts, no correlation was observed between the number of mismatched antigens and the incidence of ≥grade II acute GVHD, nor between mismatch and risk of graft rejection.[28,30-32] Recently, Sykes *et al.* reported sustained mixed chimerism and a GVT effect in a patient after 1-2 antigen-mismatched allo-SCT following non-myeloablative conditioning.[33]
A promising method by which the apparent immunological action of bone marrow allografts against tumour cells could be augmented is allo-SCT followed by delayed infusion of lymphocytes from the same donor. In human trials, donor lymphocyte infusion (DLI) following allo-SCT is being investigated as being a potential therapy for leukaemia (in particular CML),[10,34-36] breast cancer,[37] multiple myeloma[9,38] and renal cell carcinoma,[8] providing further evidence for the existence of a GVT effect.[11] An effect of DLI in lymphoma patients has been reported by Mandigers *et al.*,[39] although it appears to be limited.[10] However, only a small amount of data is available on this group of patients.[40]
In regular DLI, lymphocytes are transplanted that are directed against a wide variety of antigens. Tumour cells could, however, possibly be attacked more specifically by infusion of tumour-specific T lymphocytes.[41] This concept has been proven in a patient with CML relapsing after allo-SCT and DLI. Infusion of allogeneic cytotoxic T cells selected for their reactivity predominantly directed against recipient CML cells induced a haematological response and resulted in complete molecular remission from day 140 without reinforcing GVHD.
In our own laboratory as well as others, investigations are currently being carried out to determine whether it is possible to vaccinate cancer patients with dendritic cells that are loaded with tumour antigens. In the future, this vaccination might be performed after an allo-SCT to overcome the apparent incompetence of the recipient immune system to eliminate the tumour, which is possibly caused by tumour-induced anergy.
In conclusion, there are several promising procedures under investigation that aim to augment the antitumour effect elicited by the allogeneic immune system that is created by allo-SCT. For most techniques, engraftment of allogeneic stem cells is necessary before they can be applied successfully. Therefore, making allo-SCT available with minimal toxicity and TRM, for as many patients as possible, should be a major goal in transplantation research. Although thus far TRM in non-myeloablative SCT is still considerable, similar disadvantages of regular therapies as well as promising results with mini SCTs certainly make it worthwhile to keep on trying. Not only patients with haematological malignancies, but also patients with solid tumours or non-malignant (autoimmune) diseases might very well benefit from every bit of progression that is being made.

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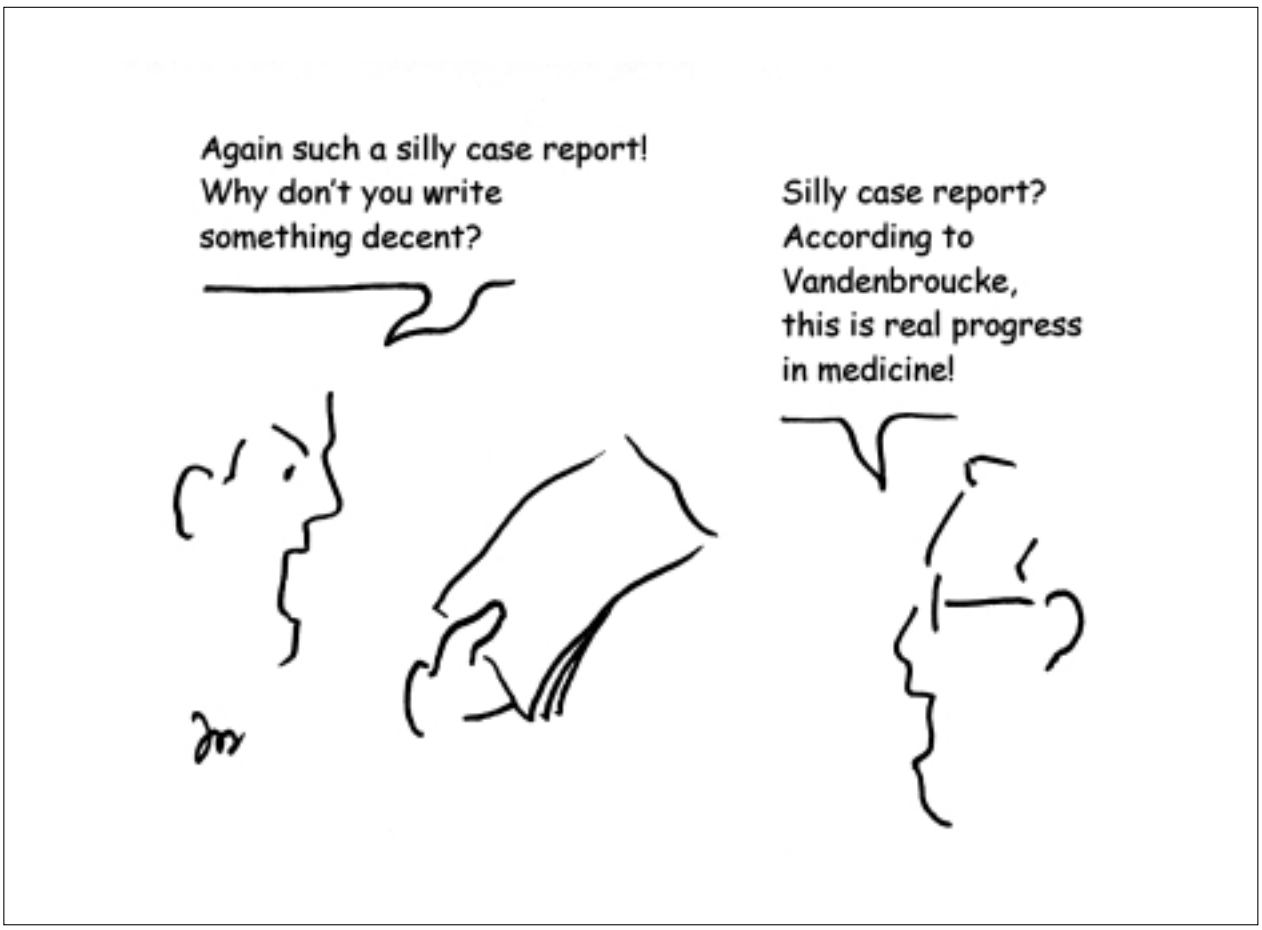

Huugen, et al. Advantages and limitations of (non-)myeloablative allogeneic stem cell transplantation.

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ORIGINAL ARTICLE

Non-myeloablative allogeneic stem cell transplantation in patients with solid tumours and patients with a haematological malignancy

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ABSTRACT

Allogeneic stem cell transplantation (allo-SCT) is a treatment option for several haematological tumours, not only to regain haematopoiesis after myeloablative chemotherapy and/or radiotherapy, but also for its apparent antitumour effect. This so-called graft-*versus*-tumour effect might not only be effective against haematological tumours, where it has best been proven, but also against solid tumours. To reduce morbidity and treatment-related mortality of allo-SCT, efforts are being made to establish engraftment of allogeneic stem cells after a non-myeloablative conditioning regimen and create a 'mini transplantation'. Such a therapy relies more heavily on the graft-*versus*-malignancy effect than on the antitumour effect exerted by the chemotherapy/radiotherapy.

Here, we report the outcomes of 15 patients with haematological disease or solid tumours who underwent an SCT in the University Hospital Maastricht after a non-myeloablative fludarabine/cyclophosphamide conditioning regimen. Although results are promising, adjustments will be needed to ensure long-term stable engraftment and optimise the antitumour effect.

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) preceded by myeloablative conditioning, i.e. high-dose treatment completely ablating the recipient's bone marrow, is a treatment option for several haematological malignancies, including non-Hodgkin's lymphoma, multiple myeloma and various forms of leukaemia or myelodysplastic syndrome.[1-3] Unfortunately, the considerable morbidity and treatment-related mortality (TRM; up to 25%) caused by graft-*versus*-host disease (GVHD), infections and direct toxicity of the conditioning regimen makes it a high-risk procedure that can only be offered to young patients without significant comorbidity.

