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EDITORIAL

Monoclonal antibodies in the treatment of non-Hodgkin's lymphoma: moving targets

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In 1997 rituximab, a chimeric monoclonal anti-CD20 antibody, was the first monoclonal antibody to be approved by the US Food and Drug Administration (FDA), based on the results of a rather limited phase II trial in 166 patients with relapsed/refractory follicular lymphoma. In the last decade the impressive results of a substantial number of randomised clinical trials have totally changed treatment paradigms in B-cell non-Hodgkin's lymphomas. Importantly, its widespread use and associated commercial success have also given an enormous boost to the development of other monoclonal antibodies.

As of 2009, the combination of rituximab and chemotherapy (R-Chemo) is the standard of care for remission induction treatment for both follicular lymphoma and diffuse large B-cell lymphoma because it results in a significantly better progression-free and overall survival than chemotherapy alone.[2-5] In relapsed follicular lymphoma, this should be followed by rituximab maintenance treatment.[6] However, resistance develops in about 50% of previously sensitive patients. Possible mechanisms of rituximab resistance are shown in *table 1*. Two interesting papers address novel treatment options for rituximab-resistant patients.[7,8] In the July/August issue of the *Netherlands Journal of Medicine*, Meerten and Hagenbeek discuss the second- and third-generation anti-CD20 antibodies. These antibodies differ from rituximab in their capacity to differentially activate the three possible effector mechanisms of monoclonal antibodies: antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and direct induction of apoptosis. The key question of course is whether these antibodies are effective in patients not or no longer responding to rituximab. If so, they offer an important expansion of our salvage treatment options. The data from the first clinical trials addressing this issue are promising, notably for ofatumumab (a fully human anti-CD20 antibody with strong complement-activating properties) and GA-101 (a humanised anti-CD20 antibody, engineered to improve direct apoptosis induction and antibody-dependent cellular cytotoxicity). Furthermore these antibodies might be superior to rituximab in non-resistant patients. Obviously this should be demonstrated by head-to-head comparison in randomised phase III trials.

In this issue, Czuczman and Bhat discuss the state of the art as to novel monoclonal antibodies against antigens other than CD20. The list is already quite impressive with 17 antibodies, two of which are directed against T-cell specific antigens (CD2, CD4). From their overview it is clear that the data are still rather limited with the exception of the anti-CD52 monoclonal antibody alemtuzumab. However, thus far the results obtained in the phase I/II trials using these novel antibodies as monotherapy appear to be less impressive than those with the anti-CD20 antibodies. Thus, the logical next step will

Table 1. *Possible mechanisms of rituximab resistance*

	Primary mechanism	Consequence
Tumour related	• Loss of CD20 expression	• No binding of antibody
	• Increased expression of complement inactivating molecules (e.g. CD55 and CD 59)	• Decreased complement dependent cytotoxicity
	• Intrinsic apoptosis resistance (molecular mechanisms largely unknown)	• No antibody-induced direct apoptosis
Host related	IgG Fc-gamma-receptor IIIA polymorphism	Decreased antibody-dependent cytotoxicity

be to study their capacity to improve the treatment results in combination with chemotherapy. Importantly, most of these antibodies have the same favourable toxicity profile as known from rituximab.

Another interesting option is the use of radiolabelled monoclonal antibodies, often referred to as the 'magic bullets'. At present two of these, both targeting CD20, have been approved for relapsed or refractory follicular lymphoma. A theoretical advantage of radiolabelled antibodies is the phenomenon of cross-fire, i.e. their capacity to kill CD20-low or -negative lymphoma cells (insensitive to non-radiolabelled antibodies) in close proximity to the CD20-positive lymphoma to which the radiolabelled antibody has bound. In Europe only the yttrium-90 labelled ibritumomab tiuxetan (Zevalin®) is available. In a direct comparison, this antibody proved to be more effective than rituximab monotherapy in patients with relapsed or refractory follicular or transformed lymphoma.[9] In addition, they show efficacy in rituximab-resistant follicular lymphoma patients.[10] Its role in diffuse large B-cell lymphoma is still under investigation. A recent randomised study showed that, when used after frontline chemotherapy, ibritumomab tiuxetan improved the complete remission rate and progression-free survival in patients with follicular lymphoma.[11] It remains to be seen whether similar results can be obtained in patients treated with the R-chemo remission induction treatment that is now standard.

Both papers make it very clear that the field of immunotherapy of non-Hodgkin's lymphoma is really booming and that rituximab has been the fascinating start but that it certainly will not be the end.[12]

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REVIEW

Novel antibodies in the treatment of non-Hodgkin's lymphoma

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ABSTRACT

Monoclonal antibodies (mAbs) have revolutionised the treatment of malignancies, especially non-Hodgkin's lymphoma (NHL). Antibody-based therapies target tumour cells expressing a specific antigen while sparing the majority of normal cells leading to a decrease in treatment-associated toxicity. Rituximab, a monoclonal antibody directed against CD20 on B cells, was the first monoclonal antibody to be approved by the US Food and Drug Association (FDA) in 1997 for the treatment of patients with relapsed/refractory, follicular or low-grade NHL. However, it was soon realised that not all patients respond to rituximab therapy and close to 60% of patients with follicular lymphoma who were previously sensitive to rituximab become 'resistant' to repeat rituximab therapy. This led to further attempts to improve the antitumour activity of anti-CD20 mAbs (i.e. 2nd/3rd generation anti-CD20s), and to identify additional potential targets on lymphoma cells other than CD20. A number of these antibodies directed against lymphoma cell targets other than CD20 are now undergoing development, many of which are currently in clinical trials. This manuscript focuses on an overview of these 'non-anti-CD20' novel mAbs for NHL.

KEYWORDS

CD20, lymphoma, monoclonal antibodies

MONOCLONAL ANTIBODIES

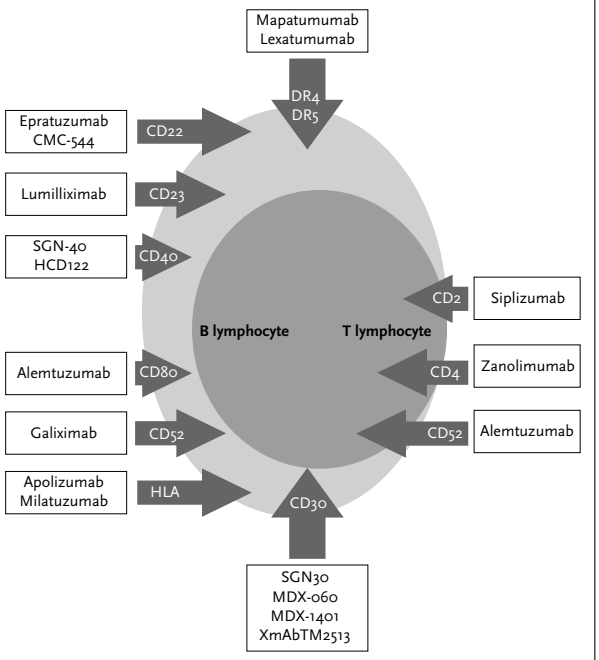
Monoclonal antibodies (mAbs) have revolutionised the treatment of malignancies, especially non-Hodgkin's lymphoma (NHL). Monoclonal antibodies were first described in 1975.[1] The first mAbs generated were non-human murine antibodies developed by fusing B cells from human lymphoma cell-immunised mice. However, humans often developed human antimouse antibodies (HAMA) to these agents, which can be associated with allergic reactions (sometimes severe), as well as a decrease in treatment efficacy by binding mAbs in the circulation prior to their reaching tumour target sites.[2] Developments in biotechnology have led to the development of chimeric antibodies (which are 65 to 90% human), partially humanised antibodies (which are 95% human) and, now, fully humanised antibodies.[3,4] Antibody-based therapies target tumour cells expressing a specific antigen while potentially sparing the majority of normal cells leading to a decrease in treatment-associated toxicity. The availability of mAbs has revolutionised the treatment of lymphomas since these cells express a number of potential target antigens (*figure 1*).

The properties of an ideal target antigen for antibody-based therapy are that: 1) it is selectively and highly expressed on neoplastic cells; 2) it is not secreted as free antigen in the blood; and 3) it does not undergo modulation following binding by the antibody. These characteristics allow recruitment of natural effectors and, subsequently, immunological attack against targeted tumour cells in the form of antibody-dependent cellular cytotoxicity (ADCC). Other antibody-associated mechanisms of antitumour activity include complement-dependent cytotoxicity (CDC), possible vaccine-like effect, and direct apoptosis.[5]

Rituximab, a chimeric monoclonal antibody composed of antigen-binding murine variable regions that are linked to a human backbone directed against CD20 on B cells, was the first monoclonal antibody to be approved by the US Food and Drug Administration (FDA) in 1997 on the basis of a study of 166 patients with relapsed or refractory, follicular or low-grade NHL.[6]

Although rituximab revolutionised the treatment of B-cell NHL, many patients do not respond to rituximab therapy: approximately 50% of patients with relapsed/

Figure 1. *Lymphocyte surface antigen targets*



refractory CD20+ follicular lymphoma (FL) previously treated with chemotherapy failed to respond to initial treatment with rituximab.[6] It has also been reported that close to 60% of previously rituximab-sensitive FL patients became 'resistant' to repeat rituximab therapy.[7] Ongoing attempts to improve the antitumour activity of anti-CD20 monoclonal antibodies include binding to a different epitope than that of rituximab; binding more tightly to CD20; increasing activation of ADCC and/or facilitating apoptosis. A number of second- and third-generation anti-CD20 candidate agents are currently in development. These 'newer' anti-CD20 antibodies may potentially prove to have augmented antitumour activity against CD20-+ B-cell neoplasms compared with rituximab; clinical trials with these novel agents are ongoing. Researchers have also identified several additional potential targets on lymphoma cells other than CD20, fostering the continued and concurrent development of a number of 'other' targeted antibodies, many of which are currently in clinical trials (*table 1*). This manuscript focuses on an overview of these non-anti-CD20 novel mAbs for NHL.

ANTIBODIES AGAINST TARGETS OTHER THAN CD20

CD22 is widely expressed on normal and malignant B cells, and its function appears to relate to B-cell activation and adhesion, modulation of antigen-receptor signalling, and cell-surface-receptor circulation.

EPRATUZUMAB

Epratuzumab (Immunomedics, Inc.) is a humanised IgG1 anti-CD22 antibody associated with both ADCC and direct cytotoxicity in preclinical studies. Phase I/II studies demonstrated objective responses across various dose levels in both relapsed/refractory follicular lymphoma (24%)[8] and diffuse large B-cell lymphoma (DLBCL, 15%).[9]
Toxicities were manageable and consisted primarily of infusion-related reactions; no dose-limiting toxicity was observed. Epratuzumab has also been combined with rituximab in phase II studies showing at least an additive

Table 1. *Novel antibodies for the treatment of non-Hodgkin lymphoma*

Antibody	Target	Type	Source	Trials
Epratuzumab	CD22	IgG1	Humanised	Phase I/II
CMC-544	CD22	IgG4	Humanised	Phase III
Lumiliximab	CD23	IgG1	Chimeric (macaque-human)	Phase III
SGN-30	CD30		Chimeric	Phase I/II
MDX-060	CD30	IgG1κ	Humanised	Phase I/II
MDX-1401	CD30		Humanised	Phase I
XmAbTM2513	CD30		Humanised	Phase I
SGN-40	CD40	IgGl	Humanised	Phase I
HCD122	CD40	IgGl	Humanised	Phase I
Alemtuzumab	CD52		Humanised	Phase II/III
Galiximab	CD80	IgG1λ	Chimeric (macaque-human)	Phase III
Siplizumab	CD2	IgG1κ	Humanised	Suspended
Apolizumab	HLADR	IgG1	Humanised	Suspended
Milatuzumab	CD74		Humanised	Phase I
Mapatumumab	DR		Humanised	Phase I/II
Lexatumumab	DR		Humanised	Phase I/II
Zanolimumab	CD4	IgG1κ	Humanised	Phase III

benefit while toxicities of the combination were comparable with those of single-agent rituximab.[10] In a recent international, multicentre trial[11] evaluating rituximab plus epratuzumab in patients with postchemotherapy relapsed/refractory, indolent NHL, an objective response (OR) was seen in 54% FL patients, (including 24% complete responses (CR) (CR/unconfirmed CR [CRu])), whereas, 57% small lymphocytic lymphoma (SLL) patients had ORs, (including 43% with CR/Cru). Rituximab-naive patients had an OR rate of 50%, whereas patients who previously responded to rituximab had an OR rate of 64%. An OR rate of 85% was observed in patients with FL who had Follicular Lymphoma International Prognostic Index (FLIPI) risk scores of 0 or 1, whereas 28 patients with intermediate or high-risk FLIPI scores (≥2) had an OR rate of 39%. The median duration of response was 13.4 months in patients with FL, and that duration increased to 29.1 months for ten patients who had a CR/CRu, including four patients who had durable responses with remissions that continued for >4 years. In patients with SLL, the median duration of response was 20 months, including one patient who had a response that continued for >3 years. Thus, the combination of epratuzumab and rituximab induced durable responses in patients with recurrent, indolent NHL. Epratuzumab is also being evaluated in combination with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and as a therapy in other B-cell neoplasms.[12]

CMC-544

CMC-544 (inotuzumab ozogamicin) is an immunoconjugate of calicheamicin and inotuzumab (humanised anti-CD22 antibody-G5/44). Calicheamicin is a potent antitumour antibiotic.[13] It acts by binding to the DNA and causing double-strand DNA breaks and is already being used in the treatment of acute myeloid leukaemia when conjugated to an anti-CD33 mAb. CMC-544 has shown preclinical efficacy against B-cell lymphoma both in *in vitro* and *in vivo* models. It inhibited the *in vitro* growth of a number of CD22+ cell lines (IC50s 6-300 pM) which was more potent than unconjugated calicheamicin alone, consistent with the active CD22-mediated cellular internalisation of the conjugate. It was also found to be more active than conjugation of calicheamicin to rituximab (i.e. which is not an 'internalising' mAb).[14] In *in vivo* models of human B-cell lymphomas, CMC-544 caused dose-dependent regression of B lymphoma xenografts.[15] It led to long-term survival of SCID mice with systemically disseminated human B-cell lymphoma.[16] Also, when suboptimal doses of CMC-544 were used in combination with suboptimal doses of rituximab, superior antitumour activity was derived by the combination than either drug administered alone.[17] These preclinical results were confirmed in a phase I study of inotuzumab ozogamicin adding further evidence to its efficacy. In this trial, 34 patients with relapsed/refractory B-cell NHL, excluding Burkitt's and lymphoblastic lymphoma, were enrolled who had an average of four prior therapies. Inotuzumab ozogamicin was administered every three to four weeks at five different doses. The most common adverse effect was thrombocytopenia with the nadir of the platelets occurring about nine days after administration of inotuzumab ozogamicin; it appeared to be related to calicheamicin. The overall response rate (ORR) was 28%; CRs and PRs were observed in all except one cohort.[18] CMC-544 is currently being evaluated in phase III clinical trials in patients with non-Hodgkin's B-cell lymphoma.

ANTI-CD23 MAB LUMILIXIMAB

CD23 is a low-affinity IgE receptor found to be highly expressed on chronic lymphocytic leukaemia (CLL) cells. Lumiliximab (Biogen Idec) is a genetically engineered primatised chimeric macaque-human anti-CD23 monoclonal antibody with a macaque variable region and human IgG1 constant region. Phase I clinical trials demonstrated that this mAb has an excellent safety profile but minimal single-agent activity in patients with CLL.[19] Since preclinical studies have shown that lumiliximab enhances the antitumour effect of fludarabine or rituximab, a phase I/II trial of lumiliximab in combination with fludarabine, cyclophosphamide and rituximab (FCR) for patients with recurring CLL was conducted. In this study, an ORR of 72% (52% CR, 10% PR, and 10% unconfirmed PR) was observed. When these outcomes were compared with published results using FCR, it seems that the addition of lumiliximab to FCR was better than FCR alone.[20] A phase III clinical trial comparing lumiliximab plus FCR *vs* FCR alone is ongoing.

ANTI-CD80 ANTIBODY

CD80 is a membrane-bound immune-costimulatory molecule involved in regulating T-cell activation. It is a member of the B7 family of costimulatory molecules.[21,22] CD80 is transiently expressed on the surface of activated B cells, dendritic cells and T cells in healthy individuals.[23] In contrast, a variety of lymphoid malignancies, including FL and Hodgkin's lymphoma (HL), constitutively express CD80, making it a suitable target for therapy.[24-28] In preclinical studies anti-CD80 antibodies demonstrated inhibition of lymphoma cell proliferation and induced ADCC.[29]

Galiximab (Biogen Idec) is a primatised anti-CD80 (IgG1λ) mAb with human constant regions and primate (cynomologous macaque) variable regions.[30] A multicentre

phase I dose-escalating study examined the single-agent activity of galiximab in patients with relapsed or refractory FL receiving four weekly intravenous infusions of galiximab at doses of 125, 250, 375 or 500 mg/m^2. Safety was established with no major adverse effects observed, and no patients were noted to have developed anti-galiximab antibodies. Tumour measurements decreased in 49% of the patients (with two CRs and two PRs) with an objective ORR of 11%.[31] Some responses were delayed, and one patient achieved a CR one year after starting therapy. Unlike rituximab, which has a relatively short half-life, the galiximab half-life is long and similar to that of epratuzumab, ranging from two to four weeks. The delayed antitumour responses cannot be easily explained as being secondary to a direct passive antibody effect, raising the possibility that galiximab may induce a 'unique' immune response. Another interesting observation was that the response did not correlate with the degree of CD80 expression; the patients who responded to treatment did not have higher levels of expression of CD80 than the non-responders. Preclinical data suggest synergy between rituximab and galiximab: a phase I/II study of galiximab together with rituximab was performed. The results showed that the combination therapy was superior to single agents.[32] In a recent study of the combination of galiximab and rituximab as initial therapy for FL, the Cancer and Leukaemia Group B (CALGB) reported a response in 69% of the study participants with 41% complete remissions.[33]

ANTI-CD52 ANTIBODY

The CD52 antigen is expressed on normal and malignant B and T lymphocytes, monocytes and natural killer (NK) cells. Alemtuzumab (Bayer HealthCare Pharmaceuticals), also known as CAMPATH, is a humanised monoclonal antibody against CD52. It was approved as a treatment for CLL previously treated with alkylating agents and refractory to fludarabine showing an ORR of 56%.[34]

Alemtuzumab has also been evaluated for the management of advanced-stage mycosis fungoides/Sezary syndrome (MF/SS). In a phase II trial, intravenous alemtuzumab, 30 mg three times a week for up to 12 weeks, showed an ORR of 55%, with a median time to treatment failure (TTF) of 12 months; 32% of patients showed a CR and 23% a PR. Sezary cells were cleared from the blood in 86% of the patients.[35]

To assess the effectiveness of alemtuzumab in the treatment of relapsed or refractory peripheral T-cell lymphomas (PTCL), a total of 14 patients were treated with alemtuzumab intravenously. The ORR was 36% and three patients achieved a CR, with the durations of the CR ranging from two to 12 months.[36] Frontline treatment with CHOP plus alemtuzumab (CHOP-C) as an effective option for patients with PTCL has been investigated. A total of 20 patients were treated with CHOP, preceded on day -1 with alemtuzumab 30 mg subcutaneously. After a median follow-up of >8 months, five patients had died of lymphoma with no toxicity deaths recorded and, for the 15 of 20 patients who were still alive, eight had a CR and one had a PR suggesting that CHOP-C appears to be a feasible option for PTCL.[37]

The major adverse effect of alemtuzumab is an increased risk of infections, largely a consequence of a dramatic decrease in CD4+ and CD8+ lymphocytes during treatment and lasting up to nine months or more after completion of therapy. This risk is further increased in patients who have previously received purine analogues with their associated myelosuppression and lymphopenia. The spectrum of infections extends from bacterial infections, atypical infections including cytomegalovirus (CMV) or herpes simplex virus reactivation, *Pneumocystis (carinii) jeroveci* pneumonia, and aspergillosis. Thus, prophylaxis is recommended with agents such as co-trimoxazole (trimethoprim/sulphamethoxazole), valaciclovir, and fluconazole.