Recent publications suggest that allo-SCTs could be performed more safely after a non-myeloablative conditioning regimen.[4-6] Apparently, not the myeloablative function of pre-transplant conditioning, but merely its immunosuppressive effect is important for the engraftment of donor stem cells. The decreased antitumour effect of such a reduced intensity conditioning regimen is supposed to be overcome by a graft-*versus*-malignancy effect exerted by the allogeneic transplant.[7] Furthermore, there is accumulating evidence that this effect is not limited to haematological malignancies alone, but is also effective against solid tumours such as renal cell carcinoma[8] and breast cancer.[9-11] Here, we report our first experiences with non-myeloablative mini SCTs in 15 patients with haematological disease or solid tumours.

MATERIAL AND METHODS

Up until now, data from a 90-day follow-up can be given for 15 of the patients included in this trial: eight females and seven males with a median age of 52 years (range 42-60 years). Six of them had metastatic breast cancer, one had melanoma, another had renal cell cancer and the other seven had a haematological malignancy (see *table 1*).

Table 1
Mini SCT for haematological malignancy and metastatic breast cancer in Maastricht

Indication (number of patients)	Haematological malignancy (7) Metastatic breast cancer (6) Melanoma (1) Renal cell cancer (1)
Pre-transplant conditioning	Fludarabine 25 mg/m² on day (-5, -4,) -3, -2, -1 CTX 60 mg/m² on day (-2,) -1
Early recovery (donor or recipient)	100%
Chimerism on day 90 (%$^{\text{donor cells}}$ in number of patients/number assessed)	>90%: 6/11 10-30%: 3/11 1%: 2/11
GVHD >grade I (number of assessable patients)	Acute: 1/14 (one patient excluded death day 7) Chronic: 2/13 (two patients excluded death day 7 and day 39)
TRM at day 100	2/15
Tumour response (PR or CR)	3/12

CTX = cyclophosphamide, GVHD = graft-versus-host disease, TRM = treatment-related mortality, PR = partial response, CR = complete response.

To be included cancer patients had to suffer from chemotherapy-sensitive disease, except for the patients with renal cell cancer and melanoma. Patients with haematological disease were only included if they were denied a regular allo-SCT because of comorbidity or age, although their disease as such implied a positive indication for allo-SCT. In the University Hospital Maastricht, regular allo-SCT is an option for patients less than 55 years of age with various forms of (pre-)leukaemia, non-Hodgkin's lymphoma or multiple myeloma. The maximum age to be included in this trial was 60 years.
Initially, conditioning consisted of fludarabine 25 mg/m² for either three days (seven patients) or five days (four patients) on days -5, -4, -3, -2 and -1 and cyclophosphamide (CTX) 60 mg/m² on day -1, in an effort to establish a minimally myeloablative regimen. After 11 patients had been included, conditioning was intensified to fludarabine 25 mg/m² on days -5, -4, -3, -2 and -1 and CTX 60 mg/m² on days -2 and -1, because of the high rate of autologous recovery. Stem cells were obtained from HLA-identical donors by leukapheresis after stimulation with G-CSF (13 patients) or by bone marrow harvesting (two patients). The median number of $CD34^+$ cells obtained by leukapheresis was 2.5 x 10.6 $CD34^+$ cells/kg of the recipient (range 0.9-5.6). No T-cell depletion was performed. Methotrexate and cyclosporin-A were given for GVHD prophylaxis.
If chimerism analysis showed <90% donor chimerism or disease progression, donor lymphocytes (DLI) were administered according to the protocol. The first DLI consisted of 10.7 $CD3^+$ T cells, a further DLI of between 2-3 x 10^7 T cells. In total 10/15 patients received DLI: four patients once, five patients twice, and one patient three times. Two patients who died early and two patients with acute GVHD did not receive DLI. Finally one patient received a full second transplant and no DLI.

RESULTS

In this group of 15 patients, treatment-related mortality (TRM) was 3/15. One patient died as early as day 7 after transplantation due to sepsis, possibly in combination with cardiotoxicity due to cyclophosphamide. For this patient the allo-SCT procedure was a second transplant (recurrence of non-Hodgkin's lymphoma after autologous transplantation). A second patient died on day 37 due to liver toxicity and terminal kidney failure. These two patients received two days of CTX. A third patient died because of lethal GVHD after DLI on day 138 after transplantation. Of the patients surviving day 100, the transplant-related non-haematological toxicity was grade III/IV in only 1/13 patients. With early engraftment, autologous recovery occurred in all patients indicating that the regimen is not myeloablative. Chimerism on day 30 showed donor chimerism (>10%) in 9/11 patients measured. On day 60 figures were identical (11/13 samples available). Chimerism analysis in patients surviving day +90 showed >90% donor chimerism in six patients, 10-30% donor cells in three patients and <5% in another two patients. One patient had 60% donor cells on day +60, as this was the last sample available.
A total of ten patients received donor lymphocytes. In one patient no donor cells were given, since he was suffering from severe grade III GVH, despite a low number of donor cells (10%). Five patients received DLI because of incomplete donor chimerism: the level of donor chimerism has not increased in any of these patients thus far. Five patients received DLI because of disease progression. Disease response was observed in one patient with CLL and two patients with breast cancer. The patient with CLL had a grade I acute GVH. He received no DLI and donor chimerism was 100% at day 330. He is now free of disease with a follow-up of over three years. The two patients with breast cancer obtained a partial response after being treated with donor lymphocyte infusion because of progressive disease. Nine patients (four with breast cancer, two with non-Hodgkin's lymphoma and three with multiple myeloma) showed no tumour response and died because of progressive disease. The patient with renal cell cancer is suffering from progressive disease, despite GVHD. Median follow-up of the total group is 328 days (range 127-1085).

DISCUSSION

Here, we report the first results of a phase I/II trial on non-myeloablative allo-SCT in patients with haematological malignancies or solid tumours. Initially, in an effort to establish a minimally myeloablative and toxic regimen, conditioning first consisted of three or five days of fludarabine and only one day of CTX. However, the high rate of autologous recovery (5/11 persons had less than 20% donor cells in peripheral blood) demonstrated that this regimen is not adequate to establish donor chimerism and therefore to expect an antitumour effect in a large group of patients. One day of cyclophosphamide and three or five days of fludarabine appeared not to be as immunosuppressive as was needed. It was therefore decided to intensify the conditioning to a five-day fludarabine/two-day CTX regimen. Despite this regimen one patient still had only 25% donor chimerism at day 90. One patient had 95% donor chimerism and two patients died too early to measure donor chimerism. It is therefore too early to judge the more intensified schedule. Another way to increase the level of donor chimerism might be to increase the number of stem cells donated.[12] The number of stem cells was relatively low in our group of patients (median 2.5 x 10^6/kg). We have now increased the minimal number of stem cells needed for a transplant to 4 x 10^6 $CD34^+$ cells.

It is unclear whether DLI will really influence the level of donor chimerism in this protocol. Ten patients underwent between one and three DLI procedures, five of them for inadequate donor chimerism. We did not observe an increase in donor chimerism in any of these patients. It seems that a loss of transplant (<10% donor chimerism) cannot be corrected by DLI alone. It should be mentioned, however, that thus far no data are available on a correlation of level of donor chimerism and clinical response and therefore the need to obtain full donor chimerism.

As was expected from the literature, incidence and severity of GVHD as well as TRM are low compared with regular allo-SCT.[13] However, since donor chimerism was not present in all patients, these patients are not at risk for GVHD. Skipping the two patients with early death (day 7 and 39). 13/15 patients were actually at risk for GVHD. Of these, 11/13 had donor chimerism of 10% or more. It is not yet clear from the literature how many donor cells are needed to obtain GVHD. In our situation one patient with only 10% donor cells suffered from severe (grade III) GVHD. One patient developed lethal GVHD after donor lymphocyte infusion. If, indeed, the incidence of GVHD is lower in non-myeloablative transplantation, protocols need to be established in larger series. The same holds true for TRM. In our hands it was 2/15 before day 100, including one patient with a second transplant, and one patient at day 138 due to GVHD. The figure of 3/15 deaths (20%) is comparable with TRM in full allo-SCT. Therefore it is too early to conclude whether mini transplantation is indeed as safe as has been suggested.

Although tumour response was achieved in some patients, results are still unsatisfactory. This might be caused in part by autologous recovery, but progressive disease has also been observed in patients with nearly full donor chimerism. Therefore, establishment of donor chimerism per se is not enough to establish antitumour effects in all patients. Interestingly, we observed three patients (two with breast cancer, one with myeloma) in which progression of disease took place together with loss of donor chimerism. It is, however, not clear whether there is a correlation between tumour progression and graft rejection and, if so, in what direction. Two patients with progressive breast cancer received DLI, after which a partial response could be demonstrated. At the time of DLI, there was already a substantial percentage of donor chimerism. These data indicate a possible immune effect of donor T cells, also in breast cancer.