In an attempt to maintain effectiveness while decreasing toxicity, a reduced dosage of alemtuzumab was examined in a phase II study. Alemtuzumab 10 mg intravenously three times a week for four weeks was administered to patients with relapsed/refractory T-cell lymphoma. Results from ten patients receiving this protocol have demonstrated an OR of 60% with CMV reactivation in one patient.[38] Altering the route of administration has been examined as a strategy to reduce the risk of acute infusion reactions while maintaining effectiveness. Ther are data on subcutaneous administration of alemtuzamab, using a dose-escalation scheme of 3 mg, 10 mg, 30 mg for the first week, and then 30 mg subcutaneously three times a week for up to 12 weeks. Twenty patients were given this regimen (13 patients with CLL, one with CLL/acute myeloid leukaemia (AML), three with cutaneous T-cell lymphoma (CTCL), and three with PTCL), While CMV reactivation, bacterial pneumonia and herpes zoster still occurred, grades 3 and 4 infusion reactions were notably less than with intravenous administration and the ORR to therapy was 60%.[39]

Thus, alemtuzumab has documented clinical efficacy for the treatment of relapsed/refractory MF/SS and PTCL, although infusion-related toxicities and infectious adverse effects are common.

CD2 ANTIBODIES

CD2 is a transmembrane glycoprotein with a dual role as an adhesion molecule and a costimulatory molecule via its actions with its ligand CD58. It is important in both T-cell and NK-cell functions. CD2 antigen is thus a potential

target for the treatment of T-cell lymphoma.[40] Siplizumab (MEDI-507) is a humanised IgG1κ monoclonal antibody against CD2 antigen. Preclinical studies demonstrated that siplizumab induces ADCC.[41] This antibody was being evaluated in a phase I study in patients with adult T-cell leukaemia and peripheral T-cell lymphoma. However, recently an increased incidence of Epstein-Baar virus (EBV)-induced B-cell lymphoproliferative disease (LPD) in patients treated with siplizumab has been reported. Although initial responses were encouraging, four (13.7%) patients developed EBV-LPD and the trial was stopped. In those patients developing EBV-LPD, a significantly greater reduction in NK cell number and CD2 expression on T cells was seen.[42]

CD4 ANTIBODIES

Zanolimumab (Genmab) is an anti-CD4 human monoclonal IgG1κ antibody (also known as HuMax-CD4). The CD4 receptor is expressed on most T lymphocytes and to a lesser degree on macrophages. It is also highly expressed on malignant T-cell lymphoma cells. This antibody interferes with interaction between the CD4 receptor and the major histocompatibility complex (MHC) class II molecule preventing T-cell activation. Currently, it is under investigation for the treatment of $CD4^+$ malignancies, mainly CTCL in early and advanced stages and other noncutaneous PTCL. *In vitro* studies demonstrated that zanolimumab depleted CD4+ T cells via ADCC.[43] In a phase II trial in refractory CTCL a safe toxicity profile and a favourable response of 40% were observed.[44] Another phase II trial of HuMax-CD4 in noncutaneous PTCL presented by D'Amore *et al.*[45] demonstrated an ORR of 62.5% in the first eight patients enrolled in the trial with only one case of febrile neutropenia. In two open-label phase II clinical trials evaluating the efficacy of zanolimumab in early and late-stage CTCL, 38 patients with mycosis fungoides (MF) and nine patients with Sezary syndrome (SS) were treated with zanolimumab. Objective responses were seen in 15% patients with MF receiving low-dose zanolimumab (280 mg). An increased response rate of 56% was observed with high-dose treatment (560 mg or 980 mg). In this high-dose group, responses occurred early with 90% of responses already present within eight weeks of treatment initiation. These responses were durable with a median response duration of 81 weeks. Zanolimumab demonstrated a favourable safety profile with the most frequent AEs being inflammatory skin reactions and low-grade infections.[46] Based on these results a blinded, randomised phase III trial comparing two different dosings of zanolimumab (8 mg/m^2 *vs* 14 mg/m^2) in previously treated MF was initiated.[47]

TNF RECEPTOR FAMILY

The tumour necrosis factor (TNF) family of proteins is implicated in the regulation of essential cell processes such as survival, proliferation, differentiation and cell death. Altered expression of TNF family members is often associated with pathological conditions such as autoimmune disease and cancer.[48]

CD30 ANTIBODIES

CD30 is a member of the TNF family of proteins. Its expression is restricted in normal healthy individuals to a small number of activated B and T lymphocytes. CD30 is expressed on Reed-Stemberg cells of classical Hodgkin's lymphoma (HL) and anaplastic large cell lymphoma (ALCL).[49] Patients with high-risk, relapsed or refractory HL, systemic anaplastic large-cell lymphoma, and primary cutaneous CD30-positive disorders have CD30 as a common marker, which can serve as a therapeutic target.

SGN-30 (Seattle Genetics, Inc.) is a chimeric antibody against CD30. There are *in vivo* and *in vitro* data that SGN-30 may be synergistic with chemotherapy.[50] A phase I study confirmed the safety and tolerability of SGN-30.[51] Preliminary results of phase II studies of SGN-30 and MDX-060 confirm some efficacy of these anti-CD30 monoclonal antibodies in HL and ALCL. Encouraging results were seen in patients with relapsed or refractory systemic anaplastic large cell lymphoma with objective responses in both systemic and cutaneous variants of the disease.[52-54]

MDX-060 (Medarex, Inc.) is a human anti-CD30 IgG1κ monoclonal antibody that inhibits growth of CD30-expressing tumour cells in preclinical models. Phase I and II studies were performed to determine the safety and efficacy of MDX-060 in patients with relapsed or refractory CD30+ lymphomas.[55] MDX-060 was well tolerated at doses up to 15 mg/kg, and a maximum tolerated dose was not identified. Only 7% of patients experienced grade 3 or 4 treatment-related adverse events. Among the 72 patients treated, clinical responses were observed in six. Twenty-five patients had stable disease, including five who remained free-from-progression one year after treatment, although MDX-060 demonstrated limited activity as a single agent. The minimal toxicity observed and the significant proportion of patients with prolonged stable disease suggest that further study of MDX-060 in combination with other therapies is warranted.

MDX-1401 is a nonfucosylated fully human monoclonal antibody that binds to human CD30; it was compared with MDX-060 *in vitro* and *in vivo*.[56] MDX-1401 greatly improved ADCC activity as evidenced by a decrease in half-maximal effective concentration (EC50) and an increase in

maximum cell lysis when compared with MDX-060. Increased ADCC activity was observed among a panel of cell lines, including one with very low CD30 antigen expression in which parental antibody failed to induce any detectable ADCC. Thus, the low doses of antibody required for ADCC activity irrespective of donor genotype, the ability to mediate ADCC in target cells expressing low levels of CD30, and increased *in vivo* efficacy support the development of MDX-1401 for treatment of malignant lymphoma. Preliminary data from an ongoing phase I clinical trial of MDX-1401 in patients with relapsed or refractory HL was presented at the recent Annual Meeting of the American Association for Cancer.[57]

XmAb™2513 is a novel humanised monoclonal antibody that binds to CD30 and demonstrates anti-proliferative activity against CD30-positive (CD30+) cell lines. XmAb2513 also has an engineered Fc region to enhance cell killing activity via recruitment of effector cells through increased binding affinity to Fcγ receptors. Consequently, XmAb2513 exhibits superior antibody-dependent cell mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP), when compared with a native IgG1 (unengineered) version of the antibody.[58] Xencor, Inc., a company developing protein and antibody therapeutics, has initiated a phase I clinical trial with its lead product candidate XmAb™2513 in patients with HL and anaplastic large cell lymphoma (ALCL).

ANTI-CD40 ANTIBODIES

CD40 is also a member of the tumour necrosis factor receptor family. CD40 is expressed by normal B lymphocytes, monocytes and dendritic cells, as well as some epithelial and endothelial cells. B- and T-cell lymphomas, Hodgkin and Reed-Sternberg cells and several types of carcinomas also express CD40. CD40L (CD154) is predominantly expressed by activated T lymphocytes. The various biological functions of CD40L include priming dendritic cells to activate CD8-cytotoxic T cells, B-cell selection and survival, and switching of immunoglobulin isotype. However, CD40L is less frequently expressed by activated B lymphocytes, NK cells, monocytes, eosinophils, basophils, dendritic cells, platelets, and endothelial and smooth muscle cells. Soluble CD40L (sCD40L) can be detected in the serum of patients with lymphoma, CLL, essential thrombocythaemia and autoimmune diseases.[59] The role of sCD40L has not been completely determined. Preliminary data have shown that high levels of sCD40 appear to be an independent risk factor for a poor prognosis in multiple myeloma and acute myelogenous leukaemia, but not in mantle-cell lymphoma.[60]

Two antibodies targeting CD40 (SGN-40 and HCD122 [Chirl2.12]) are currently being evaluated in clinical trials. SGN-40-Dacetuzumab (Seattle Genetics) is a humanised IgGl antihuman CD40 antibody. Preclinical data have shown SGN-40 to cause potent inhibition of proliferation, and induction of apoptosis and ADCC in high-grade B-cell lymphoma lines. Activity similar to that of rituximab was seen in xenograft CD40 tumour models treated with SGN-40.[61] Preliminary data from phase I trials in NHL show that disease was stabilised in one of six patients treated with doses of 2 mg/kg/week for four weeks, while another patient showed symptomatic improvement.[62] Another humanised IgGl anti-CD40 monoclonal antibody, HCD122 (Novartis), is also being tested and has *in vitro* activity in both CLL and NHL cells. Interestingly, when rituximab and HCD122 were compared for their ADCC activity using malignant human B-cell lymphoma lines expressing CD20 and CD40, HCD122 was superior.[63] HCD122 is currently being investigated in phase I trials for the treatment of B-cell CLL.

TRAIL RECEPTOR

Tumour necrosis factor-related apoptosis-inducing ligand or Apo2 ligand (TRAIL/Apo2L) is also a member of the tumour necrosis factor (TNF) superfamily of proteins that induces apoptosis upon binding to its death domain-containing transmembrane receptors: death receptors 4 and 5 (DR4, DR5). Importantly, TRAIL preferentially induces apoptosis in cancer cells while sparing normal cells.[64] This preferential killing is partly due to the differential expression of its receptors. Normal tissues do not usually express the death receptors TRAIL-Rl and TRAIL-R2 and, therefore, are protected from TRAIL-induced apoptosis. In contrast, most tumours express TRAIL-Rl and TRAIL-R2, making them more sensitive to TRAIL-induced apoptosis. Agonistic monoclonal antibodies targeting TRAIL-death receptors (TRAIL-Rs) have been developed and are currently being used in clinical trials. Binding of these antibodies to TRAIL-R1 and TRAIL-R2 results in death-inducing signalling complex (DISC) formation and induction of apoptosis. These novel fully humanised compounds have been combined with conventional agents in the treatment of advanced solid malignancies, including different types of lymphoma.[65] Preclinical studies and clinical trials using TRAIL/Apo2L ligand or anti-TRAIL-Rl (mapatumumab [HGS-ETRl]) and -R2 (HGS-ETR2) fully human monoclonal antibodies are ongoing. Phase Ia studies indicate that mapatumumab is well tolerated, and the maximum tolerated dose (MTD) has yet to be reached.[66,67] A phase II study using mapatumumab as a single-agent in NHL has yielded 8% objective responses.[68] This monotherapy was well tolerated in the phase II setting, with a single drug-related serious adverse event (i.e. vomiting) reported. Similar results have been seen with single-agent lexatumumab,

with several patients experiencing stable disease in a phase Ia study, although no objective tumour responses have been observed to date.[69,70]

Interim results from a phase Ib study of rhApo2L/TRAIL ligand plus rituximab in patients with low-grade NHL who had previously failed rituximab-containing therapy have shown the combination to be well tolerated and active, with two (25%) complete responses, one (13%) PR, and five (63%) SDs achieved.[71]

HLA CLASS II ANTIGENS

HLA class II antigens are expressed on B cells throughout differentiation and play a key role in cell cycling and proliferation. Anti-class II antibodies inhibit B-cell proliferation and induce apoptosis, in part through induction of the Fas–Fas ligand pathway or activation of Akt. Apolizumab (Hu1D10) is a humanised anti-HLADR antibody capable of inducing complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and programmed cell death. Its limited clinical activity and unexpected increased risk for clot development suspended its further development.[72] Other anti-HLA antibodies are currently in development. CD74 is an integral membrane protein that functions as a major histocompatibility complex class II chaperone. It has recently also been shown to have a role as an accessory-signalling molecule and has been implicated in malignant B-cell proliferation and survival. These biological functions combined with expression of CD74 on malignant B cells and limited expression on normal tissues implicate CD74 as a potential therapeutic target. The anti-CD74 monoclonal antibody LL1 has been humanised (hLL1: milatuzumab or IMMU-115) and can provide the basis for novel therapeutic approaches to B-cell malignancies, particularly because this antibody shows rapid internalisation into CD74+ malignant cells. Studies show that unconjugated hLL1 and conjugates of hLL1 constructs with radioisotopes, doxorubicin, and frog RNase have high antitumour activity. CD74 is a new candidate target for the immunotherapy of neoplasms expressing this antigen, which can be exploited using either a naked antibody or conjugating it to isotopes, drugs, or toxins.[73] Phase I trials of milatuzumab are now underway in human subjects with lymphoma and multiple myeloma.

TARGETING ANGIOGENESIS

Tumour-induced angiogenesis is necessary for their growth and metastasis. Of the different inducers of tumour-induced angiogenesis, vascular endothelial growth factor A (VEGF) plays the most important role.[74] Increased VEGF expression has been found in many tumour types, including NHL.[75-77] Elevated levels of VEGF correlate with a worse overall survival and increased aggressiveness in patients with NHL.[77-79] Bevacizumab is a humanised monoclonal antibody that recognises all known isoforms of VEGF.[80] Southwest Oncology Group (SWOG) initiated the phase II study, S0108, to test biweekly intravenous bevacizumab as single agent therapy for patients with relapsed, aggressive NHL.[81] Bevacizumab as a single agent was not effective therapy in this population. Results from animal models and clinical trials suggest this agent is best utilised when combined with other effective antitumour therapies.[82,83] A clinical trial testing standard CHOP-rituximab (R-CHOP) therapy with bevacizumab in 13 patients with DLBCL has been published documenting the tolerability and feasibility of this treatment.[84] SWOG has recently completed S0515, a phase II trial of R-CHOP plus bevacizumab in 70 patients with untreated, advanced DLBCL. A phase III international trial is also currently enrolling patients to R-CHOP with or without bevacizumab in *de novo* patients with DLBCL. These trials will determine the ultimate role of this agent in aggressive NHL.

RADIOIMMUNOTHERAPY

Radioimmunotherapy with radiolabelled mAbs is an emerging and promising treatment option for non-Hodgkin's lymphoma and has lately gained momentum due to results from recent trials. In his original work, DeNardo *et al.* provided an early example of this concept, targeting HLA antigens in aggressive non-Hodgkin's lymphoma with radiolabelled monoclonal antibodies against Lym-1 showing some complete responses.[85] Convincing data supporting the benefits of radiolabelled consolidation immunotherapy are available in FL. Other lymphomas, such as relapsed diffuse large B-cell lymphoma,[86-90] mantle-cell lymphoma,[91-94] HL[95] and marginal zone lymphoma[96] have also been evaluated with positive results.

At present, two radioimmunoconjugates that target CD20 are approved for use in patients with relapsed or refractory follicular or low-grade lymphoma: yttrium-90 (90Y) labelled ibritumomab tiuxetan (Zevalin, Cell Therapeutics) and iodine-131 (131)-labelled tositumomab (Bexxar, GlaxoSmithKline). Other radiolabelled immunotherapies currently being evaluated in B-cell NHL, include: LL2 anti-CD22, conjugated to either 131I or 90Y; Lym-1 HLA-DR, conjugated to 90Y or 67Cu; rituximab anti-CD20, conjugated to 211At, 186Re, or 227^{Th}; or B4 anti-CD19, conjugated to 90Y.[97-106] Witzig *et al.*[107] demonstrated that radioimmunotherapy with 90Y-ibritumomab tiuxetan produces statistically significantly higher overall response rates (80 *vs* 56%, p=0.002) and complete response rates (30

vs 16%, p=0.04) compared with standard immunotherapy using rituximab. Myelosuppression was the primary toxicity noted which was reversible. In a recent meta-analysis[108] of patients with recurrent/refractory NHL treated with 90Y-ibritumomab tiuxetan, long-term response, defined as time to treatment progression of 12 months or longer, was seen in 37% of patients. At a median follow-up of 53.5 months the median duration of response was 28.1 months and the median TTP was 29.3 months. One-third of these patients had been treated with three or more earlier therapies, and 37% had not responded to their last therapy. An interesting finding was that a single dose of 90Y-ibritumomab tiuxetan yielded durable responses and prolonged overall survival in a substantial number of patients not responding to earlier therapies.

The use of 90Y-labelled ibritumomab tiuxetan is being evaluated in aggressive lymphomas. It induced high response rates in relapsed DLBCL and in patients refractory to CHOP chemotherapy; interestingly lower responses were observed after failure of R-CHOP than after failure of CHOP alone.[109] In a recent phase II study of CHOP chemotherapy followed by 90Y-ibritumomab tiuxetan as frontline therapy in patients with DLBCL[110] ORR to 90Y-ibritumomab tiuxetan was 100%, including 95% CR and 5% partial remission, and four of the five patients who achieved less than a CR with CHOP improved their remission status after 90Y-ibritumomab tiuxetan therapy. In another study, consolidation of frontline chemotherapy with 90Y-labelled ibritumomab tiuxetan improved the rate of complete remission and prolonged progression-free survival.[111] 90Y-labelled ibritumomab is also being evaluated prior to transplantation in NHL patients.[112-114]

131I-labelled tositumomab is a conjugate of the murine anti-CD20 antibody tositumomab and iodine-131. In one study, two thirds of patients with chemotherapy-refractory low-grade non-Hodgkin's lymphoma had a response with 20% complete remissions.[115] Among patients with rituximab-refractory lymphoma the rate of response to 131I-labelled tositumomab was 63% with 29% having complete remissions.[116] In another study, 95% of patients with newly diagnosed non-Hodgkin's lymphoma had responses to 131I-labelled tositumomab used as frontline treatment, including 75% complete remissions.[117] This treatment has also been administered as consolidation after chemotherapy, resulting in durable responses with conversion of partial remission to complete remissions.[118]

Radioimmunotherapy options for T-cell lymphomas (T-NHL) are limited. Anti-CD45-RIT is being evaluated in human and murine T-NHL. CD45 was shown to be highly expressed on T-NHL patient samples. This high CD45 expression of T-NHL may allow reliable tumour targeting and disease control supporting anti-CD45 RIT for T-NHL patients.[119]

CONCLUSIONS

Therapeutic monoclonal antibodies have provided significant benefit for patients with NHL. Virtually all patients with B-cell lymphoma receive rituximab at variable times over their treatment course. Radiolabelled antibodies may be effective in rituximab-resistant and chemotherapy-resistant disease, but their clinical use is much more limited today compared with the use of unlabelled mAbs. While significant efforts continue in this area, the logistics, haematological toxicity and other factors have limited the use of concurrent chemotherapy plus radioimmunotherapy; however, recent data suggest that sequential radioimmunoherapy following chemotherapy may have significant clinical value.[111] Novel anti-CD20 agents offer the potential for enhanced activity relative to that of rituximab, while agents directed against unique non-CD20 targets offer the possibility of combining mAbs against CD20 and other antigens concurrently. However, many challenges exist in the clarification of the optimal use of such novel agents. Whether new anti-CD20s are better than rituximab requires randomised comparative trials or definitive demonstration of improved effectiveness in rituximab-refractory patients. The promise of antibody-based therapeutics in lymphoma has already been demonstrated and suggests that the further development of such agents offers the potential for increasing clinical benefit for NHL patients and improving outcomes with less toxicity than that associated with historical therapy.

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REVIEW

Assisted reproductive technologies to establish pregnancies in couples with an HIV-1-infected man

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ABSTRACT

For HIV-1-infected men and women the introduction of highly active antiretroviral therapy (HAART) in 1996 led to a spectacular increase in life expectancy and quality of life. In Western society where HAART is readily available, HIV-1 is now considered to be a chronic disease and as a consequence quality of life is an important aspect for men and women with HIV-1. Many of them express the desire to father or mother a child. Assisted reproductive technologies, including intrauterine insemination (IUI), *in vitro* fertilisation (IVF) and intracytoplasmatic sperm injection (ICSI) in combination with semen washing have been used to decrease the risk of HIV-1 transmission in HIV-1-infected discordant couples with an HIV-1-infected man. This article aims to summarise the current state of the art of assisted reproductive technologies for couples with an HIV-1-infected man and to discuss current trends and dilemmas in the treatment of these couples.

KEYWORDS

Assisted reproductive techniques, HIV-1, reproduction

INTRODUCTION

From its initial presentation in the early 1980s until 1996, HIV-1 infection almost inevitably led to AIDS, which was a death sentence. Life expectancy after the diagnosis was on average only ten months in 1987, and only 20 months after the introduction of zidovudine in 1990.[1] Because of such a short life expectancy, patients were advised not to get pregnant.[2] Couples with one HIV-1-infected partner, i.e. HIV-1-discordant couples, had a high risk of horizontal transmission of the virus to their uninfected partner, and HIV-1-infected women had a high risk of vertically transmitting the virus to their child.[2,3] As a consequence, these couples were advised to always use condoms, irrespective of other contraceptives. If women nonetheless did become pregnant, they were advised to undergo first-trimester abortion.[4]

In 1990, in an era where data on HIV-1 in semen or spermatozoa were not yet available, the Italian gynaecologist Semprini started carrying out intrauterine inseminations (IUI) of HIV-negative women with processed semen from their HIV-1-infected partners, in order to reduce the risk of horizontal HIV-1 transmission.[5] In the hope of selecting HIV-1-free motile spermatozoa, Semprini processed semen from HIV-1-infected men by combining density-gradient centrifugation with swim-up of spermatozoa. After negative testing for HIV using immunofluorescence with monoclonal antibodies against HIV p17, the final sperm fraction was used for IUI, in which processed semen is inserted directly into the uterine cavity with a syringe. For more than ten years, Semprini remained the only clinician providing fertility care for HIV couples, but he received a lot of criticism from his colleagues.[6] Arguments against IUI with processed sperm during that time were 1) the short life expectancy of the future father, 2) the very low sensitivity and therefore a high chance of a false-negative result of the test that was used to detect any residual HIV in processed semen before insemination, and 3) the report by the Centre for Disease Control (CDC) of one case of HIV-1 transmission after IUI with processed

semen in 1990, although in this specific case the semen did not undergo the combination of density-gradient centrifugation and swim-up of spermatozoa that was used by Semprini and was not tested for the presence of HIV before insemination.[7]

In 1996, the introduction of highly active antiretroviral therapy (HAART) led to a spectacular increase in life expectancy, AIDS-free survival, and quality of life of HIV-1-infected men and women with access to this therapy.[8] These radically changed clinical circumstances led to the publication of numerous debates in authoritative journals with a plea to reconsider the ban on reproduction for HIV-1-infected couples.[9-11] The first argument to offer HIV-1-infected couples artificial reproductive technologies was 'harm minimisation'. IUI with processed semen seemed to be safe, as seroconversions of the treated women or their offspring after IUI with HIV-negative sperm had never been described.[12] Withholding these techniques could lead patients to practice unprotected intercourse with an unknown but presumably higher risk of HIV-1 infection. Second, in 1998 the US Supreme Court declared that an asymptomatic HIV-1 infection should be considered a handicap falling under the protection of the Americans with Disabilities Act (ADA).[13] As discrimination of people with any handicap under the ADA is unlawful, it was felt that the categorical exclusion of people with an HIV infection from assisted reproductive technology programmes was also unlawful.[14] The third –moral– argument was that medical interventions should not be discriminatory. Couples with HIV infection were not essentially different from couples with other chronic diseases or couples with an increased chance of having offspring with anomalies, for whom there was no ban on assisted reproductive technologies, for instance couples with diabetes, and women in their forties who have an increased chance of having a child with Down's syndrome. The final argument was that a doctor has to respect a patient's autonomy when the risks seemed acceptable, even if the patient's values, preferences and decisions conflicted with the values of the doctor.

These debates gradually changed the initial unwillingness to accept HIV-1-infected couples into assisted reproductive technologies programmes. In addition, the improved prognosis of patients with HIV-1 infection following the introduction of combination antiretroviral therapy had led to more HIV-1-discordant and HIV-1-concordant couples wishing to mother or father a child.[15] Furthermore, far more sensitive polymerase chain reaction-based methods to detect the presence of HIV-1 not only in blood but also in other body fluids including semen had become available.[16] Following this mind shift, Semprini's method has been copied and refined by many others, and assisted reproductive technologies are now increasingly being offered to these couples around the globe.[17,18] The Academic Medical Centre in Amsterdam is currently the only hospital in the Netherlands providing fertility care for HIV-1-discordant and HIV-1-concordant couples. The current state of the art of assisted reproductive technologies for couples with an HIV-1-infection is summarised below and current trends and dilemmas in the treatment of these couples are discussed.

ASSISTED REPRODUCTIVE TECHNOLOGIES

At present, the rationale of assisted reproductive technologies in HIV-1-infected couples can be threefold: to overcome subfertility for the same indications as in non-HIV-1-infected couples, to minimise the risk of HIV-1 transmission in case of an HIV-1-serodiscordant couple with an HIV-1-infected man or to prevent HIV-1 superinfection with a different HIV-1 strain in seroconcordant couples (*table 1*). A treatment algorithm has been published to guide in the careful evaluation of these couples.[19]

The basic principle underlying assisted reproductive technologies in HIV-1-discordant couples with an HIV-1-infected man is the processing of semen, during which HIV-1-free, motile spermatozoa with a normal morphology are separated from seminal plasma and other non-seminal cells. This is achieved by combining density gradient centrifugation with swim-up, and testing of the spermatozoal fraction for HIV-1, using PCR-based methods.[20] After a negative test, the remaining spermatozoa

Table 1. *Artificial reproductive techniques in human immunodeficiency virus type-1 (HIV-1) discordant and concordant couples*

Man	Woman	Risk for (super)infection partner	Primary goal of artificial reproductive techniques	HIV semen processing
HIV+	HIV-	Yes	Prevent HIV-1 transmission	Yes
HIV-	HIV+	No[a]	Overcome subfertility	No
HIV+	HIV+	No[b]	Overcome subfertility	No
HIV+	HIV+	Yes[b]	Prevent HIV-1 transmission	Yes

[a]When self-insemination is used; [b] risk of superinfection depends on whether or not partners are infected with the same HIV strain, and whether or not one or both partners are being treated with HAART.

can be used for assisted reproductive technologies such as intrauterine insemination (IUI), *in vitro* fertilisation (IVF) or intracytoplasmatic sperm injection (ICSI). Since the lower limit of detection of the PCR tests used is never nil, the risk of HIV-1 transmission by assisted reproductive technologies can never be completely eliminated. These assisted reproductive technologies in HIV-1-discordant couples should therefore be considered risk-reduction and not risk-elimination strategies.

INTRAUTERINE INSEMINATIONS IN THE ACADEMIC MEDICAL CENTRE

In 2003 the AMC started a programme offering assisted reproductive technologies for HIV-1-discordant couples with an HIV-1-infected man. Both therapy-naive men and men receiving HAART are eligible for the programme.

A standardised fertility work-up is performed to assess possible fertility problems. In addition, HIV-1-semen processing is done to ascertain whether two million spermatozoa remain after processing, for both the PCR test and the actual insemination, since this number is a prerequisite for treatment.

During IUI treatment mild ovarian hyperstimulation takes place with recombinant follicle stimulating hormone (FSH). On the day of insemination the semen is produced in the morning, processed, and half of the spermatozoa fraction with at least one million spermatozoa is tested for the presence of HIV-1 RNA. The test, which includes both positive and negative internal controls, was validated in our own hospital and has a lower limit of detection of 10 HIV-1-RNA copies per portion of one million spermatozoa. IUI is only performed in the afternoon with the remaining part of the spermatozoa fraction, which contains at least one million spermatozoa, when HIV-1-RNA tests in the spermatozoa fraction are negative. The woman undergoes standard HIV testing every three IUI cycles or at 4, 12 and 24 weeks gestation. The child undergoes an HIV test at the age of six months.

Since the start of the programme, 61 HIV-1-discordant couples have been accepted (*table 2*). These 61 couples underwent 266 IUI cycles. In 174 cycles (65%) IUI was performed, and in 92 cycles (35%) the insemination was cancelled. In 46 cycles the insemination was cancelled before ovulation, because of a risk of multiple pregnancy (more than two dominant follicles on trans-vaginal ultrasound), ovulation during the weekend (no possibility to perform the PCR test) or for personal reasons. In 46 cycles the insemination was cancelled after ovulation, because the number of spermatozoa was lower than two million spermatozoa after semen processing on the day of insemination, the HIV-1 RNA test after processing was positive or not reliable, or because of other reasons.

Table 2. *Results of intrauterine inseminations in human immunodeficiency virus type-1 (HIV-1) discordant couples with an HIV-1-infected male partner in the Academic Medical Centre from 2003- 2008*

Results	**N (%)**
Couples	61
Cycles	266
Cancel insemination	92 (35)
Inseminations:	174 (65)
• Clinical pregnancies	32 (52)
• Miscarriage	6
• Ectopic pregnancies	1
• Ongoing pregnancies	25 (41)
• Twins	5
• Babies born	30
• Seroconversions	0

Thirty-two women became pregnant (52%), 25 of these women had an ongoing pregnancy (41%), i.e. a viable pregnancy on ultrasound at 12 weeks gestation, of whom 20 were singletons and five were twin pregnancies. The percentage of clinical pregnancies, i.e. a pregnancy visible on ultrasound, and the percentage of ongoing pregnancies, was 12 and 9%, respectively, per IUI cycle, and 18 and 14%, respectively, per insemination. As of June 2009, 30 children have been born; none of the mothers or children have seroconverted for HIV-1.

Ten couples returned for second children, thus far nine of these women have an ongoing pregnancy.

Our clinical pregnancy rate for first children (18%) is comparable with the 15.1% pregnancies per intrauterine insemination (IUI) which were reported in the largest reported series of IUI in discordant couples with an HIV-1-infected man. This series described pooled data of 2840 IUI cycles carried out in Europe.[17] Seroconversion for HIV-1 did not occur in either of the HIV-1 negative women or babies.

INFLUENCE OF HIV INFECTION AND HAART ON SEMEN QUALITY

IUI is noninvasive and less costly than IVF or ICSI.[21] However, many HIV-1-infected men are excluded from IUI, because their semen qualities are poor, already prior to the semen processing, or after the intensive semen processing due to its low efficiency (5-10% recovery rate, unpublished data). As a result, only men with good semen quality can opt for IUI. In the AMC, out of 177 men who underwent a semen processing test, 56 (32%) had less than two million spermatozoa after processing.

In a longitudinal cohort study involving 55 men not yet receiving antiretroviral therapy, we found that once these men were chronically infected with HIV-1, semen parameters were not affected by ongoing HIV-1 infection during the

observation period of 77 weeks on average.[22] We observed in this study that delaying treatment in HIV-1-infected patients until CD4 cell counts reached around 200 cells/mm^3 had no adverse effect on semen quality. Ongoing HIV-1 infection, therefore, probably does not appear to affect the chance to qualify for IUI. In contrast, the percentage of progressively motile spermatozoa decreased significantly from 28 to 17% during 48 weeks of follow-up in another longitudinal cohort study involving 34 men who started first-line HAART.[23] The negative effect on the percentage of progressively motile spermatozoa by HAART may thus have a negative effect on the chance to qualify for IUI. The impact of HAART on semen quality becomes more relevant in view of guidelines increasingly recommending earlier initiation of HAART.[24,25] As a result, more men will use HAART for a longer period during their HIV-1 infection. The impact of this policy on semen quality has not yet been studied, but according to the outcome of our study on HAART and semen quality, it may be associated with a negative impact on the percentage of progressively motile spermatozoa.

The consequences of the observed reduction in the percentage of progressively motile spermatozoa during HAART on IUI outcome remain unknown. Although data acquired from a non-HIV-1-infected population show that progressively motile spermatozoa determine the chance to conceive successfully by IUI,[26,27] the only study that described predictors of success in HIV-IUI was flawed by the *a priori* inclusion of men with good semen qualities only.[28,29]

ASSISTED REPRODUCTIVE TECHNOLOGY OF CHOICE: IUI, IVF OR ICSI

There is no uniformity in assisted reproductive technologies that are offered by various centres around the world to HIV-1-discordant couples.[17] Most centres perform IUI, as HIV-1-infected couples are not infertile unless proven otherwise, so ICSI should not be used routinely.

In men with *a priori* lower semen qualities or a sperm yield lower than two million spermatozoa after semen processing, ICSI is the only realistic treatment option.[18,30-34] In ICSI a single spermatozoon is injected directly into an oocyte, during which the pellucid zone of the oocyte is penetrated artificially. So far, the results of over 1300 cycles of ICSI have been published and not a single case of HIV-1 transmission to the woman or the child has been reported.[17,18,30-36] The largest numbers of ICSI cycles have been published in Europe and the USA. In Europe, clinical pregnancy rates, and live birth rates per ICSI cycle of 31 and 16% respectively have been reported.[17] In the USA, clinical pregnancy rates and live birth rates per ICSI cycle of 36 and 29%, respectively, have been published.[36]

Despite the lack of scientific evidence, some authors even advocate the sole use of ICSI to prevent HIV-1 transmission irrespective of semen quality.[21] Arguments used in favour of ICSI are that pregnancy rates are generally higher with ICSI than with IUI, and thus less cycles of ICSI are needed to achieve pregnancy, with less exposure to possibly HIV-1-contaminated spermatozoa, but randomised studies that compare pregnancy rates in ICSI and IUI are lacking.[36] A second argument in favour of ICSI is that, in contrast to IUI, only a single spermatozoon in minute amounts of medium is used, thus decreasing the likelihood of contamination with HIV-1.[31,33] As no HIV-1 infections have ever been observed after IUI this argument lacks validity

At present, the joint perspective of the Dutch Society of Obstetrics and Gynaecology, the Dutch Society of Clinical Embryologists and the Dutch Working Group of Clinical Virologists is not to perform ICSI in HIV-1-infected men and women, reasoning that the injection of a single spermatozoon, potentially carrying an HIV-1 particle, directly into an oocyte may lead to incorporation of the viral genome into the future embryo, with unknown but possible catastrophic consequences, for instance iatrogenic HIV-1-infected children.

NATURAL CONCEPTION IN DISCORDANT COUPLES WITH AN HIV-1-INFECTED MAN

Some infectious disease specialists, ethicists, and fertility specialists feel that HIV-1-discordant couples should not only be informed about assisted reproductive technologies, but also about the possibility of natural conception when they request reproductive advice.[37,38]

The argument in favour of natural conception is that the estimated risk of HIV-1 transmission in HIV-1-serodiscordant couples is lower than 1/1000 unprotected intercourses at blood plasma HIV-1-RNA concentrations lower than 1700 copies/ml,[39] and is estimated to be even lower during successful HAART,[40] when the blood plasma HIV-1-RNA concentration, the most important predictor of sexual HIV-1 transmission, decreases to below the limit of detection.[41]

In these couples, natural conception should only take place after optimisation of factors that limit the chance of HIV-1 transmission and improve the chance to conceive. This would include: 1) a fertility screen, with couples diagnosed as infertile being offered assisted reproductive technologies, 2) the initiation of HAART, 3) the exclusion or treatment of genital tract infections, and 4) the avoidance of unprotected intercourse other than around the established time of ovulation –timed intercourse– and immediate abstinence from unprotected sex as soon as pregnancy is achieved.[37,38,42,43] In the Kantonsspital St.

Gallen, Switzerland, HIV-1-discordant couples with an HIV-1-infected man on HAART are currently offered three cycles of timed, unprotected intercourse with pre-exposure prophylaxis with tenofovir 245 mg provided to the woman, 36 and 12 hours before intercourse (http://www.creathe.org). The results of this strategy in terms of pregnancy rates and seroconversions have not yet been published.

The concept of unprotected intercourse is heavily debated for several reasons. First, the safety of natural conception will be difficult to prove due to the low seroconversion rate during unprotected intercourse.[39] A 3.8% HIV-1-transmission rate in pregnancy was observed in 1997 in previously HIV-negative women who conceived naturally from their HIV-1-infected male partners.[44] However, in this study only 20% of men were using antiretroviral therapy and none of them were using HAART. A recent retrospective study did not report HIV-1 transmission in HIV-1-discordant couples achieving pregnancy when the HIV-1-infected man or woman had an undetectable blood plasma HIV-1-RNA level under HAART.[42] Unfortunately, both studies are flawed by the inclusion of successful pregnancies only, couples who were unsuccessfully trying to conceive were not included and both studies do not mention the number of unprotected coital acts needed to achieve pregnancy.[42,44] The actual seroconversion rate during natural conception may thus have been higher. Second, the exact chance of HIV-1 transmission in an individual couple is difficult to predict, as HIV-1 may be intermittently present in the male and female genital tract at variable concentrations, sometimes irrespective of HAART or genital tract infections and is detectable in seminal plasma in 5% of men who are using HAART for at least six months.[45,46] Third, we have recently reported that although the risk of HIV-1 transmission is reduced to almost nil by assisted reproductive technologies, the fear for HIV-1 transmission among these couples is not proportionally reduced, and anxiety for HIV-1 transmission is still present among these couples.[47] To our knowledge, there are no data on the psychological impact of natural conception in HIV-1-discordant couples with an HIV-1-infected man.

RECOMMENDATIONS

In our view four issues in the use of assisted reproductive technologies for HIV-1-infected men deserve priority to be clarified further. First, it is unclear at present whether natural conception is a safe strategy. With the knowledge we have today, a randomised controlled trial comparing natural conception with pre-exposure prophylaxis to IUI with semen washing could demonstrate feasibility, safety and effectiveness. Second, HIV-1-discordant couples' attitudes towards natural conception must be explored. Third, the consequences on IUI outcome of the observed reduction in the percentage of progressively motile spermatozoa during HAART are unknown. It is therefore important to identify prognostic factors for IUI outcome, and to adjust assisted reproductive technology protocols accordingly. Fourth, despite the wide application of ICSI in HIV-1-discordant couples, its safety has not yet been proven. The safety of ICSI is currently being studied in *in vitro* studies in the Academic Medical Centre in Amsterdam, the Netherlands. The objectives of these studies are to investigate whether human oocytes can become infected via intracytoplasmic injection with HIV-1.

As fertility care for HIV-1-infected couples is complex, it is crucial that HIV physicians, reproductive gynaecologists, reproductive biologists and virologists work together on the fertility treatment of HIV-1-infected patients and extend their knowledge on reproductive issues, in order to offer these couples up-to-date assisted reproductive support.

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REVIEW

Where the immune response meets the vessel wall

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ABSTRACT

Immune-mediated inflammatory diseases (IMIDs), including rheumatoid arthritis and spondyloarthritis, are associated with increased cardiovascular morbidity and mortality, independent of the established cardiovascular risk factors. The chronic inflammatory state, a hallmark of IMIDs, is considered to be a driving force for accelerated atherogenesis. Consequently, aggressive control of disease activity has been suggested to be instrumental for cardiovascular risk reduction. Specific guidelines for cardiovascular risk reduction in patients with IMIDs, particularly rheumatoid arthritis, are lacking, largely due to the absence of randomised clinical trial data. In this review, we focus on pathophysiology and observational evidence of cardiovascular risk in different prototypes of IMIDs.

KEYWORDS

Atherosclerosis, cardiovascular disease, immune-mediated inflammatory disease

INTRODUCTION

Immune-mediated inflammatory diseases (IMID), including atherosclerosis, Sjögren's syndrome, systemic lupus erythematosus (SLE), spondyloarthritis (e.g. ankylosing spondylitis (AS) and psoriatic arthritis (PsA)), and rheumatoid arthritis (RA), are characterised by common inflammatory pathways leading to inflammation and accompanied by increased cardiovascular morbidity and mortality. Chronic activation of innate and adaptive inflammatory pathways that provide an essential defence against 'foreign' substances, ranging from bacterial products to endogenous oxidised lipids, may contribute to atherosclerotic plaque progression, destabilisation, and ultimately rupture with subsequent clinical sequelae such as myocardial infarction or stroke. In fact, both localised and systemic infections involving respiratory or urinary tract transiently increase the risk of cardiovascular events.[1,2] A marked association has been reported between poor dental health and myocardial infarction, regardless of traditional cardiovascular risk factors,[3] which may be ascribed to bacterial components from the dental cavity, which directly affect endothelial integrity, blood coagulation and platelet function.[3] Thus, a wide array of infectious diseases may trigger manifestations of atherosclerosis and contribute to the onset of acute cardiovascular events. During the last decade, the role of chronic inflammatory disorders in the onset of cardiovascular events has increasingly been recognised. The nature of the inflammatory response preceding vascular events may have a profound impact on the final outcome.[4]

In this review, we address the impact of chronic inflammation on the cardiovascular risk profile by focussing on highly prevalent IMIDs, such as RA and SLE. Identifying critical inflammatory pathways involved in progression and destabilisation of pre-existing atherosclerotic plaques may help to define novel therapeutic strategies to further reduce cardiovascular disease burden, particularly in patients with chronic inflammatory disorders.