Based on the results so far we will extend the number of patients treated according to the protocol described above. Larger series will give more precise information on TRM and disease response. At present, non-myeloablative allo-SCT should, however, be performed only in trials until major clinical relevant end-points are established and successfully achieved.

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ABOUT THE COVER

Woodcut 'Chairs'

JOSÉ VEUGEN

This month's cover shows a woodcut by José Veugen. José works in Nijmegen, the Netherlands. After having successfully concluded her Social Sciences studies at the Nijmegen University, she attended the Academy of Art in Arnhem. She exhibits her work at many individual and group exhibitions in the Netherlands, such as Galerie Stagione d'Arte in Amsterdam, Watertoren in Aalsmeer, Stadsgalerie in Gouda, and Bergkerk in Deventer. She teaches art in Nijmegen and runs painting classes in Morocco. Nowadays, her art mainly depicts chairs, since – as she puts it – chairs have an outspoken function in space: a chair is a spot to rest and sit, but also to observe the surrounding and continuously moving world. The chairs depicted are imaginary and represent not only rest, but also poetry. A limited edition of original prints (size 37.5 x 57 cm) of the woodcut on this month's cover is available at a price of € 200. You can order the print at Galerie Unita, Rijksstraatweg 109, 6573CK Beek-Ubbergen, the Netherlands or by e-mail: galerie-unita@planet.nl.

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CASE REPORT

Acute idiopathic thrombocytopenic purpura in adults following viral infection: report of two cases

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ABSTRACT

Idiopathic thrombocytopenic purpura (ITP) can follow a viral infection. We describe severe thrombocytopenia in two adult patients preceded by varicella zoster and Epstein-Barr virus infection, respectively. The differences between acute and chronic ITP are discussed, as well as therapeutic options.

INTRODUCTION

Acute idiopathic thrombocytopenic purpura (ITP) following a viral infection is a well-known disease in children, but is a rare cause of thrombocytopenia in adult patients. Contrary to chronic ITP, acute ITP is a self-limiting disease, relapses occur infrequently, and therapy is only necessary in case of bleeding. We describe two patients with severe thrombocytopenia following varicella zoster virus and Epstein-Barr virus infection, respectively.

Case 1

A 34-year-old woman was admitted to our hospital with severe hypermenorrhoea, gingival bleeding and spontaneous haematomas. Two weeks before admission she suffered from a febrile illness with generalised vesicles, probably chickenpox. She was not taking any medication.
On admission she had a temperature of 37.8 °C, a regular pulse of 90 beats/min and a blood pressure of 120/70 mmHg. Petechiae were seen on the palate and multiple ecchymoses on her arms and legs. Centripetally located, crusted and purpuric chickenpox lesions were observed. Lymph nodes and spleen were not enlarged.
Laboratory investigation showed an ESR of 7 mm/h, a haemoglobin level of 7.0 mmol/l, a WBC count of 8.1 x 10^9/l with a normal differential count and a platelet count of 1 x 10^9/l with a mean platelet volume (MPV) of 15.2 fl (normal: 6-8 fl). Serum thrombopoietin (TPO) was 6 E/ml (normal: 4-32 E/ml) and antiplatelet antibody tests were negative. Varicella zoster virus (VZV) IgG and IgM antibodies were present. The complement-fixing antibody test showed a titre of 1:2048. EBV, CMV, HSV and HIV serology were negative. Her bone marrow was normocellular and showed no abnormalities. Numerous megakaryocytes were seen.
She was diagnosed as having acute ITP following VZV infection. She was treated with intravenous methylprednisolone 500 mg/day for three days, followed by 20 mg prednisone daily. After an initial rise in platelet count there was a relapse. The prednisone dose was raised to 60 mg daily, which produced a sustained response. Her platelet count normalised within seven weeks and the prednisone was stopped. Since then, there has been no thrombocytopenia for two and a half years.

Case 2

A 26-year-old man presented with haematuria and spontaneous haematomas. For four weeks he had been suffering from a febrile illness with loss of appetite and myalgia. He was not taking any medication.
On admission he had a temperature of 38.3 °C, a regular pulse of 100 beats/min and a blood pressure of 110/70 mmHg. There were scattered ecchymoses all over his body and petechiae on the palate. Lymph nodes and spleen were not enlarged.

Laboratory investigation showed an ESR of 7 mm/h, a haemoglobin level of 10.2 mmol/l, a WBC count of 28.1 x 10^9/l with numerous atypical lymphocytes and a platelet count of 7 x 10^9/l with an MPV of 15.2 fl. Serum TPO level was 86 E/ml. Antiplatelet antibody tests were positive for anti-Ig and anti-IgG. Epstein-Barr virus serology was positive for IgM and IgG. HIV and CMV serology were negative. Bone marrow showed no morphological abnormalities and a normal megakaryocyte count.
He was diagnosed as having acute ITP following Epstein-Barr virus infection. Treatment was started with intravenous human immunoglobulin (IVIg) 1 g/kg/day for two days. There was a rapid rise in platelet count with normalisation within four weeks. Since then, there has been no relapse for two years.

DISCUSSION

Acute idiopathic thrombocytopenic purpura (ITP, also known as immune thrombocytopenic purpura) following viral infection is a relatively common disease in childhood but very rare in adults. In adults, ITP is typically a chronic disorder with frequent relapses without an association with infection. Differentiating acute and chronic ITP is useful because they differ in incidence, cause, prognosis and therapy. Some distinguishing features are summarised in *table 1*.
Acute ITP is characterised by thrombocytopenia for less than six months, frequently preceded by viral infection or vaccination. It usually resolves spontaneously. Common preceding infections in children are rubella, EBV, VZV, influenza and HIV infection, although non-specific viral infections still predominate.[1] In adults, EBV-associated ITP with severe thrombocytopenia has been reported incidentally.[2] ITP associated with VZV infection in adults is extremely uncommon.

Table 1

FEATURES	ACUTE ITP	CHRONIC ITP
Peak incidence (age)	2-6 years	20-40 years
Sex predilection	None	3:1 female:male
Antecedent infection	Common 1-3 weeks before	Unusual
Onset of bleeding	Abrupt	Insidious
Platelet count	<20,000/ul	30-80,000/ul
Eosinophilia/ lymphocytosis	Common	Rare
Duration	2-6 weeks; rarely longer	Months or years
Spontaneous remissions	Occur in 80% of cases	Uncommon

Viral infection may induce thrombocytopenia in several ways. In the early stage of infection, virus can invade megakaryocytes and thereby interfere with platelet production.[3,4] Virus antigens can attach to platelets and promote intravascular aggregation[5] or trigger clearance by the spleen.[6] There may be a direct toxic effect on platelets.[7] Furthermore, virally induced vasculitis can give rise to microangiopathic thrombocytopenia. Immune-mediated thrombocytopenia characteristically appears seven to ten days after the primary symptoms of the infection have been fully established, but it can develop any time thereafter.[8]
Central in the genesis of ITP is that platelets sensitised by autoantibodies or antibody-containing immune complexes are cleared by phagocytic cells in the reticuloendothelial system, in particular the spleen. Antibodies responsible for virus clearance may cross-react with antigens naturally present on platelets, in particular glycoproteins (GP). These antibodies are frequently of the IgM type[9] in contrast to chronic ITP where antibodies are always of the IgG type. Disappearance of IgM antibodies from the circulation may explain the self-limiting nature of acute ITP.[10] Furthermore, the antibodies may be part of immune complexes which bind to platelet-Fc receptors. Levels of immune complexes have been shown to correlate negatively with platelet count in HIV infection.[11]
When acute ITP is suspected, other causes of immune-mediated thrombocytopenia have to be excluded, such as pregnancy, SLE, use of medication (heparin, sulpha) and recent blood transfusion. Physical examination should focus on the extension of bleeding and spleen size (usually normal). Complete blood count, platelet count in citrate blood and examination of the peripheral smear should exclude pseudothrombocytopenia and leukaemia. In acute ITP, atypical lymphocytosis or eosinophilia can be seen and usually the mean platelet volume (MPV) is elevated. LE serology should be taken and HIV test requested. Serum thrombopoietin (TPO), which is mainly produced by the liver at a constant rate, irrespective of the platelet count, regulates megakaryocyte development and platelet production. TPO is bound to platelets and is removed together with platelets in the spleen. Serum (free) TPO level rises if platelet production is depressed and less TPO can be bound, or when there is intravascular destruction of platelets. If the low platelet count is caused by accelerated destruction in the spleen as occurs in ITP, TPO levels are in the normal range or slightly above. Therefore, estimation of TPO level may help to distinguish depressed platelet production from ITP.[12] Antiplatelet-antibody tests are neither specific nor sensitive enough for routine clinical use. Furthermore, due to severe thrombocytopenia in acute ITP, it is often not possible to collect enough platelets to measure platelet-associated immunoglobulin. However, when detected, platelet antigen-specific antibody supports the diagnosis of ITP. According to the guidelines of the

American Association of Hematology, bone marrow aspiration is mandatory in adults above 60 years in order to exclude myelodysplasia. Under this age it is questionable. Abdominal ultrasound is advised when splenomegaly is suspected on physical examination.

Whether or not to start therapy in acute ITP is still subject to debate. Two studies[13,14] show a favourable outcome in 221 children who were not treated initially. Only 0.9% had fatal bleeding and 87% had a complete remission. In one prospective study, 16 children with severe thrombocytopenia (platelet count <20 x 10^9/l) were not treated. They had a platelet count of >50 x 10^9/l in a median of 16 days and no fatal outcomes.[15] The lack of consensus regarding therapy is illustrated by a recent world-wide survey in the Lancet. Of 1976 children with ITP, 31% were not treated, 29% received corticosteroids and 33% intravenous immunoglobulin. The outcome at six months did not seem to be affected by initial treatment strategy.[16]

It is advised to treat patients with significant mucous membrane bleeding and a platelet count <20 x 10^9/l or those with minor purpura and a platelet count <10 x 10^9/l. Oral prednisone 1-2 mg/kg/day results in a more rapid platelet count recovery than no treatment.[15] The duration of treatment is usually limited to 21 days. High-dose methylprednisolone (10-50 mg/kg/day) for several days has equal results. Intravenous immunoglobulin induces a quick rise in platelet count, comparable to high-dose iv methylprednisolone.[17] A single dose of 0.8 g/kg IVIg seems to be sufficient.[18] However, both for IVIg and for glucocorticoids there is no evidence that treatment diminishes mortality or morbidity. Finally, no randomised studies have been performed in adults with acute ITP. As our first patient showed, a short course of prednisone (high dose for three days, followed by 20 mg/day) was not sufficient.

Idiopathic thrombocytopenia purpura in adults is usually a chronic disorder. However, as these two cases demonstrate, it can occur acutely in the course of a viral illness. This acute variant of ITP runs a benign course and is often self-limiting. Therapy is not always warranted. If treated, glucocorticoids and IVIg seem to be equivalent.

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CASE REPORT

Recurrent staphylococcal bacteraemia and subhepatic abscess associated with gallstones spilled during laparoscopic cholecystectomy two years earlier

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ABSTRACT

This is the first report of *Staphylococcus aureus* bacteraemia and subhepatic abscess in association with intraperitoneal gallstones spilled during laparoscopic cholecystectomy two years earlier. Spilled gallstones can lead to abscess formation in the late postoperative period by acting as foreign bodies that can become infected during bacteraemia and then become a source of recurrent bacteraemia.

INTRODUCTION

Laparoscopic cholecystectomy (LC) is nowadays considered a safe procedure for treatment of symptomatic cholelithiasis. The hospitalisation period and cosmetic results compare favourably with the classical transabdominal approach,[1] but bile duct injury occurs more frequently than during open surgery. One complication specifically associated with LC is intraperitoneal spill of bile and gallstones, which can lead to abscess formation.[2-9] The interval between LC and abscess formation associated with dropped gallstones may vary from several days to several years, but an interval exceeding one year has only been reported infrequently. The micro-organisms cultured from gallstone-associated abscesses are those normally found to colonise the gastrointestinal tract.[3,7,8] We describe here a patient who presented two years after LC with staphylococcal bacteraemia and a subhepatic abscess associated with spilled gallstones. To our knowledge, this is the first report of late *S. aureus* infection associated with spilled gallstones.

CASE REPORT

In November 1995, a 48-year-old woman was admitted with attacks of pain in the upper right abdominal quadrant, accompanied by nausea, vomiting and pruritus. She had noticed brown-coloured urine and pale-coloured stools for one day. On examination, she was afebrile, slightly jaundiced, with abdominal tenderness but without signs of an acute abdomen. Laboratory tests indicated moderate cholestasis. On echography, the gallbladder was filled with stones but the gallbladder wall, bile ducts and liver appeared normal. Endoscopic retrograde choledochopancreaticography (ERCP) with papillotomy was performed and one stone was removed from the common bile duct. During LC the following day, the gallbladder was found to adhere to the surrounding tissues. Rupture of the gallbladder during removal led to intraperitoneal spill of bile and gallstones. No antibiotics were given but the postoperative course was nevertheless uneventful.
Three months later the patient complained of ructus, pyrosis and anorexia. Duodenoscopy, echography, CT and MRI examinations suggested the presence of a haemangioma

in the left liver lobe, which had probably been overlooked during the previous echography because the attention was then focused on cholelithiasis. On the CT scan, several surgical clips from the previous LC were visible. The haemangioma was removed surgically and no signs of intraperitoneal inflammation were observed during operation. The postoperative course was again uncomplicated. Histology of resected material revealed a cavernous haemangioma with thrombosis and fibrosis.

In May 1996, three months after the last operation, the patient presented with fever and suppurative arthritis of the right knee. In the weeks before admission, the knee had been punctured several times because of recurrent swelling without other signs of inflammation. In the absence of any other predisposing factor for septic arthritis, such as intravenous drug use, the infection was thought to have occurred secondary to the punctures of a previously sterile effusion. Microscopy of the knee effusion revealed many granulocytes, but no bacteria, crystals or findings indicative of rheumatoid arthritis. Cultures of the pus yielded *Staphylococcus aureus*, sensitive to flucloxacillin. No blood cultures were taken at that time. Treatment consisted of arthroscopic drainage and high-dose flucloxacillin intravenously for four weeks. Complete recovery was achieved after further treatment with flucloxacillin 500 mg q.i.d. orally for an additional two weeks.

The patient then remained free of symptoms until November 1997, when she presented with pain in the upper right abdominal quadrant and fever up to 40 °C. Abnormal laboratory results were an elevated erythrocyte sedimentation rate (ESR) and C-reactive protein and abnormal liver function indicating cholestasis. Two blood cultures yielded *S. aureus*, with an identical antibiogram as had been obtained from the knee isolate 18 months earlier. Nasal swabs were negative for *S. aureus*. The patient had no prosthetic material. There were no clinical signs of infection of the right knee, and a radiograph showed no indication of osteomyelitis or Brodie's abscess. A skeletal scintigraphy showed moderately increased uptake of radiolabelled bone tracer in the medial side of the right knee, consistent with arthrosis, and an otherwise normal distribution of the tracer, essentially excluding a skeletal focus of infection. Cardiac echography gave no indication of endocarditis. Evaluation of the oral cavity and the ear, nose and throat region revealed no source of the bacteraemia either. However, a CT scan of the abdomen was suggestive of a subhepatic abscess measuring 4.8 cm in diameter and a linear density was seen in the abscess cavity, which was thought to be vascular clips but without indication of stones. ERCP showed no abnormalities. Without prior aspiration of the abscess, treatment with high-dose flucloxacillin intravenously for four weeks resulted in clinical recovery, decrease in size of the abscess and normalisation of the C-reactive protein.