RHEUMATOID ARTHRITIS

For many years, RA has been recognised to increase cardiovascular morbidity and mortality, irrespective of established cardiovascular risk factors.[5-7] In the early 1990s, it was shown that having RA may shorten overall survival with mortality rates exceeding a twofold increase.[8] This increased cardiovascular event rate was largely restricted to patients with marked disease activity. At that time, the five-year survival in RA patients with the highest disease activity proved comparable to that of individuals with three-vessel coronary artery disease or stage 4 Hodgkin's

disease.[9] Subsequently, the increased cardiovascular disease among RA patients has been corroborated in a large number of studies.[5,9-17] In recent years, tighter control of the inflammatory process in RA has been held accountable for a significant reduction in atherosclerosis-related death rates, as elegantly demonstrated in a cohort of nearly 3900 patients, followed from 1980 to 1997.[18] Determinants that probably have beneficial effects on the cardiovascular risk profile involve potent suppression of inflammation, diminished use of non-steroidal anti-inflammatory drugs, higher functional status and increased physical activity.[19,20]

Vascular function changes and atherogenesis

One of the earliest changes in the onset of atherosclerosis pertains to loss of vascular protection against 'atherosclerotic insults' (≈endothelial dysfunction). The severity of endothelial dysfunction has been shown to predict future cardiovascular events.[21] Active RA has been consistently associated with endothelial dysfunction, which can be reversed by retraction of the inflammatory insult.[22] Thus, acetylcholine-mediated vasodilation, as measured by venous plethysmography, was reduced in newly diagnosed RA patients, indicative of decreased vascular nitric oxide (NO) bioavailibility.[23] Six months of routine anti-inflammatory therapy effectively mitigated clinical and biochemical markers of inflammation in these patients, with ensuing improvement of endothelial vasomotor function. Similar findings have been confirmed in small-scale studies, where improvement of endothelial function paralleled the reduction in systemic inflammation and disease activity scores (DAS28) upon tumour-necrosis factor α (TNFα) blockade or treatment with the anti-CD20 monoclonal antibody rituximab.[24-26] Taken together, these data imply that endothelial dysfunction is an integral part of the early disease process in RA.

Vascular structure changes and atherogenesis

Morphological changes in the carotid artery wall, visualised as increased intima-media thickening (IMT) by B-mode ultrasonography, are reported to precede the development of overt atherosclerotic lesions by at least one to two decades.[21] In this regard, carotid IMT proved thicker in RA patients, compared with controls[29] and smoking inclined towards higher carotid IMT compared with non-smokers.[27] Additional studies revealed increased prevalence and severity of coronary-artery calcification in RA patients.[28,29] Furthermore, the prevalence of carotid atherosclerotic plaques was threefold higher in 94 RA patients compared with matched controls, and was associated with age, hypertension and TNFα-blocking therapy, most likely confounded by a more severe subgroup of patients.[30] Once cardiovascular disease becomes manifest, RA patients exhibit a higher prevalence of multivessel disease and greater need for revascularisation than age- and sex-matched controls.[31] Consistent with this notion, angiographic scores indicate that RA patients have an increased risk for multivessel disease compared with matched controls.[32,33] Taken together, it seems safe to assume that RA can be considered a high-risk condition for cardiovascular events; once cardiovascular disease is present it may carry a poorer prognosis in the post-event period.

PSORIATIC ARTHRITIS

Hitherto, several studies provide support for augmented cardiovascular risk, represented by both functional and structural arterial wall changes in association with PsA. Flow-mediated dilation (FMD) was significantly impaired in 50 PsA patients without traditional cardiovascular risk factors or cardiovascular disease compared with matched controls (mean, [range] 6.3%, [0.3-13.4%] *vs* 8.2%, [0.0-21.2%]). In this report, a significant correlation between inflammation indices (i.e. C-reactive protein and erythrocyte sedimentation rate) at the time of diagnosis and FMD was found.[34] In addition, a cohort of 59 PsA patients showed increased carotid IMT compared with healthy controls.[35] Another study also showed a higher prevalence of subclinical atherosclerosis, as measured by carotid IMT, among 82 PsA patients compared with matched controls, even after adjusting for traditional cardiovascular risk factors.[36] In this report, independent variables that significantly correlated with subclinical atherosclerosis include increased glucose and triglyceride levels. In accordance with these data, a recent case control study showed higher carotid IMT and carotid plaque index in 40 patients with PsA than in matched controls, whereas PsA status as well as age and triglyceride levels correlated with carotid plaque presence.[37] There was a trend suggesting that other traditional risk factors were also more prevalent among patients with PsA. As in RA, cardiovascular diseases and their risk factors including hyperlipidaemia, diabetes mellitus and hypertension were more common in patients PsA than in matched controls.[38] Taken together, having PsA is associated with increased cardiovascular risk, particularly in the presence of risk factors that relate to the metabolic syndrome.

ANKYLOSING SPONDYLITIS

A recent study demonstrated impairment of endothelial function, as measured by FMD, in 54 patients with AS compared with healthy controls. FMD did not correlate with known risk markers such as age, serum lipids, smoking habits or inflammatory indices and disease activity scores.[39] The findings of a recent study show that coronary flow reserve, reflecting coronary microvascular function, and left ventricular diastolic function are impaired in AS, possibly pointing at an early manifestation of cardiac involvement in patients with AS.[40]

The severity of these impairments correlated with inflammation indices, including C-reactive protein. Referring to structural changes, another study found significantly increased carotid IMT in 60 AS patients compared with healthy controls, whereas carotid IMT was positively correlated with smoking habits, waist-hip-ratio and blood pressure.[41]

Other studies, however, failed to demonstrate increased cardiovascular risk among AS patients. A cross-sectional study in 28 AS patients showed that carotid IMT and parameters related to arterial elastic properties in young AS patients free of traditional cardiovascular risk factors were not different from those in healthy controls.[42] Two additional studies also failed to observe difference in subclinical atherosclerosis, as detected by carotid IMT, between AS patients compared with matched controls,[39,43] although there was a higher prevalence of metabolic syndrome among AS patients. Based on the foregoing, the evidence for increased cardiovascular risk in AS is still inconclusive and needs further clarification.

SYSTEMIC LUPUS ERYTHEMATOSUS

Epidemiological reports from the early 1970s unambiguously showed that cardiovascular disease contributes significantly to morbidity and (premature) mortality in SLE.[44-49] In a retrospective study on a cohort of 498 women with SLE that was followed for 14 years, the age-specific incident rates of cardiovascular events, including myocardial infarction and angina pectoris, was 50-fold higher compared with age-matched controls.[50] Combining data from 263 participants in two SLE registries, retrospective assessment demonstrated a relative risk of 10.1 for nonfatal myocardial infarction (95% CI 5.8-15.6), 17.0 for death due to coronary heart disease (95% CI 8.1-29.7), 7.5 for overall coronary heart disease (5.1-10.4), and 7.9 for stroke (4.0-13.6) after a mean follow-up of 8.6 years.[51] These findings could only partially be attributed to traditional Framingham risk factors. Accordingly, a population-based estimation of the relative prevalence of myocardial infarction, congestive heart failure, or cerebrovascular accident among young women with SLE, using the California Hospital Discharge Database, revealed that the frequencies of hospitalisation due to these cardiovascular 'entities' was increased by 2.3 times (95% CI 1.1-3.5), 3.8 times (2.4-5.2), and 2.1 times (1.2-2.9), respectively, compared with controls.[52]

Vascular function changes and atherogenesis

FMD was impaired in 62 SLE patients compared with controls (median, 3.6 *vs* 6.9%; p<0.01).[53] In a study comprising 111 SLE patients, lupus was associated with significant endothelial dysfunction compared with healthy controls.[54] Recent studies have corroborated these findings, showing endothelial dysfunction and increased arterial stiffness in patients with SLE compared with matched controls,[55] which correlates with disease activity.[56,57]

Vascular structure changes and atherogenesis

A large case-control study showed that preclinical carotid atherosclerosis was more prevalent among SLE patients than in the controls (37.1 *vs* 15.2%, p<0.001), whereas older age, the presence of SLE (odds ratio 4.8; 95% CI 2.6-8.7), and higher serum cholesterol were independently related to the presence of plaque.[58] Similar findings were achieved in other studies, where a higher prevalence of carotid plaques was observed among patients with SLE compared with controls, and carotid IMT was higher in the former.[54,59] Additional studies revealed increased carotid IMT in patients with SLE,[55,60,61] particularly among those with a history of cardiovascular disease[62] and in the presence of nephrotic-range proteinuria.[50] Last, a longitudinal study of 217 women with SLE from the Pittsburgh Lupus Registry showed that carotid plaque progression rate was higher than the control group, whereas the IMT progression rate was similar.[63] Noticeably, SLE patients showed a higher prevalence of coronary-artery atherosclerosis (calcification), as measured by electron-beam computed tomography, compared with controls, whereas the age at onset was reduced.[64]

Despite a few studies that failed to show evidence for early carotid atherosclerosis,[65] available data clearly indicates that SLE similar to RA potentiates the risk of cardiovascular disease in a young, predominantly female population without common risk factors.

MECHANISMS CONTRIBUTING TO ACCELERATED ATHEROGENESIS

Accelerated atherogenesis in IMID may involve many inflammation-related and non-inflammatory factors. Due to space limitations, we forego a detailed discussion of all factors described and provide the reader with a brief insight into the most common mechanisms involved.

Innate immunity

Monocytes have been firmly implicated in the pathogenesis of atherosclerosis, starting from adhesion to the endothelium and their migration into the intima early in atherogenesis, followed by differentiation into macrophages. Macrophages that are crucial to all IMIDs[66-68] are phenotypically polarised by specific signals and secrete a variety of proinflammatory cytokines, and proteinases that may accelerate atherosclerosis progression. Among many proatherosclerotic activities TNFα, a key inflammatory mediator, induces potent atherogenic effects on the arterial wall, involving cell apoptosis, upregulation of adhesion molecules[69] and endothelial cells adopting

a more procoagulant[70] and vasoconstrictor phenotype.[71] Matrix metalloproteinases are proteolytic enzymes that can degrade collagen and render the growing plaque's cap thin and susceptible to rupture.[72] Neutrophils that infiltrate the atherosclerotic plaque and express destabilising factors such as myeloperoxidase, gelatinase-associated lipocalin, proteolytic enzymes and tissue factor, have been increasingly linked to accelerated atherogenesis, as has been shown elegantly in experimental atherosclerosis.[73,74] With regard to inflammatory proteins, the acute phase reactant CRP has emerged as a direct partaker in atherosclerosis, and we have shown that infusion of CRP in humans renders the endothelium dysfunctional with ensuing procoagulant responses, particularly under hypercholesterolaemic conditions.[75] The atherogenic role of the terminal complement has been confirmed recently by showing that the absence of CD59, a key regulator of the complement membrane attack complex assembly, accelerated and a neutralising anti-mouse C5 antibody attenuated atherosclerosis in experimental atherosclerosis.[76]

Adaptive immunity

Peripheral blood $CD4^+$ T-cell subsets that lack expression of the CD28 molecule may contribute to increased cardiovascular risk.[77] Individuals with RA, in whom expansion of this T-cell subset with a proinflammatory phenotype and tissue damaging potential[78, 79] is detected, show impairment of FMD and increased IMT compared with those without, and TNFα-blockade induces CD28 reappearance on the $CD4^+$ cell surface.[77] Additional T-cell activities that have been linked to atherogenesis involve IL-17 production with interferon-γ by coronary artery-infiltrating T cells, inducing proinflammatory responses in vascular smooth muscle cells.[80] Further, CXCR6, a chemokine receptor expressed on a subset of $CD4^+$ T helper 1 cells and natural killer T cells, has been implicated in lymphocyte homing and the local immune response within the vessel wall.[81] Furthermore, dendritic cells at the media-adventitia junction were identified to have an important role in immune-sensing and T-cell-stimulatory functions, modulating wall-infiltrating T cells to display vessel-specific activation profiles including that of atherosclerosis with differential production of CD40L, lymphotoxin-α, and interferon-γ in medium and large human arteries.[82]

Non-inflammatory factors

Referring to non-inflammation-related factors, few IMIDs such as RA have been associated with an atherogenic lipid profile, i.e. elevated levels of apolipoprotein-B containing lipoproteins and low HDL, which is reversible upon effective treatment and correlates significantly with clinical scores and inflammatory activity.[83-85] Improvement of lipid profile is accompanied by atheroprotective alterations in high-density lipoprotein composition upon tumour necrosis factor blockade. As indicated above, IMID patients exhibit a higher prevalence of the metabolic syndrome, a cluster of cardiovascular risk factors including dyslipidaemia, insulin resistance, elevated blood pressure and abdominal obesity.[86] Another mechanism focuses on endothelial progenitor cells (EPC), of which the numbers inversely correlate with cardiovascular risk factors, and thus constitute a biomarker for vascular malfunction.[87] In RA, peripheral numbers of EPCs have been found to correlate inversely with disease activity.[88] Statin therapy, at least in the experimental adjuvant-induced arthritis model of RA, appears to have favourable effects on the presence of EPCs.[89]

Collectively, the mechanisms by which chronic inflammation in IMIDs may contribute to the pathogenesis of atherosclerosis are multifactorial by nature. High disease activity promotes an inflammatory endothelial and leucocyte phenotype combined with a proatherogenic lipid profile that in conjunction stimulates plaque growth and destabilisation, ultimately culminating into an acute clinical event.

CONCLUSION

Over the past two decades it has become increasingly clear that chronic inflammation is an independent risk factor for cardiovascular events, with an impact over and above established risk factors. Since IMIDs are protracted disorders, the focus on adequate cardiovascular prevention in these patients is long overdue. Pathophysiologically, chronic inflammation provides a direct link between IMIDs and accelerated atherogenesis. Therefore, proper management of cardiovascular risk, first and foremost, requires aggressive control of disease activity. Yet, guidelines for optimal cardiovascular risk reduction in patients with IMIDs are lacking, largely due to the absence of randomised clinical trial data. As implicated by this review, the need to adapt cardiovascular risk calculators is growing to better accommodate the impact of chronic inflammatory disease over and above established risk factors to predict cardiovascular risk in the individual patient with an IMID.

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PHOTO QUIZ

Intermitting umbilical pain, fever and weight loss in an otherwise healthy 65-year-old male

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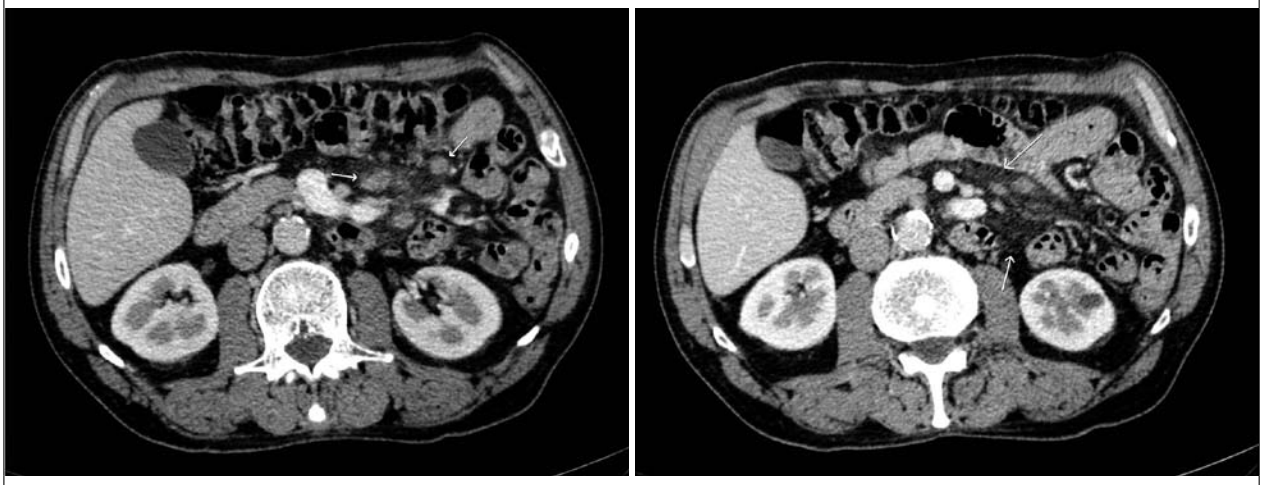
CASE REPORT

A 65-year-old male was evaluated because of umbilical abdominal pain with intermittent diarrhoea and fever accompanied by 12 kg of weight loss. Medical history only noted a one-year history of type 2 diabetes mellitus, for which he used metformin 500 mg once daily. He denied changes in stool colour or melena. Lab tests showed slightly elevated erythrocyte sedimentation rate (ESR) (26 mm/h) and increased liver enzymes (γGT 682 U/l, alkaline phosphatase 317 U/l, aspartate aminotransferase 73 U/l, alanine aminotransferase 255 U/l, bilirubin total 10 µmol/l, and bilirubin direct 3 µmol/l). Abdominal ultrasound and chest radiograph were unremarkable. CT scan showed mesenteric fat infiltration and lymphadenopathy around the mesenteric vessels (*figure 1*). At follow-up visit four weeks later, the patient had fully recovered with no signs of abdominal pain. He gained 5 kg in weight. Liver function and ESR were fully normalised. The patient refused further work-up including biopsy of the process.

WHAT IS YOUR DIAGNOSIS?

See page 335 for the answer to this photo quiz.

Figure 1. *CT scan*



Left panel: shows focal infiltration of mesenteric fat with enlarged lymph nodes (white arrows) around the branches of the superior mesenteric artery and superior mesenteric vein. There is no tethering of the nearby bowel.
Right panel: the mesenteric fat shows infiltration (white arrows) slightly more dense compared to normal fat around the liver and retroperitoneal fat.

ANSWER TO PHOTO QUIZ (PAGE 334)

INTERMITTING UMBILICAL PAIN, FEVER AND WEIGHT LOSS IN AN OTHERWISE HEALTHY 65-YEAR-OLD MALE

DIAGNOSIS

Sclerosing mesenteritis (synonym mesenterial lipodystrophy) is a rare idiopathic autoimmune disease with fibrosis and inflammation of the intra-adominal fat (preferential localisation around mesenteric arteries).[1] Differential diagnosis includes lymphoma, constipation, inflammatory bowel disease (Crohn's disease or ulcerative colitis) or solid tumours (colon cancer or liposarcomas). The prevalence is unknown (but likely to be very low), and diagnosis is made by abdominal CT scan (fat ring sign and tumour pseudocapsule), confirmed by biopsy.[2] It predominantly affects males around 50 to 70 years of age and presents with intermittent abdominal pain, fever, diarrhoea, nausea and dysphagia and subsequent weight loss. In a retrospective study of 98 subjects, 50% of patients presented with a palpable abdominal mass.[3] Interestingly, 41% of these subjects underwent abdominal surgery (e.g. cholecystectomy, appendectomy, hysterectomy or colectomy) in the previous years before developing sclerosing mesenteritis, emphasising the inflammatory basis of the excessive fibroblast growth found in biopsies. Moreover, 50% of these subjects develop either extra-abdominal malignancies (e.g. lymphoma, breast cancer or bronchial carcinoma) or another autoimmune disease such as thyreoiditis or primary sclerosing cholangitis after diagnosis.[4] The prognosis is not well known but in general a benign slowly progressive course is seen, yet self-limiting or a spontaneously resolving disease has been described. Progression of abdominal symptoms may require 'tailor-made' therapeutic intervention with prednisone or tamoxifen for a period of six months.[3] In refractory cases, thalidomide had equivocal success.[5] Surgery is not warranted unless sclerosing mesenteritis is dominated by focal intestinal obstruction requiring bypass procedure of the bowel with leaving primary panniculitis in place.[6]

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PHOTO QUIZ

An ulcer of the foot

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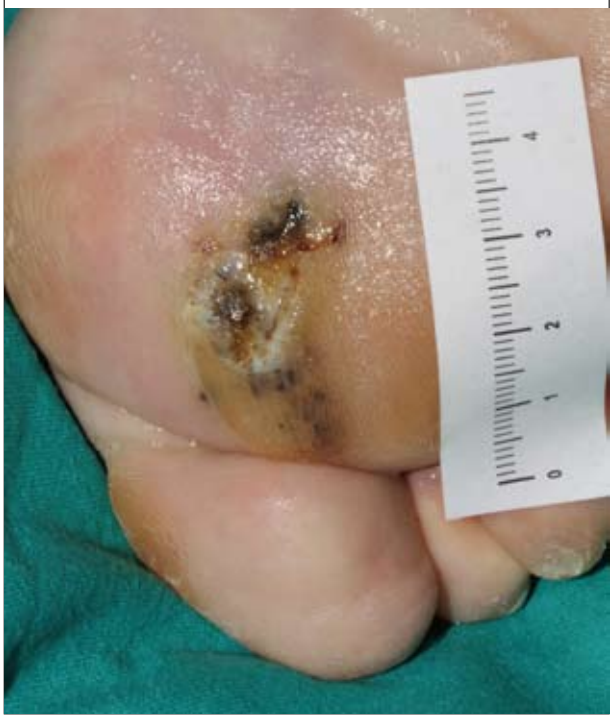
CASE REPORT

A 58-year-old woman sought medical attention for a non-healing and slow-growing lesion present for approximately seven months on her left foot sole. Her medical history was remarkable for diabetes mellitus that had been poorly controlled during the past 11 years. The patient specifically denied prior trauma or radiation to the affected area. The patient had been seen several times by a primary care physician and received topical antibiotic treatment for the presumptive diagnosis of mal perforans from a diabetic neuropathy.
When examined, a 4 x 1.5 cm slightly hyperpigmented nodule was found with a central ulceration located on the distal portion of the left foot sole. An incisional biopsy was obtained which included a focus of dark pigmentation (*figure 1*).