In February 1998 the patient was readmitted with high fever and abdominal pain, with four out of four blood cultures growing *S. aureus* with the same antibiogram as all previous isolates. Echography and a CT scan of the abdomen again revealed an abscess in the same region as in the previous episode, containing solid structures (*figure 1*). A review of all previous CT scans, including the one made before the resection of the haemangioma, confirmed the presence of intra-abdominal stones, which had been misjudged as surgical clips. Laparotomy was performed on the assumption that the abscess was related to the presence of foreign-body material such as surgical clips or dropped gallstones. A smooth-walled abscess containing 27 pigmented gallstones was drained surgically and cultures were positive for *S. aureus*, but no other micro-organisms. Besides the gall-

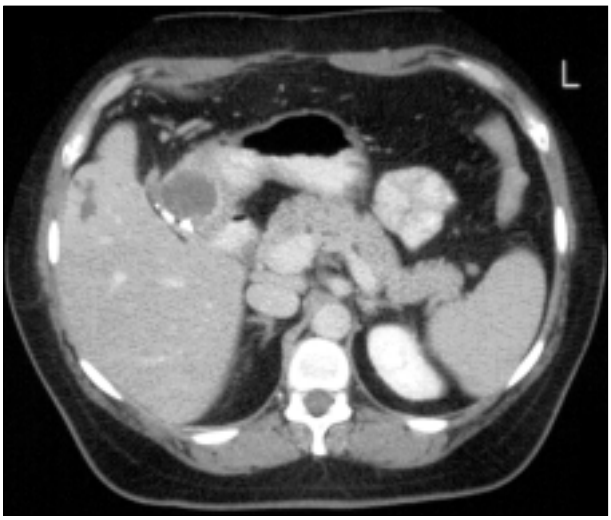

Figure 1
CT scan of the abdomen in February 1998 shows an abscess in the liver hilus with several solid structures

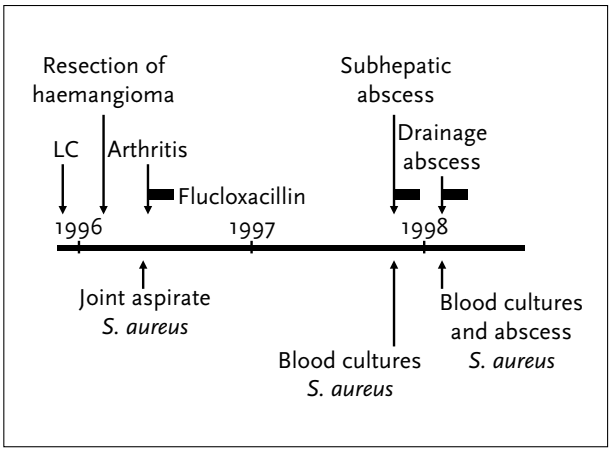

Figure 2
Course of events in the patient described in this report

strones, no surgical clips or other material related to the previous operations were found. Treatment with high-dose flucloxacillin for four weeks now resulted in complete recovery and up until the time of this writing she has remained free of symptoms. The course of events in this patient is summarised in *figure 2* on page 178.

DISCUSSION

Abdominal abscess formation in association with intraperitoneal spill of gallstones is a specific complication of laparoscopic cholecystectomy, second in frequency only to bile duct injury. The outcome of unretrieved gallstones in the peritoneal cavity was recently reviewed.[3] Estimates of the frequency of rupture of the gallbladder during LC vary from 5% to 36%.[3,5,7,9] In a retrospective analysis of 10,174 patients undergoing LC, spillage of gallstones occurred in 5.7% and formation of intra-abdominal abscesses requiring reoperation was seen in 7/547 (1.5%) of the patients with dropped stones, representing 0.08% of all patients.[7] In two other studies, 0.3% of all patients who underwent LC developed abscesses.[8,9] In a review of 31 patients with abscesses associated with dropped gallstones from 25 publications, the median interval between LC and diagnosis was five months (range ten days to five years) and an interval exceeding two years was seen in only one patient.[8] Abscess formation associated with a dropped surgical clip has been described once. In the Netherlands, around 15,000 laparoscopic cholecystectomies are performed each year, corresponding to a frequency of one LC per 1000 inhabitants per year (J. Ringers, personal communication). With the estimated risk of abscesses associated with dropped gallstones varying from 0.08% to 0.3%, this complication would be expected to occur in the Netherlands in 12 to 45 persons yearly and extrapolating these figures to the US population the annual incidence would be 240 to 900 persons.

Risk factors for preoperative gallbladder perforation and formation of abscesses associated with dropped gallstones were male gender, older age, high body mass index,[5,7] adhesions[9] and acute cholecystitis as the indication for the LC.[5] Stone composition also seems a risk factor for abscess formation. In a review of 31 cases, pigmented stones were found in 14 out of 16 patients for whom stone composition was reported,[8] which exceeds the frequency of pigmented stones in general. Compared with cholesterol gallstones, pigmented stones are commonly small-sized and multiple in number often leading to inflammation and formation of adhesions with an increased risk of perforation during LC and difficulties with retrieval of dropped pigmented stones.

Stone-associated abscesses are most frequently located intraperitoneally in the subhepatic space or the retroperitoneum, but have been found infrequently at other locations, including the umbilicus, trocar sites, the right hemithorax, and abdominal wall hernias.[3,7,8]

When culture results from stone-related abscesses were mentioned, the isolated micro-organisms were *Escherichia coli, Klebsiella pneumoniae, Streptococcus milleri, Pseudomonas aeruginosa, Serratia marcescens, Enterobacter cloacae, Hafnea alvei* or *Enterococcus faecalis*.[3,7,8] These micro-organisms are normal inhabitants of the intestinal tract, suggesting that spilled bile or gallstones were already contaminated at the time of spillage. This would be in accordance with the hypothesis of gallstone formation stating that bacteria facilitate gallstone formation and actually make up a significant portion of gallstones. In one case report, cultures from the initial LC and the subsequent abscess were available and yielded identical micro-organisms.[10] To our knowledge, this is the first report of *S. aureus* as the cause of late abscesses in association with spilled gallstones. While blood cultures had not been taken at the time of the arthritis in this patient, they should be part of the work-up of any patient suspected of bacterial arthritis. However, in view of the high virulence of *S. aureus*, we think that the presence of prolonged latent infection between the arthritis and the episodes characterised by bacteraemia and subhepatic abscess would be highly unlikely. A more plausible course of events in our patient would be that the dropped gallstones were secondarily infected during a transient bacteraemia, after which the stones acted as infected foreign bodies leading to recurrent bacteraemia. Although the *S. aureus* strains that were cultured from the knee in 1996 and from the blood and the abscess in 1997 and 1998 had identical antibiograms, genotypic identity or dissimilarity could not be proved as the strain from 1996 was no longer available.

As was the case in our patient, dropped gallstones associated with late abdominal abscesses can be overlooked during radiological investigations although they were often seen in retrospect.[6] A high index of suspicion for the presence of dropped stones is therefore justified in every patient with intra-abdominal abscesses and a history of LC, irrespective of the operation report or radiological results. In most patients an open procedure with drainage of abscesses and removal of all stones was required to achieve a complete cure,[6-8] although successful percutaneous removal of stones has been reported.[10] Late abscesses were not observed if rupture of the gallbladder with spill of bile and stones was followed by conversion to an open procedure,[8] or when a meticulous strategy was followed at retrieval of stones by large bore suction.[5] Retrieval of all dropped stones, therefore, seems worthwhile to prevent the development of infectious complications, but this should be weighed against the low incidence of stone-related infectious complications and the increased morbidity associated with open procedures.

In conclusion, intra-abdominal abscesses can be associated

with gallstones that were spilled during laparoscopic cholecystectomy even if the LC was performed years earlier. Although this complication is rare, spilled gallstones should be considered in patients who have previously undergone an LC, and present with bacteraemia without an obvious source.

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CASE REPORT

Echocardiographic diagnosis in carcinoid heart disease

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ABSTRACT

In this case report the typical echocardiographic features of carcinoid heart disease are presented. Newer treatments such as the use of a somatostatin analogue, interferon and hepatic de-arterialisation have improved the prognosis in patients with carcinoid syndrome. Nevertheless this syndrome portends a poor prognosis in patients with cardiac involvement. Cardiac lesions are mainly located in the right side of the heart. Regurgitation and stenosis of the tricuspid and pulmonary valve, leading to right heart failure, are the most common cardiac manifestations of the disease. Elevated levels of serotonin are probably responsible for the development of these cardiac lesions. Despite treatment resulting in significant reductions of urinary levels of 5-HIAA, regression of the cardiac manifestations in carcinoid syndrome has not been observed. Two-dimensional and Doppler echocardiography are the main tools to establish the diagnosis and severity of carcinoid heart disease. Cardiac surgery for carcinoid heart disease might improve symptoms and longevity, but the scarce data report on early mortality of over 35%.