Figure 1. *Ulcer of the left foot*



WHAT IS YOUR DIAGNOSIS?

See page 337 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 336)

AN ULCER OF THE FOOT

DIAGNOSIS

Histological examination showed tumoral melanocytic cell nests filling and expanding the papillary dermis with atypical mitosis and melanoma cells infiltrating up through the epidermis. Ulceration was present (*figure 2*). On the basis of the clinical and histological findings, a diagnosis of acral lentiginous melanoma (ALM) was made. The patient was sent to the Plastic Surgery Department for complete excision. Histopathological examinations confirmed the ALM diagnosis. Tumour thickness was estimated to be 5.25 mm and there was invasion into the reticular dermis (Clark's level IV). Sentinel lymph node biopsy was negative. Radiological evaluations and analytical studies revealed neither bony involvement nor metastatic disease. After an 18-month follow up, no evidence of extracutaneous illness was found.

ALM constitutes a small proportion of all melanomas found in fair-skinned persons, although this type of malignant melanocytic neoplasm comprises the majority of melanomas among those who have darker skin tone.[1] Acral lentiginous melanoma can be seen on the digits, palmar or plantar sites. The plantar region is the most frequently seen site on the foot, with the dorsum of the foot, subungual region and digits less commonly involved. Because of its unusual sites and atypical clinical morphologies, ALM is frequently misdiagnosed and may receive prolonged courses of inadequate therapy.[2,3] Delay in the diagnosis of acral melanoma is greater and misdiagnosis is more frequent than with other subtypes. A study on the delay in diagnosis of ALM revealed that 17 (52%) of 33 subungual melanomas and 10 (20%) of 50 palmoplantar melanomas had been clinically misdiagnosed by physicians.[3] According to this study, misdiagnosis caused a median delay of 12 months in the diagnosis of palmoplantar melanomas and 18 months in the diagnosis of subungual melanomas.

Acral lentiginous melanoma can be mistaken for a variety of alternative diagnoses, including verruca, corn or callus, eccrine poroma, pyogenic granuloma, ischaemic ulceration, mal perforans from a peripheral neuropathy, gangrene, superficial fungal infection, traumatic residual, foreign body, and benign nevus. Foot lesions are often entirely overlooked by both patient and physician. Even if discovered, both patients and their healthcare providers may not readily think of melanoma as likely diagnosis.[4] This multifactorial delay may lead to months or years of inadequate therapeutic intervention affecting the overall patient prognosis. Pedal lesions require close observation and early biopsy if any clinical uncertainty exists or when therapeutic interventions fail.

Figure 2. *Histological finding of the lesion (see explanations in the text)*

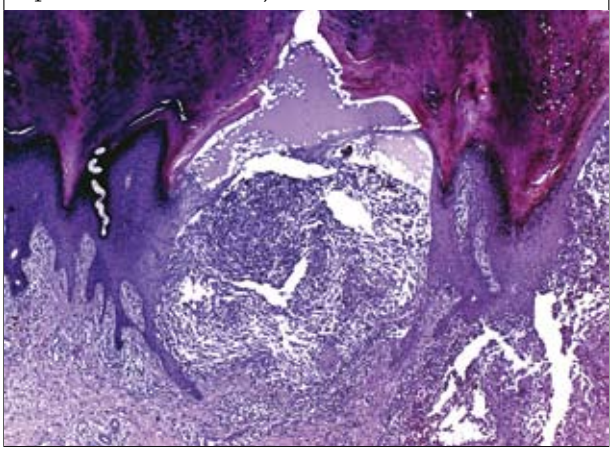


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PHOTO QUIZ

Periorbital oedema

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CASE REPORT

A 15-year-old girl presented with a three-day history of progressive bilateral eyelid swelling despite antihistamines prescribed by her general practitioner under suspicion of an allergic reaction. There was no burning, itching or pain in the eyes. She denied use of topical medications, cosmetics and contact lenses. She also complained of painful and swollen cervical lymph nodes and fatigue during the last 24 hours. On physical examination she was not ill and was afebrile. She had bilateral periorbital oedema (*figure 1* and *2*). There were no overlying skin changes or red eyes. Throat examination showed no pharyngitis. Tonsils were absent after tonsillectomy 11 years earlier. Tender lymphadenopathy was present in the cervical and inguinal regions. The liver and spleen were not palpable. Except for the eyes there was no peripheral oedema.

WHAT IS YOUR DIAGNOSIS?

See page 339 for the answer to this photo quiz.

Figure 1 and 2. *A 15-year-old girl with progressive bilateral eyelid swelling*

Pictures are published with explicit permission of the patient.

ANSWER TO PHOTO QUIZ (PAGE 338)

PERIORBITAL OEDEMA

DIAGNOSIS

C-reactive protein was 20 mg/l (<5). White cell differential showed 20% atypical lymphocytes. The platelet count was 118 x 10^9/l (150 to 350) and transaminase levels were slightly elevated. Urine dipstick test was negative for red cells and protein. Suspicion of Epstein-Barr virus (EBV) mononucleosis was confirmed by a positive monospot test and anti-EBV-viral capsid antigen (VCA) IgM. IgG against EBV-VCA and EBV nuclear antigen (EBNA) was absent. Because anti-EBV-VCA IgG is usually present at the onset of clinical disease and since the IgM and monospot test can be falsely positive, a polymerase chain reaction (PCR) assay for EBV DNA quantification was performed. This test showed 306,700 EBV DNA copies/ml, confirming the diagnosis of infectious mononucleosis.

Subsequently she experienced some days of fever, chills and malaise. The periorbital oedema resolved spontaneously within a week. After four weeks all symptoms had disappeared.

Typical symptoms of infectious mononucleosis include fever, tonsillopharyngitis, lymphadenopathy and splenomegaly. Bilateral periorbital oedema is a less common clinical feature,[1] although it has been reported in up to one third of patients.[2] Periorbital oedema usually develops early in the course of infectious mononucleosis and can be the presenting manifestation of the disease.[3,4] Then it can be mistaken for angio-oedema, cellulitis, contact dermatitis, nephrotic syndrome or thyroid disease. In the present patient the initial diagnosis was an allergic reaction. Eyelid oedema in mononucleosis is usually not accompanied by conjunctivitis, inflammation or tenderness of the eyelids.[3] A distinct atypical lymphocytosis is frequently present in these cases.

The aetiology of the oedema is unknown, but nasopharyngeal replication of virus, lymphoproliferation, or lymphatic obstruction may be contributing factors.[5]

This case emphasises that infectious mononucleosis should be included in the differential diagnosis of periorbital oedema.

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PHOTO QUIZ

Purple urine bag syndrome

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CASE REPORT

A 72-year-old man was admitted to the internal medical ward with the suspicion of a sepsis on top of his metastasised bladder cancer. The home care nurse reported that during the days before admission he had not been eating and barely drinking. Because of increased back pain he had used more morphine, without being able to take laxatives. At physical examination the patient had fever (38.6°C) and a blood pressure of 90/64 mmHg with a pulse rate of 104 beats/min. He was cachectic, dehydrated and constipated and had no clinical focus of infection. Laboratory investigations revealed a white blood cell count of 15.5 x 10^9/l, C-reactive protein 37 mg/l, urea 31.6 mmol/l and creatinine 189 µmol/l. A chest X-ray did not show pulmonary infiltrates. He received an indwelling urinary catheter, intravenous fluids and, after blood and urine cultures had been taken, cefuroxime.
The morning after admission an intense purple discolouration of his urine and the drainage system was noted (*figure 1*).

Figure 1. *Purple urine discolouration*

WHAT IS YOUR DIAGNOSIS?

See page 341 for the answer to this photo quiz.

ANSWER TO PHOTO QUIZ (PAGE 340)

PURPLE URINE BAG SYNDROME

DIAGNOSIS

The urine culture showed a mixture of several bacteria, one group was determined as *Klebsiella pneumoniae*. The patient had not eaten red beets, nor had mitoxantrone chemotherapy, which can both change the colour of the urine into purple.

We diagnosed the purple urine bag syndrome, in this patient presumably caused by a urinary tract infection with *Klebsiella pneumoniae*.

The aetiology of the purple colour of the urine: is as follows. Bacteria such as *Klebsiella pneumoniae*, *Escherichia coli* and *Providencia stuartii* have an enzyme called indoxyl sulphatase/phosphatase. This enzyme transforms urinary indoxylsulphate, a residual product of metabolised dietary tryptophan, into indigo (blue) and indirubin (red), in combination purple.[1] The combination of urine and indoxyl sulphatase/phosphatase producing bacteria is sufficient to colour agar culture plates purple. The intensity of the colour varies and depends on individual characteristics of the patient and bacteria and increases with constipation (longer resorption time of tryptophan) and with alkaline urine.[2] Furthermore, an interaction of the bacteria, urine and the polyvinylchloride of the urine bag can enhance the purplish colour. The purple colour can disappear spontaneously or after treatment of the urinary tract infection. In our patient it disappeared after the start of cefuroxime. We would like to stress that asymptomatic patients with bacteriuria do not need antibiotic treatment: The urine of many asymptomatic patients with an indwelling urinary catheter is colonised with bacteria, often resistant to multiple antibiotics. The prevalence of purple urine bags in patients with a chronic indwelling catheter varies from 8 to 16%.[3] It has most often been observed in older patients, who have the highest prevalence of indwelling urinary catheters, constipation and urinary tract infections. A short look at the urine bag can draw attention to a urinary tract infection[4] and explanation of the phenomenon to patients and carers can reduce unnecessary concerns and investigations.

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ORIGINAL ARTICLE

Blood pressure response to moderate physical activity is increased in obesity

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ABSTRACT

Objective: To test the hypothesis that in young, normotensive obese subjects, physical activity at a fixed, moderate workload, causes a more pronounced hypertensive effect than in lean subjects.

Patients and methods: 24 subjects (12 with BMI >30 kg/m², 12 with BMI <25 kg/m²), underwent a moderate-intensity physical activity protocol (cycling at 100 W). Blood pressure and oxygen consumption were monitored continuously.

Results: In the obese subjects, physical activity caused a more pronounced increase in both systolic blood pressure (increase of 40.4 ± 15.3 mmHg *vs* 21.2 ± 10.2 mmHg in lean subjects; p=0.001) and diastolic blood pressure (17.5 ± 17.9 mmHg *vs* 3.2 ± 8.1 mmHg in lean subjects; p=0.02). In regression analyses, these differences were only partly explained by small differences in resting blood pressure.

Conclusion: Healthy obese subjects show an enhanced prohypertensive response of both systolic and diastolic blood pressure to moderate-intensity physical activity.

KEYWORDS

Blood pressure, exercise, obesity

INTRODUCTION

Epidemiological studies demonstrate a close association between obesity and increased blood pressure, and hypertension is considered to be a major contributor to cardiovascular risk in obese subjects.[1] The association between the degree of obesity and blood pressure appears to be linear and extends into the non-obese range of the body mass index (BMI).[2] Although the strength of the association between obesity and blood pressure varies among different racial and ethnic groups,[3-6] estimates obtained from the Framingham Heart Study suggest that overweight contributes to the aetiology of 'essential' hypertension in 75% of hypertensive males and 65% of hypertensive females.[7] Hence, a sharp increase in the incidence of hypertension can be anticipated as a consequence of the unfolding obesity pandemic. Studies have identified multiple alterations in homeostatic mechanisms acting simultaneously to increase blood pressure. The main factors appear to be increased sympathetic nervous system activity in renal and peripheral soft tissues, and increased activity of the renin-angiotensin-aldosterone system.[8] Even in young, normotensive obese subjects, subtle prohypertensive alterations of this kind can be observed already.[8]

An understudied aspect of obesity-related hypertension is the role of physical activity. Any degree of physical activity increases (mainly systolic) blood pressure.[9] Given that several prohypertensive homeostatic alterations are present, even in young, normotensive obese subjects, physical activity could conceivably cause a more pronounced hypertensive effect in obese subjects. From a clinical perspective, this could be relevant, as it would imply that office blood pressure under resting conditions would underestimate the daily blood pressure load in obese individuals more than in lean individuals, particularly in physically active people. In addition, a recent study demonstrated that the increase in diastolic blood pressure during low-intensity exercise is an independent predictor of incident cardiovascular disease.[10] Under experimental conditions of local exercise (i.e. handgrip test), a more pronounced blood pressure increase to physical stress tests has indeed been shown in obese children[11] but the results of similar studies in adults are more unequivocal.[12,13] Previous experimental studies have been limited by the fact that physical stressors often did not resemble normal daily activities, such as walking, cycling, etc. Some studies using ambulatory blood pressure measurements combined with accelerometry have

observed a higher reactivity of blood pressure to regular daytime physical activity in obese subjects.[14,15] However, subjects in these studies were relatively old, and many were hypertensive and/or used antihypertensive medication. Also, physical activity estimated by accelerometry may be an inaccurate reflection of actual workload, particularly in subjects with marked differences in BMI who engage in both weight-bearing and non-weight-bearing activities.

Given the uncertainty as to whether obesity causes an enhanced hypertensive response to 'normal' physical activity, we studied, in normotensive subjects, the influence of obesity on blood pressure response to cycling; a non-weight-bearing, everyday type of physical activity. During cycling, the level of exercise was standardised at a moderate, fixed workload of 100 W. We hypothesised that, even at this matched workload level, obesity would be associated with an enhanced blood pressure increase.

METHODS

Study subjects

Subjects were recruited by advertisement in local newspapers and from university personnel. Subjects were eligible for inclusion if they were Caucasian, between 18 and 35 years old, and if they were either obese (BMI >30 kg/m^2) or normal weight (BMI <25 kg/m^2). Exclusion criteria were: hypertension (systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg), a history of smoking, use of liquorice products, history or any sign of cardiac, pulmonary, endocrine or renal disease, and use of any type of medication apart from oral contraceptives. Obese and lean subjects were matched for age, sex, and daily physical activity level, which we estimated in two different ways:

1. The NASA/Johnson Space Center Physical Activity Rating (PA-R)[16]
2. A simple, locally developed questionnaire on physical activity during commuting and leisure time (including sports) in metabolic equivalents (MET) hours per week.

Maximum oxygen consumption (VO_2) was calculated using the N-Ex BMI model,[16] in order to be able to assess whether lean and obese subjects were active at the same level relative to their estimated VO_{2max} and VO_{2max}/kg body weight. Each participant gave written informed consent. The local ethics committee approved the study.

Protocol

All subjects were asked to refrain from strenuous exercise for 24 hours prior to the study. Food intake was restricted to a light meal four hours prior to testing, which was performed between 1 and 5 pm.

After arrival in the test room, there was a 30-minute resting and acclimatisation period. Subjects then took position on a home trainer bicycle (Lode, Groningen, the Netherlands), wearing light clothes only. The test comprised five minutes adaptation (t=-10 to t=-5), five minutes of baseline recording (t=-5 to t=0) and ten minutes of cycling (t=0 to t=10) at a workload of 100 Watt.

During the test, heart rate (HR) and continuous analysis of blood pressure were monitored using beat-to-beat finger pulse wave analysis (Finapres Medical Systems, Amsterdam, the Netherlands). The hand was held at a fixed position (arm rest bar of bicycle) relative to the level of the heart. VO_2 was monitored by breathing through a mouth piece connected to a metabolic computer (Viasys, Los Angeles, USA)

Data analysis

Baseline SBP, DBP, HR and VO_2 was defined as the mean of measurements taken during the baseline recording period (t=-5 to t=0 minutes). DBP, HR and VO_2 turned out to be relatively stable during t=1 to t=10 minutes into the cycling phase. Therefore, the mean of measured values taken during this period was calculated to assess the changes between rest and physical activity. Mann-Whitney-U tests and general linear model analysis were performed to test for differences between the two groups. Multiple regression analysis was performed to adjust observed differences in blood pressure response between BMI categories for baseline blood pressure. Analyses were done with SPSS 16.0 for windows.

RESULTS

Baseline characteristics of included subjects are summarised in *table 1*. Both groups were well matched with respect to age, gender and estimated level of physical activity. As expected, baseline blood pressure was somewhat higher in the obese group. As expected, estimated absolute VO_{2max} was higher in obese, and VO_{2max}/kg was higher in lean subjects (*table 1*).

Table 1. *Baseline characteristics*

	Obese (12)	Lean (12)
Sex (male/female)	5/7	5/7
Age (years)	24.1 ± 4.6	24.0 ± 1.8
BMI (kg/m^2)	34.8 ± 2.6	21.8 ± 1.6
SBP (mmHg)	128 ± 11.7	120 ± 11.9
DBP (mmHg)	77 ± 8.5	70 ± 9.1
Heart rate (beats/min)	78 ± 7.1	72 ± 9.1
Daily activity (MET hrs/week)	22.1 ± 16.8	24.6 ± 16.9
Average PA-R rating (arbitrary units)	3.5 ± 1.1	3.7 ± 1.1
Predicted VO_{2max} (l/min)	3.37 ± 1.08	2.94 ± 0.63
Predicted VO_{2max}/kg (ml/kg/min)	32.4 ± 7.8	42.5 ± 7.4

Data are presented as mean ± SD. BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; MET = metabolic equivalents, VO_2 = oxygen consumption; VO_{2max}/kg prediction by N-Ex BMI.[16]

In response to physical activity at the fixed 100 W workload, the increase in SBP and DBP was significantly more pronounced in obese *vs* lean subjects (*table 2*; *figures 1* and *2*). In the obese, SBP tended to decrease slightly after a maximum reached during t=2 to t=4 minutes, whereas SBP recovered almost completely in lean individuals (*figure 1*). The increase in SBP was significantly (p<0.02) more pronounced in obese *vs* lean subjects at all time points after t=1 min (*figure 1*). DBP returned to values close to baseline values after eight minutes in lean subjects, but remained elevated in the obese (*figure 2*). The increase in DBP was significantly (p<0.02) more pronounced in obese *vs* lean subjects during the full exercise period (*figure 2*).

The activity-induced changes in heart rate were similar for both groups (*table 2*). Absolute VO_2 during cycling was higher (p=0.01) in obese subjects, in contrast to VO_2/kg which was higher (p=0.01) in lean subjects (*table 2*). Both groups were active at a comparable level relative to their predicted VO_{2max} (*table 2*).

Multiple regression analysis revealed that the difference in SBP response to exercise was not explained by baseline blood pressure differences. The increased DBP response in obese subjects was only partially explained by higher baseline DBP. After adjustment for baseline DBP, the difference in DBP response between obese and lean subjects was reduced from 14.3 to 9.5 mmHg. Additional analyses showed that the differences in DBP and SBP response to exercise were not explained by differences in baseline heart rate or heart rate increase during activity.

DISCUSSION

This study demonstrates that healthy obese subjects show an enhanced response of both SBP and DBP to a fixed, moderate level of physical activity.

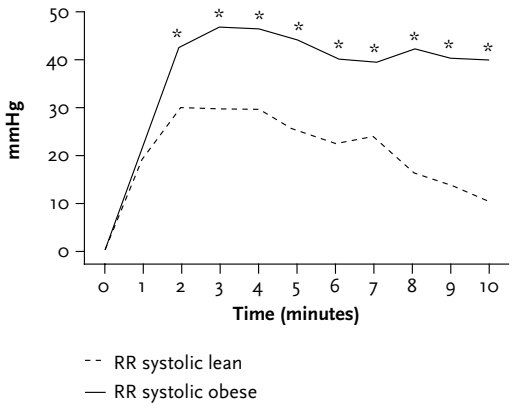
The physiological response to moderate physical activity consists of an increase in SBP and a stable or minor decrease

Table 2. *Changes in haemodynamic parameters and oxygen consumption during physical activity (t=1 to t=10 minutes) in obese versus lean subjects*

	Obese	Lean	P value
Δ SBP (mmHg)	40.4 ± 15.3	21.2 ± 10.2	0.001
Δ DBP (mmHg)	17.5 ± 17.9	3.2 ± 8.1	0.02
Δ HR (beats/min)	42.8 ± 19.2	36.9 ± 25.2	0.7
Δ VO_2 (l/min)	1.3 ± 0.3	1.2 ± 0.2	0.2
Peak VO_2 during test (l/min)	1.8 ± 0.5	1.5 ± 0.2	0.01
Peak VO_2/kg during test (ml/kg/min)	17.5 ± 3	22 ± 2.5	0.01
(peak VO_2/kg during test)/(VO_{2max}/kg)	0.56 ± 0.14	0.53 ± 0.13	0.55

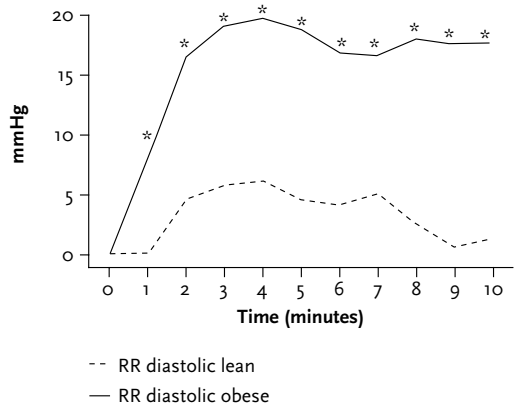
Data are presented as mean ±SD. SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; VO_2 = oxygen consumption; VO_{2max}/kg prediction by N-Ex BMI.[16] P values by Mann-Whitney test.

Figure 1. *Time trends in systolic blood pressure response to moderate physical activity in lean and obese subjects*



SBP lean = mean difference in systolic blood pressure in mmHg of the lean group; SBP obese = mean difference in systolic blood pressure in mmHg of the obese group; * = statistically significant difference p<0.05 using generalised linear models.

Figure 2. *Time trends in diastolic blood pressure response to moderate physical activity in lean and obese subjects*



DBP lean = mean difference in diastolic blood pressure in mmHg of the lean group; DBP obese = mean difference in diastolic blood pressure in mmHg of the obese group; * = statistically significant difference p<0.05 using generalised linear models.

in DBP.[9] Several recent studies, using ambulatory blood pressure measurements combined with accelerometry, have shown a correlation between mean daytime blood pressure and daily physical activity level.[14,15,17,18] Some studies have shown a more pronounced blood pressure increase to such daily activities in obese subjects,[14,15] whereas others did not observe such a difference.[18] Other factors that have been found to affect blood pressure response to physical activity include age and gender.[18] However, in all these studies, the actual workload associated with physical activity was not measured, and it is possible that the more pronounced blood pressure response to physical activity as assessed by accelerometry in the obese was the result of a higher workload, for example during weight-bearing activities such as walking, climbing stairs, et cetera.

We did not study the underlying mechanism for the increased blood pressure response to exercise in obesity. As outlined in the introduction, a variety of prohypertensive alterations in blood pressure homeostasis are found in obese people, even when they are normotensive. Increased sympathetic nervous system activity and activation of the renin-angiotensin-aldosterone axis are amongst the mechanisms that act together to increase peripheral vascular resistance and promote sodium retention. Increased muscle neurovascular sympathetic tone has in fact been demonstrated to impair the physiological exercise-induced decrease in peripheral vascular resistance in obese children and adults.[11,12] In addition to the disturbed neurovascular response to exercise, increased central arterial stiffness associated with obesity may play a role in the blood pressure response to physical activity.[19] Previous studies suggested that increased cardiac output is unlikely to contribute to the difference in blood pressure response to physical and mental stressors[11] although we did not find any studies actually measuring cardiac output in this context.

It is unlikely that our findings would be explained by obese subjects being physically active at a higher relative level compared with the lean controls. Firstly we matched subjects for daily physical activity level, thus avoiding selection of inactive obese individuals. Secondly, the heart rate increase between both groups was similar. Finally, with respect to oxygen uptake, it is well established that VO_{2max} increases with BMI, but VO_{2max} /kg body weight is lower in obesity.[20] In our study, oxygen consumption relative to calculated maximum oxygen uptake was virtually identical in both groups.

Our study is limited by the relatively small number of included subjects, and thus requires confirmation in a larger study. Such studies should also focus on the possible role of the degree of obesity and the obesity phenotype (e.g. central *vs* peripheral obesity). In addition we have not physically determined VO_{2max}, but instead used a prediction model, which may be less precise. However, as outlined above, several additional arguments suggest that exercise level was similar in both study groups. Finally, the increase in heart rate during exercise may increase finger blood pressure disproportionally, causing overestimation of the systemic blood pressure increase.[21] However, this phenomenon has not been reported to be more outspoken in obese subjects, and mainly applies to systolic blood pressure, whereas diastolic blood pressure also increased more markedly in obesity. Statistical adjustment for heart rate differences did not affect our findings. Nonetheless, a definitive study using intra-arterial blood pressure measurement would reduce any theoretical element of confounding by the measurement technique.

Finally, although blood pressure changes were directionally similar in most lean and obese individuals, the degree of interindividual variation is substantial (see standard deviations *table 2*), precluding generalisation of our findings to all obese individuals.

CONCLUSIONS

Our findings have potential relevance for how we should interpret blood pressure measurements in obese subjects. An enhanced blood pressure increase in response to moderate physical activity in obese subjects would imply that resting blood pressure underestimates the daily blood pressure load more in obese than it does in lean people, particularly in circumstances of a high daily physical activity level. In such patients, ambulatory blood pressure monitoring during periods of daily physical activity may provide information relevant for cardiovascular risk stratification. This is supported by a recent study identifying diastolic blood pressure during low-intensity exercise as an independent predictor for incident cardiovascular disease.[10] As weight loss is an effective means of resting blood pressure reduction[22] and is associated with improvement of sympathetic muscle nerve overactivity,[11,13] weight loss may well normalise the enhanced blood pressure response to physical activity in obese individuals.

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CASE REPORT

Complete remission of MDS RAEB following immunosuppressive treatment in a patient with Sweet's syndrome

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ABSTRACT

We report on a patient with myelodysplastic syndrome (MDS), classified as refractory anaemia with excess of blasts-2, and histiocytoid Sweet's syndrome. The skin lesions disappeared after initiation of corticosteroids and doxycycline. Remarkably, two months later a complete remission of the MDS occurred. Fourteen months later both the skin lesions and the MDS relapsed. Antileukaemic activity following reversion of the impaired cellular immunity due to an increased number of natural killer cells in his bone marrow may be responsible for this rare event. Inhibition of T-cell mediated myelosuppression by corticosteroids or a proapoptotic effect of doxycycline may have attributed as well.

KEYWORDS

Complete remission, corticosteroids; doxycycline, histiocytoid Sweet's syndrome, myelodysplastic syndrome, natural killer cells

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of haematological disorders characterised by dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis resulting in peripheral cytopenias and increased risk of acute myeloid leukaemia (AML). High age and poor performance status of most patients with MDS hamper allogeneic stem cell transplantation and intensive chemotherapy, which are the most effective treatment options.[1] Spontaneous remissions in MDS and AML are rare, usually not long lasting and generally occur after blood transfusions or severe infections.[2] Several different immune responses are held responsible for this phenomenon[2-5] and have been the target in clinical trials.[6]

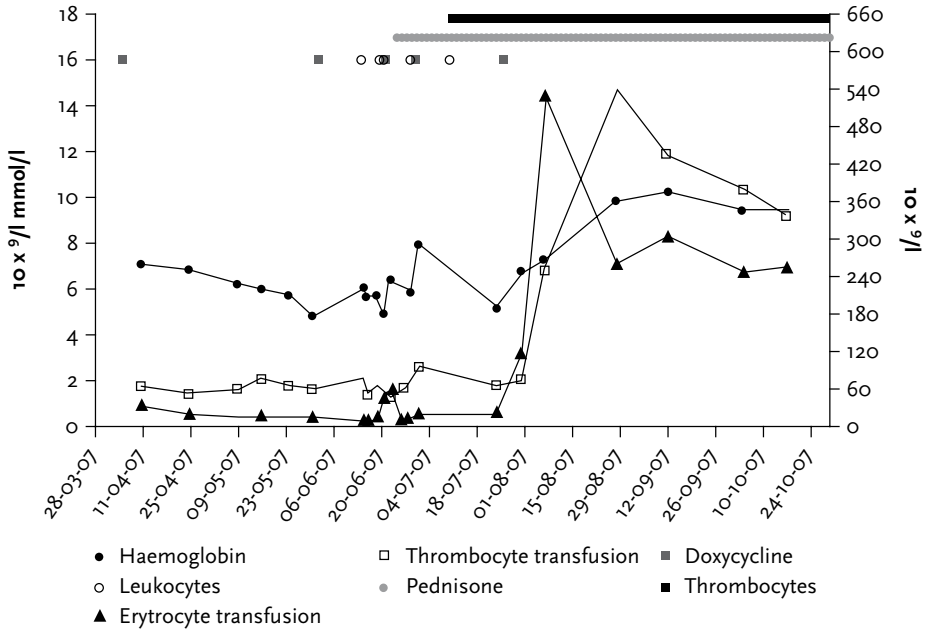
We present a patient with a complete remission of MDS in transformation to AML after treatment for extensive histiocytoid Sweet's syndrome and discuss the contributing factors.

CASE REPORT

A 73-year-old male presented with a five-day history of fever. Besides progressive non-itching lesions on his trunk and to a lesser extent on his extremities no symptoms were present. His medical history comprised endoscopic resection of bladder carcinoma two years before, for which he intermittently received BCG instillations, and in the last year a pancytopenia due to an MDS (RAEB-2 according to the WHO classification) for which he was treated with vitamin B6, folic acid, and with increasing frequency, ultimately every three weeks, supported by erythrocyte and thrombocyte transfusions. Treatment with erythropoietin did not result in any significant effect.

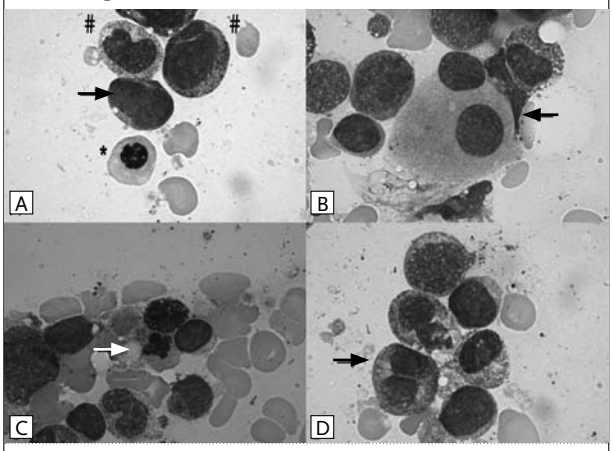
Examination of his skin showed multiple red tender plaques on his trunk and upper extremities varying in size from 0.5 to 5 cm. The blood count showed a deterioration of the pancytopenia. The haemoglobin level was 5.7 mmol/l, the white blood cell count was 1.4 x 10^9/l with 0.9 x 10^9/l neutrophils in the differentiation and the platelet count was 13 x 10^9/l (*figure 1*). The C-reactive protein was 66 mg/l. Despite four days of treatment with ceftriaxone the fever persisted and the skin lesions expanded. Blood and urine cultures were negative and there were no signs of infection

Figure 1. *The course of haemoglobin, leukocytes and thrombocytes in relation to erythrocyte and thrombocyte transfusions and the treatment with prednisone and doxycycline around the time of the complete remission*



on a chest X-ray. Skin cultures were negative for fungi and bacteria. The auramine stain was negative. A skin biopsy showed papillar oedema with a dermal infiltrate consisting of small lymphocytes in the subepithelial zone and larger mononuclear cells containing atypical nuclei with karyorrhexis and nucleoli in the deeper layers. These cells were positive for CD68/KP1, myeloperoxidase and muramidase and negative for CD34 and CD117 and subsequently proved to be (immature) myeloid cells. These cells exhibited a high proliferative activity in the MIB-1 stain. Immunofluorescence was negative for IgG, IgA, IgM, fibrinogen, albumin, C3 and C1q. This morphological and immunophenotypic picture is compatible with both histiocytoid Sweet's syndrome and differentiated myeloid sarcoma and the real nature of these lesions is debatable. Prednisone 70 mg/day as treatment for Sweet's syndrome was started and followed by immediate defervescence and disappearance of the lesions. Because of the association of Sweet's syndrome with (recurrence or progression of) solid and haematological malignancies and the possibility of myeloid sarcoma further investigations were carried out. No metastases or tumour were found on a chest and abdominal CT scan. Urine cytology was negative for malignant cells. Bone marrow aspirate (on the first day of prednisone therapy) showed myelodysplastic features with an excess of blasts (18%) (*figure 2*). Immunophenotyping of bone marrow by flow cytometry revealed 14% blasts (CD34+) and 10% natural killer cells (NK cells) (CD56/16+/CD3-). Bone marrow biopsy was hypercellular and with signs of myelodysplasia, hence MDS with excess of blasts

Figure 2. *Bone marrow aspirate showing multiple dysplastic features at the time of diagnosis of histiocytoid Sweet's syndrome*



A) An erythroblast (*), a myeloid blast (?) and two myeloid precursors (#). B) A small megakaryocyte with two nuclei and mature cytoplasm (?). C) An erythroblast in the centre with karyorrhexis (?). D dysplays a dysplastic myeloid precursor with nuclear anomaly (?)

(RAEB-2) was diagnosed. Tapering to 40 mg/day resulted in a recurrence of the skin lesions after which the dosage was increased and doxycycline was added, resulting in a remission. Six weeks after the start of the prednisone the haemoglobin level, the leucocyte and the thrombocyte counts were rising and were within normal range after eight weeks (*figure 1*). As before, he received blood transfusions in the intermediary period. Bone marrow

examination showed normocellular morphologically normal bone marrow with 2% of blasts and less than 1% of NK cells. The bone marrow cells had a normal karyotype on examination. The complete remission lasted 14 months. Corticosteroids were successfully tapered slowly to 2.5 mg every other day over six months and were stopped ten months after presentation. Treatment with doxycycline was stopped after 13 months. Unfortunately, two weeks after cessation of doxycycline similar skin lesions recurred together with low-grade fever. A skin biopsy showed a dermal infiltrate predominantly in the surroundings of hair follicles consisting of large atypical cells with large nuclei, some with nucleoli, and many mitotic figures. These cells were partly positive for CD68/KP1 and myeloperoxidase and possibly positive for CD34 and negative for CD15 and CD61. Apoptosis was abundant. No mature granulocytes were seen; some scattered lymphocytes were present. These skin changes were characterised as myeloid sarcoma. Histiocytoid Sweet's syndrome could not be excluded, but appeared less likely because of the absence of mature granulocytes and the possible presence of blasts. These lesions were accompanied by a pancytopenia: haemoglobin 8.4 mmol/l, leucocytes 1.9 x 10^9/l with 0.7 x 10^9/l neutrophils and 5% blasts in the differentiation and platelets 46 x 10^9/l. Prednisone 20 mg per day, increased to 70 mg in three weeks, and doxycycline 100 mg twice daily resulted in disappearance of the lesions in several weeks without alteration of peripheral blood cells. Two months later the same skin lesions with extended purpura recurred. The pancytopenia deteriorated and 1 to 8% of blasts were present in peripheral blood. The bone marrow aspirate was hypocellular which hampered assessment of the specimen. Six percent of myeloid blasts and a normal proportion of NK cells were present. Bone marrow biopsy contained 70% adipose tissue, but was morphologically the same as before the remission. Cytogenetic analysis revealed a deletion of chromosome 7q22q33 in combination with trisomy of chromosome 21 in the majority of the bone marrow cells. Despite a seven-day course of methylprednisolone 1 gram/day, started one day before bone marrow aspiration and biopsy, his condition deteriorated and he died of a bilateral pneumonia.

DISCUSSION

Complete remission is rare in MDS, predominantly due to the inability of the often older patients to receive intensive but potentially curative treatment. Many therapies have been tried but intensive chemotherapy and allogeneic stem cell transplantation are the only ones with a significant impact on survival.[1] As with treatment-induced complete remissions, spontaneous remissions in patients with MDS and AML are rare.[2,3] Spontaneous remissions are often, but not necessarily, triggered by severe infections and blood transfusions. Many mechanisms have been proposed: antibodies directed against the malignant clone (for example acquired through transfusion or infection), increased numbers of NK cells with subsequent cytotoxic effect for malignant cells and graft versus leukaemia effect of transfused allogeneic lymphocytes.[3-5] MDS is a clonal haematopoietic stem cell disease of which the pathophysiology is not completely elucidated. However, it is clear that altered cytokine and apoptosis rates and immune dysregulation contribute to the initiation and progression of MDS.[7-9] This role of the immune system in the pathogenesis of MDS and the contribution of immunological responses to spontaneous remissions offer explanations for the observed remission in our patient.

One of the immunological abnormalities in MDS is a defective immune surveillance caused by a decreased cytolytic function of NK cells.[10] The subsequent diminished antileukaemic immune response of NK cells seems to play a role in the progression of MDS.[9] In clinical studies aimed at induction of haematological improvement by IL-2 and other NK cell-stimulating drugs the results were disappointing.[6,11] However, some patients seem to benefit from stimulation of NK cells in MDS.[11,12]

It can be hypothesised that the increased number of natural killer cells in our case was responsible for reversing impaired cellular immunity as well as inducing antileukaemic activity, resulting in the observed remission. Alternatively, as is reported in some cases, the NK cells may be part of the MDS clone and did not contribute to the reversal of MDS.[10] After complete remission had occurred the NK cells were no longer detectable as one would perhaps expect if the NK cells played a major role in eliminating the malignant MDS clone. However, in a case report of a spontaneous remission of AML the high serum concentration of tumour necrosis factor alpha (TNFα) and IL-2 (as stimulus for activation of NK cells) declined to normal values within one month.[5] Consequently, only a temporary rise in NK cells may be sufficient to induce normalisation of haematopoiesis and is not a requisite for maintaining it.

Neoantigens on MDS cells are probably responsible for evoking a T-cell mediated suppression of haematopoiesis.[8] Increased production of TNFα and other proapoptotic cytokines and expression of transmembrane ligands result in increased apoptosis and myelosuppression in early MDS. On the contrary, in advanced disease and in case of progression to AML antiapoptotic mechanisms prevail.[7,8]

The involvement of immune system is the rationale for immunosuppressive treatment in MDS. Antithymocyte globulin, cyclosporine, thalidomide and corticosteroids have been used with variable success.[8,13] Patients with hypoplastic MDS share an overlap of characteristics with

patients with aplastic anaemia and the observed T-cell mediated myelosuppression makes them theoretically more susceptible for immunosuppressive therapy.[14] In our patient corticosteroids could have resulted in inhibiting the expansion of cytotoxic lymphocytes and in suppression of T-cell derived inhibitors of haematopoiesis like TNFα.[15]
Besides antibiotic effects, doxycycline can induce a proliferation arrest in leukaemic cells, probably by inhibition of mitochondrial protein synthesis. No *in vivo* studies concerning doxycycline as treatment for MDS or AML are available. However, human and animal *in vitro* studies show increased apoptosis in leukaemia cells, with increased caspase-3 activity after doxycycline incubation in one study.[16,17] Enhanced activity of antiapoptotic mechanisms is characteristic of high-risk MDS. Consequently, it can be hypothesised that by inducing apoptosis doxycycline may have had an (additional) effect in the occurrence of the complete remission. The occurrence of the remission after the introduction of doxycycline and the rapid recurrence of MDS after cessation of doxycycline supports this hypothesis.
After the recurrence of the MDS the same treatment was started as 16 months before. Despite increasing the dose of steroids and a higher probability of responding to immunosuppression (because of the bone marrow hypocellularity) no improvement of the pancytopenia occurred. There was no excess of NK cells this time. The cytogenetic abnormalities which were not present at the time of the remission may be responsible for treatment failure. Chromosome 7 abnormalities are associated with poor prognosis[7] and the genetic instability could have made the clonal cells unsusceptible for the treatment.

CONCLUSION

Complete remissions of high-risk MDS, both spontaneous and therapy-related, are rare. Several immunological mechanisms play a role in the pathophysiology of MDS and can be a target for treatment. These mechanisms are complex and theoretically can have counteracting effects. Increased tumour lysis by NK cells, inhibitory T-cell suppression by corticosteroids, an unidentified immune mechanism triggered by blood transfusion or a proapoptotic effect of doxycycline can be responsible for the observed complete remission lasting 14 months in this case.