INTRODUCTION

In 1907 Oberndorfer introduced the term 'Karzinoide' (meaning similar to carcinoma) to describe indolent but malignant tumours arising from the enterochromaffin cells (Kulchitsky cells), in particular from Kulchitsky cells situated in the gastrointestinal tract and the bronchi. These cells produce various biologically active amines or polypeptide hormones, including serotonin (5-hydoxytryptamine) which, when reaching the circulation, are held responsible for the occurrence of the characteristic symptoms. The classical syndrome, occurring in only 4% of the patients with a carcinoid tumour, is characterised by dermal flushing, diarrhoea, bronchospasm (wheezing) and right-sided valvular heart disease.[1,2] In the majority (95%) of patients, the carcinoid tumour arises from the gastrointestinal tract and only causes the syndrome in the presence of hepatic metastases. Serotonin is metabolised to 5-HIAA (5-hydroxyindoleacetic acid) by the monoamine oxidase enzyme in the endothelium of lung and liver vasculature. The amount of serotonin can be measured both in serum and in thrombocytes. The amount of 5-HIAA is measured in the urine.

During the past decade, several new treatments have emerged for patients with malignant carcinoid syndrome in order to eliminate systemic symptoms. Somatostatin inhibits the release of numerous peptides, such as growth hormone, insulin, glucagon and gut peptide. Because of the short half-life of the native somatostatin compound, the somatostatin analogue sandostatin (SMS 201-995) was developed and proved to be more potent and longer acting in its inhibitory effects. It has resulted in marked symptomatic improvement and improved survival in patients with the carcinoid syndrome. Hepatic de-arterialisation by ligation or occlusion has also been effective in relieving the symptoms by inducing rapid tumour shrinkage in patients with hepatic metastases. Despite the alleviation of systemic symptoms by these measures, patients in whom carcinoid cardiac involvement has already developed continue to experience progressive symptoms of right-sided heart failure and mortality from cardiac causes. Treatment

involving only medications for the relief of symptoms of heart failure results in a high mortality rate from progressive right heart failure. To improve the long-term outlook for these patients, severe haemodynamic consequences of the valvular abnormalities of carcinoid heart disease can be corrected by means of valvular surgery. It is therefore important to follow up the patients regularly and consider surgical intervention when cardiac symptoms become severe, albeit with a significant risk of perioperative mortality.
Cardiovascular involvement occurs in over half of the patients with carcinoid disease. The severity of cardiac involvement does not seem to be related to the duration of carcinoid disease, but more to the extent of the disease, i.e. higher plasma levels of serotonin and tachykinins. Cardiac lesions are pathognomonic and in over 50% of patients the cause of death.[3]
Because two-dimensional echocardiography can assess cardiac anatomy and valvular motion and Doppler examination can assess the severity of valvular stenosis and regurgitation, these procedures appear ideally suited for analysing and following the progression of carcinoid heart disease.

CASE REPORT

A 65-year-old man from the Caribbean (Dutch Antilles), who was known to have hypertension, underwent a resection of the coecum in 1985. A carcinoid tumour was diagnosed on the histology findings. Small metastases in the liver were present. In 1993 flushing, diarrhoea and oedema of the legs became manifest.
On admission to our hospital in 1996, his blood pressure was 160/100 mmHg. The jugular veins were distended. On auscultation an S3 gallop was noted; first and second heart sounds were normal. A mid frequent, holosystolic murmur accompanied by a mid frequent, decrescendo diastolic murmur, both grade 2/6, were present. No right ventricle impulse was detected. The lung fields were clear, without the presence of wheezing. The liver was palpable about 5 cm below the xiphoid process and the right costal margin. Expansive pulsations of the liver and pitting oedema on the lower legs were present.
The electrocardiogram revealed normal sinus rhythm with a left bundle branch block.
Laboratory results showed a serotonin level of 60.0 nmol/10^9 (normal: 2.8-5.4) in the thrombocytes. Urine (24 h collection): creatinine 9.9 mmol total (normal: 8.9-16.0), HIAA: 1865 mmol total (normal: 0-50). HIAA/creatinine: 188.4 mmol creatinine (normal: 0-4.4).
CT scan of the abdomen demonstrated pathological lesions in the right liver quadrant.
Transthoracic echocardiography revealed normal dimensions and wall thickness of the left ventricle with an ejection fraction of 70%. The aortic and mitral valves were normal. Both the right ventricle and right atrium were dilated. The tricuspid valve showed retraction and shortening of thickened anterior and septal cusps, leading to an immobile valve in a semi-open position. The pulmonary valve showed similar features. With colour Doppler, severe regurgitation of both the tricuspid and pulmonary valves could be demonstrated (jet area >40%). The continuous-wave (CW) Doppler signal showed tricuspid regurgitation, without severe stenosis: a characteristic 'dagger-shaped' regurgitation signal with max velocity 194 cm/sec, demonstrating rapid decline in pressure difference between RV and RA, indicating severe tricuspid regurgitation, and mild tricuspid stenosis with a mean pressure gradient of 2.4 mmHg and a pressure half-time of 128 m/sec (*figure 1*). CW Doppler of the pulmonary valve revealed severe regurgitation and mild stenosis (max velocity 167 cm/sec and a short deceleration time), indicating severe regurgitation (*figure 2*). No pericardial effusion or features of myocardial fibrosis were noted. Transoesophageal echocardiography highlighted the typical findings on the tricuspid and pulmonary valve as seen on transthoracic echocardiography (*figures 3* and *4*). No shunt could be demonstrated. No findings suggestive of myocardial metastases or endocardial fibrosis were noted.

DISCUSSION

Carcinoid heart disease, first described by Thorssen *et al.* in 1954,[2] has a prevalence of 57-77% in patients diagnosed

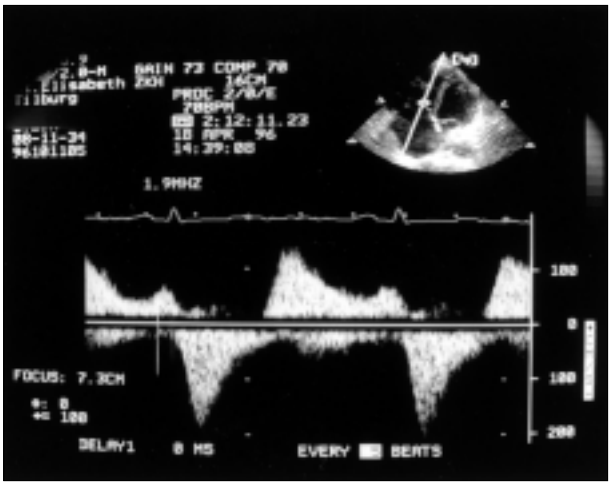

Figure 1
Continuous-wave Doppler of the tricuspid valve
The characteristic 'dagger-shaped' regurgitation signal (max velocity, 194 cm/sec) demonstrating rapid decline in pressure difference between RV and RA, due to severe tricuspid regurgitation, and only mild tricuspid stenosis with a mean pressure gradient of 2.4 mmHg and a pressure half-time of 128 m/sec.

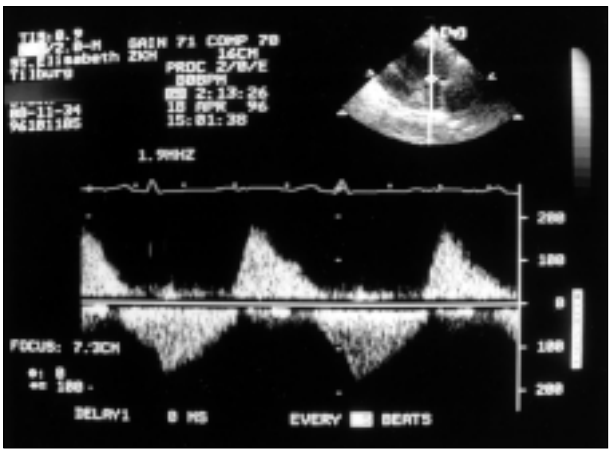

Figure 2

Continuous-wave Doppler signal of the pulmonary valve

This suggests minor pulmonary stenosis (max. velocity 167 cm/sec). The short deceleration time indicates severe regurgitation.

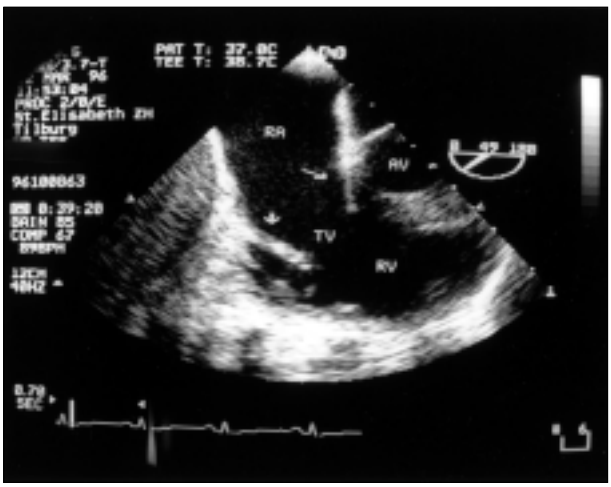

Figure 3

Transoesophageal echocardiogram of the right side of the heart with multiplane transducer (49°)

Dilated right atrium and right ventrice. In carcinoid heart disease, the tricuspid valve shows typical lesions of retraction and thickening of the anterior and septal cusp, which remain in an immobile, semi-open position. RA = right atrium, RV = right ventricle, TV = tricuspid valve, AV = aortic valve.

with a carcinoid tumour.[3,4] Histological investigation of the carcinoid lesions in the heart demonstrates white, plaque-like endocardial thickening measuring up to 2 mm. The preponderance of lesions in the right side of the heart suggests that carcinoid heart disease is related to factors secreted into the hepatic vein due to liver metastases.[5]
Both the tricuspid valve and the pulmonary valve thicken and retract, then become immobile and remain in a permanent semi-open position. Tricuspid regurgitation and pulmonary regurgitation are the result, and stenosis may also occur. These very characteristic valvular lesions eventually lead to

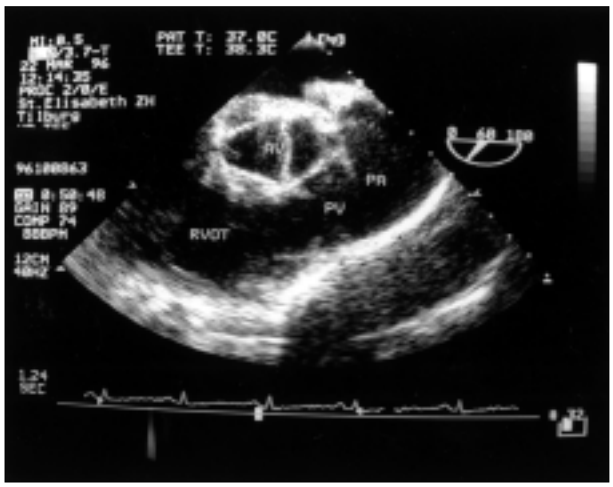

Figure 4

Transoesophageal echocardiogram with multiplane transducer (60°) of the outflow tract of the right ventricle

The thickened and shortened pulmonary valve without coaptation is barely visualised. RVOT = right ventricle outflow tract, PV = pulmonary valve, PA = pulmonary artery, AV = aortic valve.

right-sided heart failure.[1-4] Apart from the valves, similar formation of fibrosis can be seen in the intima of the great vessels, in the pleura, pericardium and retroperitoneum in carcinoid disease. Myocardial metastases have been described in the left and right ventricle as well as pericardial effusions and restrictive cardiomyopathy.
Abnormalities of the valves of the left side of the heart were described in patients who had a carcinoid tumour primarily located in the lungs and in patients who had a right-to-left shunt at the level of the fossa ovalis. The largest series of patients with carcinoid syndrome published so far is from the Mayo Clinic.[3] In this retrospective study, patients with carcinoid heart disease did not differ from patients without carcinoid heart disease regarding duration of histological diagnosis of carcinoid syndrome, age or sex, as was reported earlier.[1,3,6,7] ECG and chest X-ray abnormalities appeared to be non-specific. Repeated echocardiographic examination is essential for the diagnosis of carcinoid heart disease. Denny *et al.* suggested an echocardiographic scoring system to monitor carcinoid heart disease development and progression quantitatively.[8] Mean 5-HIAA urinary levels were significantly higher in patients with carcinoid heart disease. Despite treatment resulting in significant reductions of 5-HIAA urinary levels, regression of carcinoid heart disease has never been observed at follow-up echocardiography.
With the same treatment regimens for both groups, the three-year survival for patients with carcinoid heart disease was 31%, and 68% for patients without carcinoid heart disease. The mean duration of survival in carcinoid heart disease was 1.6 years, which is in agreement with other studies.[1]

THERAPEUTIC OPTIONS

Surgery is the best treatment for localised carcinoid tumours, although a complete cure is difficult to obtain. It can be used for liver involvement to reduce the bulk of the tumour and to reduce the symptoms. Surgical resection of liver metastases is a treatment in patients with limited hepatic disease; it will give relief of symptoms and a longer survival. Another option for liver metastases is selective embolisation of the hepatic artery, but this can be accompanied by complications, such as massive necrosis of the liver and increasing symptoms due to release of 5-HT. The duration of the endocrine response after this treatment is short.

Cytotoxic chemotherapy should be considered for patients with rapidly progressive tumours, for those with urinary excretion of 5-HIAA greater than 150 mg/24h, and for those with carcinoid-induced valvular heart disease, but it has shown only a modest benefit. The most effective options are fluorouracil and streptozocin, in monotherapy or in combination, but they also have a minimal effect. Remission varies from 13-26%.

Using interferon in patients with a metastatic carcinoid tumour decreases the urinary 5-HIAA in about 40% of patients; the effect in tumour regression is 15-20%. It has the greatest effect in controlling the symptoms, but this medication has many side effects (fever, fatigue, nausea and anorexia). Because of this and the high costs it is of limited use.

Primary tumour or metastases can take up meta-iodobenzyl guanidine (MIBG). In such cases, MIBG in therapeutic dose may be used as a palliative treatment. There is a minimal effect on tumour regression.

As for medical treatment, somatostatin and analogues play an important part in the medical treatment of metastatic carcinoid tumours. Palliation of the symptoms of carcinoid syndrome is best achieved with the long-acting analogue of somatostatin, octreotide, which can be given subcutaneously. The use of octreotide is a major advance in the management of carcinoid tumours. It also decreases the urinary 5-HIAA production. This treatment is successful in almost 80% of the patients.[5,9-11]

Therapeutic options in patients with carcinoid heart disease are limited. Whenever medical therapy of right-sided heart failure fails, surgical intervention can be considered.[5,8,12] Connolly *et al.* presented the results of the largest cardiac surgery series of patients with carcinoid heart disease studied to date.[7] In their series, 26 patients with severe symptoms of right ventricular failure were operated between 1985 and 1992. Later on, several other small studies reported on the clinical benefit of surgical intervention with valvuloplasty or valve replacement to improve right heart valvular function. Since 1966, at least 38 cases of valvular surgery for carcinoid heart disease have been reported (American Cancer Society 1997). Connolly *et al.* concluded that, even though the procedure is carried out by experienced surgeons, the perioperative mortality is extremely high (35%, mainly because of extensive pleural or pericardial plaques present at operation leading to uncontrollable bleeding). Progression of the malignancy and hepatic dysfunction due to liver metastases adds to a high short-term mortality postoperatively. The eight (30%) survivors, however, experienced a marked symptomatic improvement during follow-up (more than two years).

The timing of cardiac operation for carcinoid heart disease remains difficult and guidelines are not available as such. In our patient, sandostatin treatment was started in 1993. Until 1997 he was doing fairly well, only complaining of diarrhoea and flushing. From 1997 up to his death in 2000, he slowly deteriorated due to progressive right-sided heart failure. The echocardiogram did not show dramatic changes. Our patient refused surgery because of the high procedural risk.