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CASE REPORT

Splenectomy-induced long-term remission in a patient with multicentric Castleman's disease

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ABSTRACT

Castleman's disease (CD) is a rare lymphoproliferative disorder with a poorly understood pathogenesis. Multicentric CD can progress in different patterns, none of which can be cured with the current treatment options. We present a patient with multicentric CD in complete remission, eight years after a splenectomy without any other systemic treatment. We discuss the possible mechanism causing this long episode of complete remission in this patient.

KEYWORDS

Angiofollicular lymph node hyperplasia, Castleman's disease, human herpes virus type 8, lymphoproliferative disorders, splenectomy

What was known on this topic?
Multicentric CD can be either rapidly progressive and lethal or persistent as a chronic form. Patients are treated with corticosteroids, antiviral agents, anti-interleukin-6 antibodies, anti-CD20 antibodies (rituximab) or chemotherapy, none of which are curative. Splenectomy as a sole treatment for multicentric Castleman's has been described in only one patient with a follow-up of one year.

What does this add?
We present a patient with multicentric CD who is still in complete remission eight years after a diagnostic splenectomy without any other systemic treatment.

INTRODUCTION

Castleman's disease (CD) is a rare lymphoproliferative disorder of which the pathogenesis is still poorly understood. CD is associated with malignancies such as (non-)Hodgkin's lymphoma, Kaposi sarcoma and POEMS syndrome. The human herpes virus 8 (HHV-8) – with or without concurrent infection with the human immunodeficiency virus (HIV) – has been identified as a possible trigger of the immune activation associated with CD.[1,2]

In CD a disturbance of the lymph node architecture is seen that is both reactive and neoplastic. Three histopathological variants are recognised. Most common (±90%) is the hyaline vascular variant, characterised by a marked increase in abnormal follicles with regressed or atrophic germinal centres and broad mantle zones of small lymphocytes. The plasma cell variant (±10%) has hyperplastic germinal centres. Less common is the mixed type.[1,2]

CD comprises two different clinical presentations. Unicentric CD is an isolated benign lymphoproliferative disorder affecting young adults, which is initially asymptomatic. Surgical resection of the affected lymph node is usually curative; in case of incomplete resection radiotherapy is advised.[3,4] Patients with multicentric CD present with aspecific symptoms such as fever, weight loss, fatigue and night sweats and are usually middle-aged. Peripheral lymphadenopathy is common and can be accompanied by hepatomegaly and splenomegaly, high erythrocyte sedimentation rate (ESR), low haemoglobin and hypoalbuminaemia.[5] Multicentric CD can progress in different patterns. It can be rapidly progressive and lethal or persist as a chronic form. Treatment options are corticosteroids, antiviral agents, anti-CD20 antibodies (Rituximab), anti-interleukin-6 antibodies and chemotherapy.[6-8] None of these are curative. Splenectomy can temporarily result in improvement of symptoms.[9,10]

We present a patient with multicentric CD who is still in complete remission eight years after a diagnostic splenectomy without any other systemic treatment. The only other case ever describing splenectomy as a sole treatment for multicentric Castleman's disease was published in 1999, but follow-up was only one year.[11]

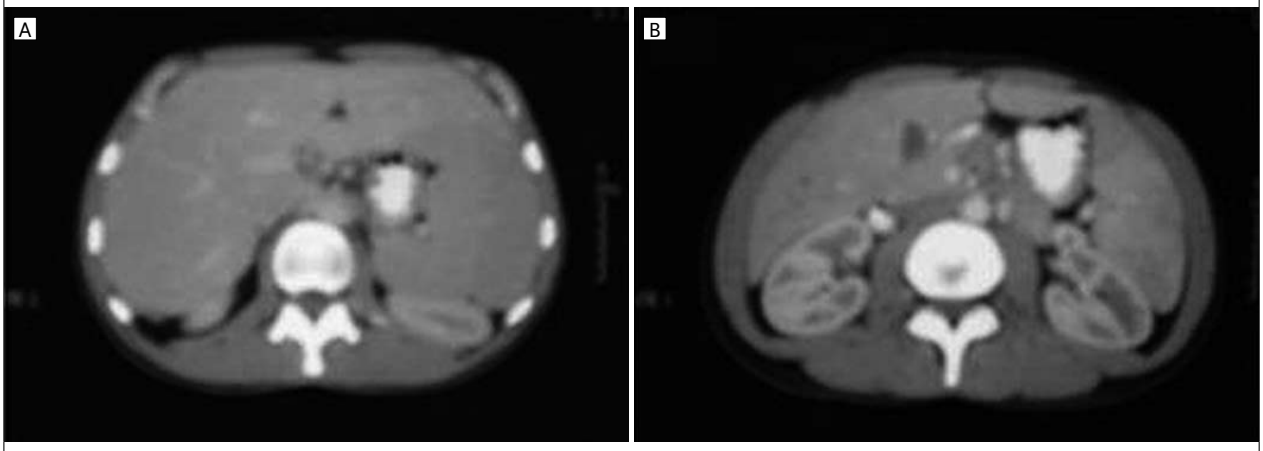
CASE REPORT

In 2001 a 29-year-old-man was first sent to our hospital because of abdominal pain, weight loss, ongoing fever and intermittent diarrhoea since a short holiday in Turkey, four weeks earlier. The previous medical history was not relevant. There were no risk factors for HIV. He did not smoke and was not using any medication. Physical examination showed a pale young man, not in distress, with a body mass index (BMI) of 21 kg/m^2. There were no palpable lymph nodes, but the liver was 1 cm palpable and the spleen reached the costal area.

Laboratory findings revealed an elevated ESR, a microcytic anaemia (mean corpus volume 57 fl), a normal leucocyte count and differentiation, a thrombocytosis (461 x 10^6/l) and macrothrombocytes in the blood smear. With an iron level of 1 µmol/l an iron deficiency was proven. Electrolytes, renal and liver function tests were normal, but a slightly elevated γ-glutamyl transpeptidase (56 U/l) and alkaline phosphatase (171 U/l) were present. The serum albumin level was low (31 g/l).

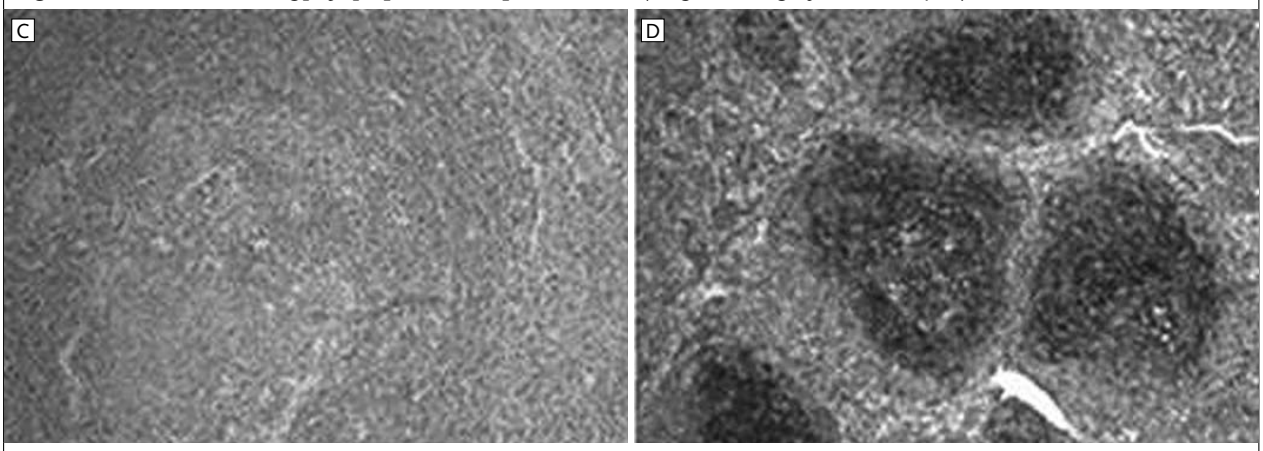
Serological tests for EBV, CMV and HIV were negative, as were stool examinations for *Giardia*, cysts and worms. Blood cultures and the tuberculin skin test were also negative. Endoscopic and radiographic examinations of the upper and lower digestive tract were within normal limits. A computed tomography (CT) scan of the abdomen showed multiple enlarged lymph nodes from the upper abdomen into the lower pelvic area, up to 5 cm in diameter. In addition, the liver (19 cm) and spleen (22 by 8 cm) were enlarged (*figures 1A* and *B*).

Figure 1A and B. *CT scan of the abdomen at presentation*



A) The spleen is enlarged measuring 8 cm transversally and 22 cm longitudinally. The liver is slightly enlarged but has a normal density.
B) Para-aortic lymph nodes (arrow) are enlarged up to 5 cm in diameter.

Figure 1C and D. *Pathology of lymph node at presentation (original magnification x 400)*



C) Enlarged lymphoid follicle with hyperplastic germinal centre and slight hyaline changes. Haematoxylin and eosin staining.
D) Immunohistochemistry with CD 23 showing compact enlarged germinal centres with compact organised dendritical cells.

Bone marrow examination showed an active haematopoesis without infiltration of malignant lymphoma or cytokeratine positive cells. At first an ultrasound-guided lymph node biopsy was performed. The pathology was suggestive but not decisive for a plasmocytic small-cell non-Hodgkin's lymphoma.

Because of the uncertainty of the diagnosis, the young age of the patient and the consequences for treatment and prognosis we decided to perform a diagnostic laparoscopic splenectomy. Both the spleen and a few enlarged para-aortal lymph nodes were removed. Particularly in the lymph nodes and to a smaller extent in the spleen, abnormal enlarged follicles with a compact and enlarged follicle centre with dendritical cells and occasionally hyaline changes were seen. Combined with interfollicular hypervascularity and a polyclonal plasmocytosis the diagnosis of multicentric plasmocellular CD was made (*figures 1C* and *D*). The pathology was reviewed by two independent pathology boards highly experienced in lymphoproliferative disorders. Serum tests for HHV8 (IgM, IgG and PCR) were negative.

Within weeks after the splenectomy our patient spontaneously recovered from his physical complaints. He gained 10 kg and the laboratory findings normalised, while the CT scan revealed a normalisation of all aspects of lymphadenopathy.

Now, eight years after the splenectomy, the patient is still without any signs of activity of Castleman's disease as proven by six monthly physical examinations, laboratory tests and a yearly CT scan.

DISCUSSION

The patient described here showed signs of malabsorption including a microcytic anaemia. After elaborate examination he was diagnosed with CD, meeting the criteria for multicentric CD (*table 1*).

Table 1. *Criteria for multicentric Castleman's disease in this patient*

Criteria for multicentric Castleman's disease	In this patient
Median age 52-64 years	-
Male sex	+
Symptoms of inflammatory illness (fever, night sweats, weight loss)	+
Peripheral lymphadenopathy	+
Hepatomegaly	+
Splenomegaly	+
Elevated ESR	+
Anaemia	+
Hypoalbuminaemia	+
Hypergammaglobulinaemia	+
Positive lymph node biopsy	+

In the treatment of multicentric CD anti-interleukin-6 antibodies, chemotherapy and steroids have been considered the mainstay, whereas lately rituximab (anti-CD-20 antibodies) is considered to be an alternative.[4,6,8,9,12,13] The disease progression in patients with multicentric CD varies from a rapid form with progression leading to death within a few weeks, an episodic relapsing form and a chronic persistent form. Regardless of the pattern, the median survival of patients with multicentric CD is 26 to 30 months.[6,9]

Our patient, meeting many criteria for multicentric CD, is still alive and well, eight years after diagnosis, without any systemic treatment. CT scans have been repeated yearly without showing significant lymphadenopathy or hepatomegaly.

This course suggests a beneficial effect of splenectomy. Previous research showed no benefit of any type of surgery in multicentric CD for the prognosis and disease progression.[9,10] In all reported cases chemotherapy was needed to improve the condition of the patient. This leaves us with the question why did splenectomy help our patient? Has the contribution of the spleen in the course of CD been underestimated until now, and/or did splenectomy result in an overall decline in interleukin-6 production due to the reduction of the tumour load? Earlier studies have linked overexpression of interleukin-6 to the systemic manifestations of CD.[14,15] Unfortunately, interleukin-6 levels were not measured in our patient. Alternatively, the beneficial outcome of this patient may at least in part be related to the fact that he is HIV and HHV8 negative. Of note, the presentation of this case of multicentric CD is atypical with regard to some aspects (*table 1*), e.g. most cases of MCD are HHV8- and HIV-related, whereas most HIV-negative patients with MCD are in their fifth to sixth decade of life.

Our patient presented with fever, abdominal pain and watery diarrhoea after a holiday abroad. Possibly, an unrecognised viral infection causing these symptoms triggered the immune system into the development of CD, similar to what has been described for HHV-8. Serology and PCR for HHV-8 were negative in this patient.

In our opinion, in a patient with multicentric CD and splenomegaly, a splenectomy could be reconsidered awaiting remission of the symptoms.

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SPECIAL REPORT

Improved survival for patients with large B-cell lymphoma after introduction of rituximab

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ABSTRACT

Background: To determine whether the reported increase in survival of patients with diffuse large B-cell malignant lymphoma (DLBCL) after the introduction of rituximab is also seen in a non-academic hospital in the Netherlands. A retrospective study.

Methods: A dataset was made containing all newly diagnosed patients with DLBCL in a period of 2.5 years before until 2.5 years after introduction of rituximab in the standard treatment. Total follow-up time was 6.5 years with a minimal follow-up of 18 months.

Results: The study population consisted of 65 patients; 32 in the prerituximab group (median follow-up time 60 months) and 33 in the postrituximab group (median follow-up time 29 months). Progression-free survival increased significantly in the postrituximab group (hazard ratio 0.31; 95% CI 0.12 to 0.78; p=0.013; log rank p=0.008). The overall survival also showed a significant increase (p=0.048). The 18-month progression-free survival increased from 59.4 to 81.8%; the overall survival at 18 months showed an increase from 65.5 to 81.8%.

Conclusion: The introduction of rituximab in the treatment of DLBCL with CHOP chemotherapy has resulted in a significantly better prognosis for patients with DLBCL, treated in the Reinier de Graaf Gasthuis in Delft.

KEYWORDS

Diffuse large B-cell malignant lymphoma (DLBCL), (R-) CHOP, rituximab

INTRODUCTION

Diffuse large B-cell malignant lymphoma (DLBCL) is the most prevalent form of malignant lymphoma and accounts for 30 to 40% of cases.[1,2] Mean age at clinical presentation is 65 years and more than 60% of patients are aged ≥60 years at the time of diagnosis.[3,4] Extranodal manifestation occurs in 40% of patients.[5]

In the group of DLBCL various subtypes are discernable, so far, however, without consequences for therapeutic choices.[1,6]

Since the mid-1970s DLBCL has been treated with combination chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine and prednisone: CHOP chemotherapy.[7,8]

More intensive treatment schemes were used later on, but a randomised trial did not show improvement for these regimens in comparison with the classical CHOP scheme.[7]

An approximation of prognosis can be made using the International Prognostic Index (IPI). Depending on the IPI score, five-year survival varied between 26 and 73% in the era of CHOP chemotherapy.[4]

Rituximab has been registered in Europe since 1998. It is a monoclonal antibody against the pan-B-cell antigen CD20.[9] Since the introduction of rituximab (R-CHOP) a revolutionary improvement in treatment outcome was achieved, especially in the low-risk group according to IPI. Several studies have shown better results in progression-free and total survival after the addition of rituximab to CHOP treatment.[10-15] The British Columbian study showed a four-year progression-free survival of 53 to 94% and a four-year survival of 55 to 94% with R-CHOP, depending on the IPI score.[10]

Using the study from British Columbia as a background, we analysed the treatment results in our clinic.

MATERIALS AND METHODS

The retrospectively formed database consisted of information on patients, included if the time of diagnosis was between February 2001 and February 2006; that is 2.5 years before and 2.5 years after the introduction of rituximab in treatment of DLBCL in our clinic.

Follow-up was achieved until September 2007 with a minimal follow-up time of 1.5 years and a maximum of 6.5 years. Thanks to the cancer registry department of the Comprehensive Cancer Centre West patients could be identified for inclusion in our study.

Treatment consisted of CHOP in standard dosage every three weeks: three cycles for stage one (in exceptional cases four cycles) and six to eight cycles for stage II-IV, depending on disease aggressiveness. Rituximab was administered in standard dosage, 375 mg/m^2, in every CHOP cycle.

In stage I disease, involved-field radiotherapy was given after the three cycles of chemotherapy. In stage II-IV disease, radiotherapy was only given in exceptional cases, in doubt about the chance of complete remission. G-CSF support was not used in a standard way, only by indication.

All patients were discussed in a regional meeting of the Comprehensive Cancer Centre West. In case of doubt about the diagnosis, pathology was reconsidered by the lymphoma panel of pathologists.

Revised International Prognostic Index (r-IPI) was used for prognostic determination, with risk factors: age over 60 years, Ann Arbor stage III/IV, increased lactate dehydrogenase (LDH), Eastern Cooperative Oncology Group (ECOG) performance status ≥2 and more than one extranodal manifestation.[4,16] Only nine patients scored no risk factors: four in the CHOP group and five in the R-CHOP group. For this reason we created two risk categories according to r-IPI: low-risk (0-2 risk factors present) and high-risk (3-5 risk factors present).

We analysed the prerituximab and postrituximab group for progression-free survival and overall survival, followed by analysis on survival per risk group.

Patients were excluded in case of central nerve system localisation of the disease at time of presentation, HIV positivity or in case of transformation from indolent lymphoma.

STATISTICAL ANALYSIS

Prognostic variables were compared using the unpaired t-test for numerical variables and χ^2 test for categorical variables. Time until progression of disease was defined as time between start of treatment and disease progression, relapse or death. Time until death was defined as time between first treatment and death.

Differences in progression free survival (PFS) and overall survival (OS) between the two groups were analysed using the Kaplan-Meier method and Cox regression analysis. The log-rank test was used to compare the Kaplan-Meier curves. Cox regression was used for analysis after stratification for risk group, according to r-IPI. Data were analysed using 'Statistical Software Package for the Social Sciences' (SPSS version 15.0).

RESULTS

Sixty-five patients were included with onset of disease between February 2001 and February 2006, of which 32 belonged to the prerituximab group and 33 to the postrituximab group. Median age was 69 years for the total patient population, with a range of 29 to 84 years and a mean age of 65 years. Clinical characteristics at time of diagnosis are listed in *table 1*. Although no significant differences were found between the two groups, the postrituximab group contained more males, more often showed stage I/II disease and low-risk according to r-IPI, were less often treated with radiotherapy (RT) and less frequently received G-CSF support. The differences in RT can be explained due to the lower number of patients with stage I disease in the postrituximab group.

The first R-CHOP chemotherapy was administered in August 2003. This date was set as the start of the rituximab era. In the postrituximab group, 88% of the patients were actually treated with rituximab. The number of cycles of chemotherapy varied between three and eight. Four patients had not been able to tolerate the entire therapy due to premature death; two patients in the prerituximab group due to progressive disease and two patients in the postrituximab group due to fatal infection.

The number of cycles of chemotherapy did not differ significantly between the two groups (p=0.301). Median follow-up time was 60 months in the prerituximab group (range 49 to 78 months) and 29 months in the postrituximab group (range 18 to 46 months). No significant difference in relevant factors (r-IPI, age and

Table 1. *Patient characteristics*

Characteristics	Prerituximab (n=32)	Postrituximab (n=33)	P value
Median age, years	67 (range 33-78)	69 (range 29-84)	0.300
Sex:			
• Male	17	23	0.170
• Female	15	10	
Nodal	22	22	0.857
Stage I/II	15	20	0.267
Stage III/IV	17	13	
Low risk (r-IPI 0-2)	20	26	0.149
High risk (r-IPI 3-5)	12	7	
LDH level:			
• Normal	15	18	0.525
• Elevated	16	15	
• Unknown	1	0	
Radiotherapy:			
• Yes	15	8	0.098
• No	16	21	
• Unknown	1	4	
G-CSF support:			
• Yes	11	4	0.053
• No	20	25	
• Unknown	1	4	

stage) was found between the two eras. Disease progression occurred in 18 patients in the prerituximab group (56.3%) against six patients in the postrituximab group (18.2%).

PFS increased significantly in the postrituximab group as compared with the prerituximab group (hazard ratio 0.31; 95% CI 0.12 to 0.78; p=0.013; log-rank p=0.008), see *figure 1*.

Progression-free survival at 18 months increased from 59.4 to 81.8% after the introduction of rituximab in the CHOP regimen. Also, OS increased significantly in the postrituximab group as compared with the prerituximab group (hazard ratio 0.39; 95% CI ow.15 to 1.03; p=0.057; log rank p=0.048), see *figure 2*. OS at 18 months increased from 62.5 to 81.8% after introduction of rituximab. Overall, 16 patients died in the prerituximab group compared with six in the postrituximab group. In the prerituximab group all deaths were related to the disease; in the postrituximab group two patients died due to the treatment, the other four due to the disease itself, see *table 2*.