CONCLUSION

Cardiac involvement in carcinoid syndrome, often combined with hepatic metastases, is nowadays diagnosed by echocardiography in over 50% of patients with this syndrome. In the last decade newer therapeutic options, such as the use of medical therapy with somatostatin analogues and interferon, embolisation of liver metastases and surgery, have improved survival in carcinoid disease. The presence of cardiac involvement in this syndrome worsens the prognosis and contributes significantly to morbidity and mortality. Right heart failure as a consequence of regurgitation and stenosis due to (sub)endocardial fibrous plaques covering the endocardium of the tricuspid and pulmonary valves is clinically most relevant. Elevation of serum serotonin levels is most likely responsible for this phenomenon. Regression of cardiac lesions by reducing the serotonin levels has not been observed. Regular echocardiographic examination is the best diagnostic tool to detect and evaluate cardiac involvement. The therapeutic options in the case of cardiac involvement are limited. If right-sided heart failure is severe, surgical intervention with valve replacement can be considered. Even in experienced centres, surgery carries a high risk, with a perioperative short-term mortality (within two years) of 70% all together.

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Netherlands
The Journal of Medicine

Capnocytophaga canimorsus sepsis in an immune-competent patient: tiny dog, major sepsis

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Dear Sir,

With great interest we read the article by Deprés-Brummer *et al.* about *Capnocytophaga canimorsus* sepsis presenting as an acute abdomen in an asplenic patient.[1] With reference to Sawmiller *et al.*, the authors focus on the atypical presentation of the acute abdominal symptoms and immuno-compromised state of their patient.[1,2] We briefly report an additional case of *Capnocytophaga canimorsus* sepsis complicated by disseminated intravascular coagulation, purpura fulminans and gangrene of the skin and subcutaneous tissues. We want to emphasise that *Capnocytophaga canimorsus* infection may rapidly lead to septic shock, multiple organ dysfunction syndrome, disseminated intravascular coagulation and massive tissue necrosis, also in the immune-competent host.[3-5]

In 1997, a previously healthy 53-year-old housekeeper was bitten by a Yorkshire terrier (*figure 1*) belonging to the house owner for whom she worked. Within one day, local erythema developed around the necrotic bite wound on the right hand and forearm (*figure 2*), followed by fever, generalised symptoms and septic shock within two days. On admission to the ICU she was in a poor clinical condition and inotropic support and mechanical ventilation were started immediately. Laboratory examinations showed acute renal failure, liver enzyme abnormalities, disseminated intravascular coagulation and rhabdomyolysis. Subsequently, she developed widespread purpura fulminans and impressive gangrene of the skin and subcutaneous tissues (*figure 3*). *Capnocytophaga canimorsus* was isolated from blood cultures. Initial antibiotic treatment consisted of amoxicillin-clavulanic acid (4 x 1.2 grams daily) and gentamicin (240 mg daily), followed by imipenem-cilastatin (4 x 1.0 grams daily) during high-volume veno-venous haemofiltration. Additional treatment consisted of vaso-pressor drugs, hydrocortisone, positive pressure ventilation and extensive surgical resection of the gangrene, followed by skin grafting procedures. After 96 days of supportive treatment she was discharged to an adjoining rehabilitation centre because of critical illness polyneuropathy and major joint contractures from fibrosis of surrounding tissues.

In a Danish study on *Capnocytophaga canimorsus* sepsis, Pers *et al.* reviewed 39 patients to determine the clinical course of the infection.[6] All cases were related to recent dog bites or other close contacts with dogs. The period from the bite to the onset of symptoms ranged from one to eight days. The mean age of the patients was 59.1 years (range 28-83 years). Underlying conditions included alcoholism and previous splenectomy. Thirteen patients (33%) had previously been in good health. Common initial symptoms were fever, malaise, myalgia, vomiting, diarrhoea, abdominal pain, dyspnoea, confusion, headache and skin manifestations. Disseminated intravascular coagulation developed in 14 patients (36%), meningitis in five (13%), and endocarditis in one (3%). Twelve of the patients (31%) died. All patients except two were treated with penicillin or ampicillin. Five patients had received antibiotics prior to admission. The incidence of this condition in Denmark is estimated to be 0.5 cases per one million people per year.

In conclusion, it should be noted that *Capnocytophaga canimorsus* sepsis might also occur in immune-competent patients, no matter the size of the dog that bit or the size of the bite wound inflicted.[3] If sepsis develops, it generally runs an overwhelming course with rapid development of multiple organ dysfunction syndrome.[3,4] In case of disseminated intra-vascular coagulation, purpura fulminans and gangrene of the skin and subcutaneous tissues may complicate the course and extensive surgery may become necessary.[5,6]

Figure 1
Yorkshire terrier, a popular pet dog

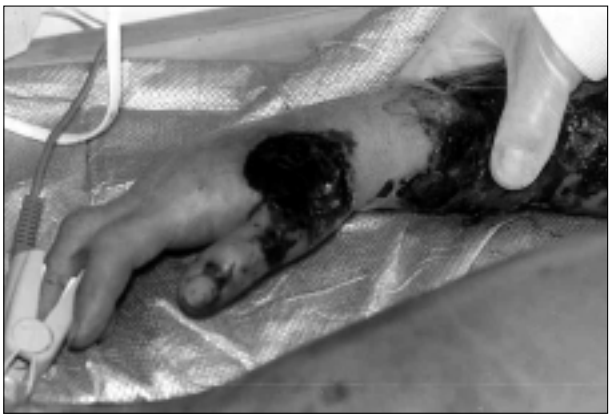

Figure 2
Site of the bite wound on the medial side of the right hand and forearm with necrosis and surrounding erythema

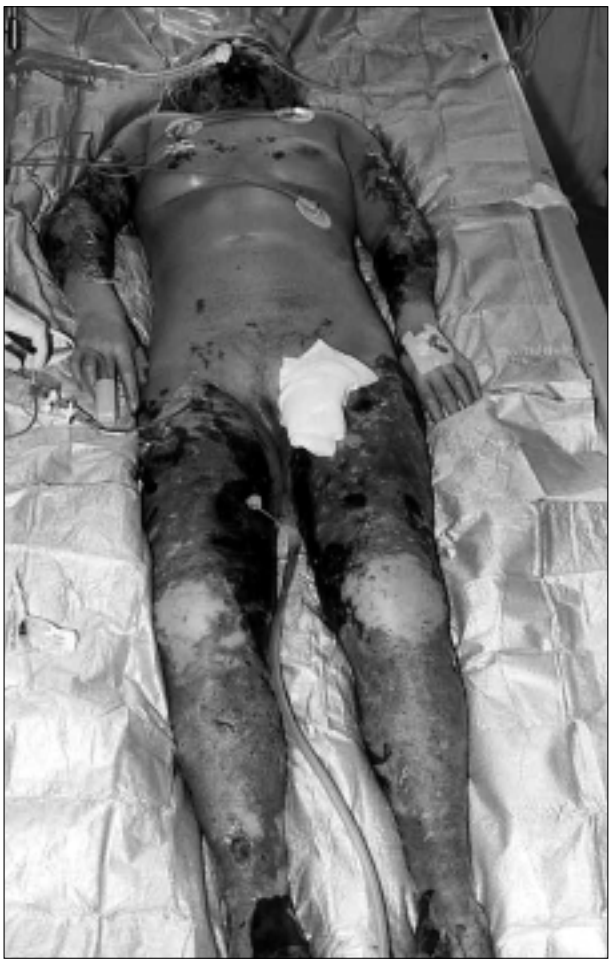

Figure 3
Widespread purpura fulminans and gangrene of the extremities

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Response

P. DEPRÉS-BRUMMER

Van der Klooster and Grootendorst present an interesting additional case report of a *Capnocytophaga canimorsus* infection leading to septic shock in an immunocompetent host. Septicaemia with this micro-organism is encountered frequently in immune-compromised patients (previous splenectomy 33%, immune suppression 5%, alcoholism 24%), but it still occurs in almost 40% of patients without predisposing conditions.[1]
As expected, a more overwhelming and more rapid development to sepsis, multi-organ failure and disseminated intravascular coagulation can be observed in immune-compromised patients.[2] A presentation with purpura fulminans or disseminated purpuric lesions in a patient with an intact spleen was described earlier.[3] Haemorrhagic skin lesions are considered to be characteristic for meningococcal infections, but similar lesions may be encountered in septicaemia due to pneumococci, staphylococci, *Haemophilus influenzae* and *Capnocytophaga canimorsus*. As Capnocytophaga species are ubiquitously present in cat and dog saliva, it seems logical that neither the size of the dog nor the size of the wound influences the clinical course.[4] Although dog bites can result in severe infection in the immune-competent patient, it is generally opinionated that routine prophylaxis with amoxicillin-clavulanic acid should be reserved for immune-compromised patients.[5]

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