Stratified analysis for risk group showed an improvement in PFS in both risk groups, but significance was only achieved in the low-risk group (low-risk group: hazard ratio 0.23; 95% CI 0.062 to 0.833; p=0.025; high-risk group: hazard ratio 0.73; 95% CI 0.193 to 2.785; p=0.648).

Figure 1. *Progression-free survival (log rank test p=0.008)*

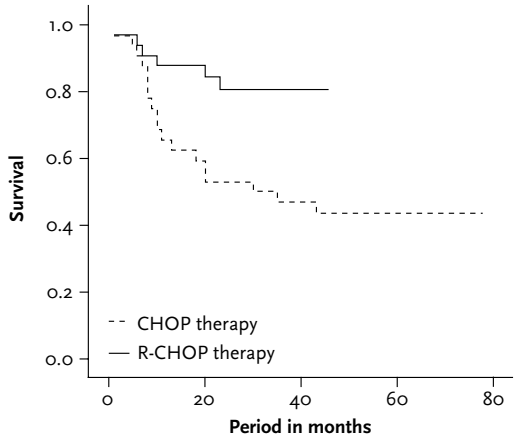

Figure 2. *Overall survival (log rank test p=0.048)*

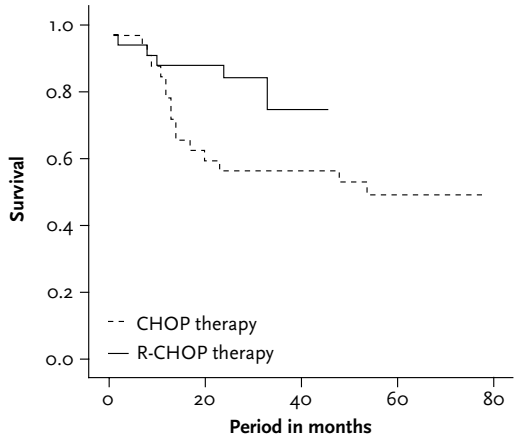

Table 2. *Survival at the end of the study period*

	Prerituximab (%)	Postrituximab (%)
Total no of patients (n= 65)	32	33
Alive without lymphoma	14 (44)	27 (81)
Alive with lymphoma	2 (6)	0 (0)
Died:	16 (50)	6 (18)
• Related to disease	16 (50)	4 (12)
• Related to treatment	0	2 (6)

DISCUSSION

Since rituximab has been introduced in the treatment of diffuse large B-cell malignant lymphoma (DLBCL) the prognosis has improved considerably. Both PFS and OS have increased significantly.[10-15,17]

This study shows the results of this new treatment with respect to PFS and OS of patients with DLBCL in the Reinier de Graaf Gasthuis in Delft since its introduction in our hospital in August 2003. The improvement of PFS (HR 0.31; p=0.008) approaches the improvement shown in the study by Sehn *et al.*[10] In this retrospective Canadian study, improvement of PFS (HR 0.56; p=0.002) and of OS (HR 0.40; p<0.0001) was shown after switching CHOP to R-CHOP. Two year PFS increased from 51 to 69% and two-year OS increased from 52 to 78%.

The first phase III randomised trial was the study by Coiffier *et al.*[11] in the elderly (age 60-80 years). Both two-year PFS (38 and 57% in CHOP and R-CHOP group respectively; HR 0.58) and two-year OS (57 and 70% in CHOP and R-CHOP group respectively; HR 0.64) had improved significantly. Five-year follow-up results confirmed this improvement of treatment.[17]

Confirmation of better treatment results with rituximab in the elderly was done by the US Intergroup (three-year PFS 35 *vs* 52% (HR 0.64) and three-year OS 58 *vs* 67% (HR 0.72) for CHOP and R-CHOP respectively).[12]

Pfreundschuh *et al.* compared treatment results within the younger population (age 18-60 years) in the MInT trial (MabThera International Trial).[14] This younger population also showed a significant increase in survival after the addition of rituximab to the chemotherapy treatment (three-year PFS 68 and 85% (HR 0.42) and three-year OS 84 and 93% (HR 0.40) in CHOP and R-CHOP respectively).

In the underlying study, especially patients with low risk according to r-IPI showed a large improvement in survival after the introduction of rituximab: HR 0.23 *vs* HR 0.73 in high-risk patients according to r-IPI. Feugier *et al.* also showed confinement of improvement especially to low-risk patients according to IPI: low-risk five-year progression-free survival 34 *vs* 69%; high-risk 29 *vs* 47% for CHOP and R-CHOP, respectively.[17]

New developments concern shortening of the interval between chemotherapy cycles from three (R-CHOP-21) to two weeks (R-CHOP-14), as has been investigated in the HOVON-46 and RICOVER-60 trials.[13,15] However, whether R-CHOP-14 is superior to R-CHOP-21 is still under study in randomised trials in France and the United Kingdom. Furthermore, within HOVON (HematoOncologieVOlwassenenNederland) the value of maintenance therapy with rituximab is being studied in the HOVON-84 study.[18]

This study shows the results of difference in treatment outcome between the two eras, before and after the introduction of rituximab. Limitations are the small number of patients per group, the slight imbalance in prognostic characteristics between the groups and the uneven follow-up periods. Bias may have occurred in the patients who were included in the postrituximab group and did not receive rituximab, but outcome for the postrituximab group could probably have been better, if these patients had been treated with rituximab.

The fact that two patients in the postrituximab group died due to infection is challenging. Is there a negative role for rituximab due to B-cell depletion? The small number of patients preclude definitive conclusions and further investigations are required to give more insight into infectious complications due to rituximab.

CONCLUSION

Treatment of patients with DLBCL in daily practice in the Reinier de Graaf Gasthuis confirms the results of better treatment outcome after addition of rituximab, as described in the literature.

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SPECIAL REPORT

The Dutch EPS Registry: Increasing the knowledge of encapsulating peritoneal sclerosis

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ABSTRACT

Encapsulating peritoneal sclerosis (EPS) is a rare condition characterised by fibrotic thickening of the visceral peritoneum, leading to encapsulating of the intestines with partial or total intestinal obstruction. EPS is a serious complication of peritoneal dialysis (PD) with high morbidity and a mortality exceeding 50%. At present, there is uncertainty concerning the incidence and the risk factors involved in the development of EPS. To address these questions a nationwide registry has been initiated.

The primary goals of the registry are to record the incidence of EPS and investigate the association of different variables, such as PD duration, medication, dialysis solutions and kidney transplantation with EPS.

The registry will improve the knowledge of EPS and will serve to develop guidelines and necessary management strategies. From the registry different research activities can be initiated. A major challenge lies in the establishment of criteria that allow a timely diagnosis of EPS. At present, there are no diagnostic tools that can accurately detect EPS at an early stage. For this reason, besides patients with proven EPS, the clinical suspicion of EPS will be a sufficient criterion for inclusion in the registry. This nationwide EPS registry is currently enrolling patients.

KEYWORDS

EPS, incidence, registry, risk factors

INTRODUCTION

Encapsulating peritoneal sclerosis (EPS) is a clinical syndrome characterised by intestinal encapsulating and subsequent obstruction of the intestinal tract.[1] EPS can be found in many different clinical settings, but the condition is most frequently encountered in patients treated with peritoneal dialysis.

Although rare, EPS has come to be recognised as a serious complication of peritoneal dialysis (PD) with a high morbidity and a mortality of approximately 50%.[2]

Reported prevalences for EPS range from 0.7 to 3.3%.[2-4] Recently, more attention has been given to this complication, as several reports have suggested an increased incidence of EPS during the last years.[5,6]

PD is an excellent modality of renal replacement therapy (RRT) and may have a superior patient survival compared with haemodialysis,[7] due to a better preservation of the renal residual function.[8] In the period 1996-2006 approximately 7800 patients with end-stage renal disease were treated with PD in the Netherlands (Renine database). However, in recent years a worldwide trend of treating fewer patients with PD has been noted. Among other reasons, an increased fear of EPS may be an incentive for the nephrologist to favour haemodialysis over PD when starting renal replacement therapy.[9]

There is much uncertainty concerning the true incidence of EPS in the Netherlands. In addition, the clinical factors associated with the development of EPS seem to differ from previous reports, as we found a substantial number of severe cases of EPS after renal transplantation.[6]

Given the severity of the condition and the current lack of data, a collaboration was started among Dutch nephrologists, which has resulted in the initiation of a nationwide registry for EPS.

DISCUSSION

Clinical spectrum of EPS

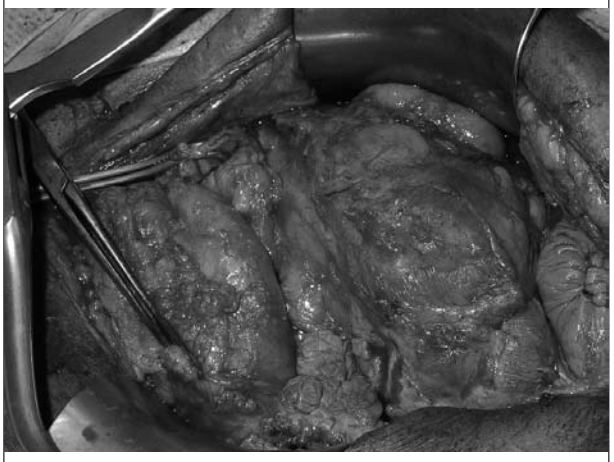
EPS, formerly known as sclerosing peritonitis, is characterised by progressive fibrosis of the visceral peritoneum resulting in a partial or total encasement of the bowel by a thickened and fibrotic membrane (*figure 1*).

The development of EPS is insidious and initially there are only vague abdominal complaints. With progressive fibrosis, symptoms as nausea, vomiting, appetite loss, weight loss and constipation appear. Usually, ultrafiltration failure has developed and signs of a systemic inflammatory syndrome may be present. Eventually, in the last stage of abdominal cocooning, there is partial or complete intestinal obstruction. At this stage there is a high morbidity and mortality. Recently, we performed a multicentre study in which we analysed the data of 2022 PD patients in the period 1996-2006. The results showed a high mortality rate for EPS, in accordance with studies from other countries (*figure 2*) (manuscript submitted).

The diagnosis of EPS is difficult because the criteria defined by the International Society for Peritoneal Dialysis (ISPD) (*table 1*) are rather aspecific.[10] The key feature of EPS is the presence of a clinical syndrome of intermittent or recurrent intestinal obstruction, with or without inflammation parameters. The existence of peritoneal thickening, sclerosis, calcifications and encapsulation is confirmed by macroscopic inspection or radiological findings.

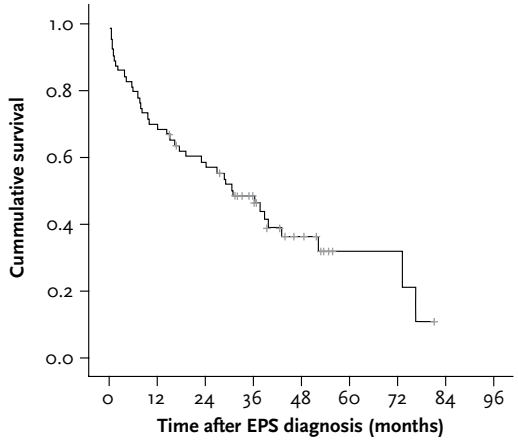
There is, however, a large overlap with simple sclerosis when patients are on PD for a longer time and CT scanning is not useful as a screening tool for early stages of EPS.[11] The use of macroscopic evidence of EPS is the only appropriate tool serving as golden standard for the diagnosis of EPS. However, this approach is not always feasible in a clinical setting and macroscopic evidence of EPS is only obtained in the minority of cases.

Figure 1. *Macroscopical encapsulating peritoneal sclerosis*



This patient with a history of PD has developed symptoms of intestinal obstruction. At laparotomy there is a clear fibrotic and thickened membrane covering the bowel. There are also extensive adhesions.

Figure 2. *Survival of patients with encapsulating peritoneal sclerosis*



In a Dutch multicentre study in the period 1996-2007 there were 63 patients with severe EPS in a total of 2022 PD patients. This figure shows the cumulative survival (Kaplan-Meier analysis) of these patients after the diagnosis of EPS was made.

Table 1. *Criteria for the diagnosis of encapsulating peritoneal sclerosis (EPS)*

ISPD[1]	**Criteria**
EPS	Intestinal obstruction[2] and radiological or macroscopical evidence[3]
EPS registry	
Macroscopial EPS (golden standard)	Intestinal obstruction[2] and macroscopically identified EPS
Clinical EPS	Intestinal obstruction[2] and radiological EPS[3]
Suspected early EPS	Intestinal obstruction or two or more findings of: • Weight or appetite loss • Bloody ascites • Radiological suggestion of EPS • Fast transport status or ultrafiltration failure
No EPS	Intestinal obstruction but other cause then EPS identified with certainty

[1]Criteria as used in the definitions by the International Society of Peritoneal Dialysis (ISPD) and the EPS registry of EPS in a patient that is currently being treated or has been treated with PD.[10] [2]Intestinal obstruction means any sign and symptom of persistent, intermittent or recurrent intestinal obstruction. [3]Radiological evidence for EPS means fulfilment of the criteria for EPS with CT scanning with findings such as peritoneal calcification, bowel thickening, bowel tethering, bowel dilatation, ascites or peritoneal thickening.[11]

Pathophysiology

The peritoneum of patients treated with PD is exposed daily to various dialysis fluids. This leads to changes of the peritoneal membrane over time characterised by mesothelial cell loss, epithelial to mesenchymal

transition of mesothelial cells, neovascularisation and vasculopathy.[12-16] These changes are probably induced by conventional dialysis fluids with bio-incompatible characteristics, high glucose concentrations, glucose degradation products, lactate buffers and acid pH. This process during long-term PD with fibrosis of parietal peritoneum is sometimes referred to as simple sclerosis.[17] It is generally assumed that the abundant fibrosis of the visceral peritoneal as seen in EPS, has a different aetiology to simple sclerosis. The complete pathophysiology of EPS is still unclear, but is probably multifactorial. The duration of PD is recognised as the single most important risk factor for EPS, as EPS within three years of treatment is rarely observed. Therefore, the most generally accepted theory assumes a progressively damaged peritoneum by prolonged use of incompatible dialysis fluids, which may be complicated by factors that aggravate the peritoneal sclerosis.[4,18] In recent years candidate factors came forth from a number of observational studies. These included cessation of peritoneal lavage,[3] peritonitis[19,20] and factors associated with kidney transplantation.[6]

Why a nationwide registry?

To date, there are still large gaps in our knowledge of EPS. This can be largely attributed to the lack of systemic prospective data collection, specifically necessary in the case of a condition encountered less than once a year in an average dialysis centre. Such a data collection is even more important as we reported a possible increased incidence of EPS.[5] Therefore, the first goal of this registry should be to record the current incidence of EPS and investigate whether it is still increasing.

Secondly, the registry needs to investigate the association of different variables, such as PD duration, medication, dialysis solutions and kidney transplantation. For instance, our case-controlled analysis of EPS cases in the Netherlands over the last ten years showed a strikingly high percentage of EPS (50%) shortly after renal transplantation and suggested that the use of icodextrin was independently associated with EPS (data unpublished). In addition, the statistical modelling indicated that a large part of the variation was not accounted for by the clinical and demographical variables used for analysis. These observations, which may have major consequences for the management of the PD patients, need to be verified in a prospective database. Furthermore, in an effort to document early stages of EPS we will also include cases of suspected EPS. This also allows identification of risk factors for progression and discovery of biomarkers for establishing EPS at an early stage.

In a recent survey among Dutch nephrologists it appeared that 16% of the responders feared EPS and subsequently considered withholding PD as a first choice of RRT.[9] Given the rarity of the disease and good overall survival on PD this decision is illogical, but illustrates the need for a registry recording data and yielding evidence-based guidelines to the treating physicians. As such, these data are currently not available and there is a lack of prospective studies on EPS. The majority of the experience comes from Japanese observational studies, where patients tend to be on PD for a longer period because the limited availability of kidney transplantation.[8] It is not clear whether the Japanese findings can be extrapolated to the PD population of Western Europe.

An important part of the guidelines is the development of an uniform management strategy for EPS. As malnutrition occurs in the presence of intestinal obstruction, supportive care with either enteral or parenteral nutrition is the mainstay of the treatment.[21] Immune suppressive medication and others agents, such as tamoxifen, have been suggested.[22-25] But the level of evidence is low as the data are from anecdotal reports or small case series. Encouraging results from Japan have been reported with surgical enterolysis, releasing the complete small intestine.[26] However, there is still little experience with this procedure in Western Europe. Finally, the registry will function as a central organisation from which different research activities, for example genetic and marker studies, can be initiated. To strengthen the importance of the registry there will be extensive collaboration within Europe, for instance with the UK EPS study group.

Design

Collaboration of all university centres and the Hans Mak Institute resulted in a steering committee, which has initiated the nationwide EPS registry. Patients with a history of PD with a diagnosis of EPS or suspicion of EPS will be prospectively included. Ideally, the registry would include all patients treated with PD. This way, all data could be accurately registered. However, given the low prevalence of EPS, inclusion of all PD patients would be time consuming and requires a very large, expensive database.

Every six months an e-mail will be sent to all Dutch nephrologists inquiring whether they can report a patient (suspected of) having EPS. In the registry patients are divided into four groups by the steering committee; macroscopically definite EPS, clinical EPS, possible EPS and no EPS, by the criteria shown in *table 1*. As multiple factors may influence the development of EPS, there will be an extensive review of all possible diagnostic, prognostic and therapeutic variables. Demographics, and factors related to PD, HD and transplantation for all included patients will be reviewed. In addition, a sample of peritoneal effluent and plasma will be taken and stored for later analysis.

An easy accessible website (www.epsregistry.com) has been developed to give more detailed information on EPS and the EPS registry. The registry is set up so that it can easily be extended to a European format.

For professionals it also has the opportunity to submit a patient with EPS. There will be a yearly update on the progress of the registry. In the future research developments and guidelines will be published on the website.

CONCLUSION

EPS is a potentially devastating disease with a high mortality. Recently, it was shown that the prevalence of EPS may increase in the Netherlands. The low prevalence of EPS has hampered the research in this area, which has resulted in a lack of knowledge about natural history, pathophysiology and risk factors, and treatment options. A nationwide registry is required to collect data prospectively. Such an EPS registry was recently initiated. The database of this EPS registry will allow establishment and monitoring of the prevalence of EPS, identifying risk factors, basic research on the pathophysiology of EPS and development of management guidelines. The EPS registry is currently enrolling patients. We kindly call upon all nephrologists to cooperate with the registry in order to obtain a representative registry and thus contribute to a better understanding of EPS.

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LETTER TO THE EDITOR

Responding to adverse events

Dear Editor,

Berend's article describes the tragic story of the prosecution of a small dialysis centre's medical director.[1] The suggested actions may help those who are being prosecuted. Some important issues like the reaction towards the patients and reactions that prevent prosecution are missing.[2] So his suggestions should be preceded by the following:

- Say 'sorry' to the patient and his relatives and prevent further harm. Offer psychosocial support to prevent a posttraumatic stress disorder or depression.[3] Be supportive, even when patients or relatives act hostile. They have – unintentionally – been harmed by those they trusted. Stay in contact to assist in the recovery. The extra costs (visits, treatment) should be compensated. If permanent disability results, a compensation should be paid apart from the question whether a mistake has been made.[4]
- Organise an in-depth investigation of the causes of the incident that will periodically be communicated. The investigation team should be trained in incident analysis techniques[5] and comes into action if a catastrophe evolves. Immediate action preserves evidence and prevents hindsight bias from those involved. A well-respected physician should lead the team, which can – depending on the issue – be extended with experts. At least one external authority should advise on, and finally approve of, the conclusions to guarantee independency.
- The team also supports the staff involved and judges whether they are emotionally stable enough to continue patient care or should be given time (and support) to recover.[6]
- Open disclosure of the findings by the leading physician to the patient, his relatives or the press is important. The key message is 'which lessons have been learned, and which actions are being taken to prevent relapse'.[4] Patients are strong about the view that they want to be informed about harmful errors and what is done to prevent recurrence.[7]

These actions should be described in a protocol presented on the website of the hospital to inform all parties.
Although open disclosure may cause complaints by patients and legal bodies and assaults by the media, these problems are less than those that arise from defensiveness.[8] Some healthcare organisations (http://www,safetyandquality.org) have moved open disclosure into an organisational policy to prevent criminal prosecution and protect health care providers and patients from future incidents and psychosocial damage. It also serves safe and patient oriented care as a moral duty.

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LETTER TO THE EDITOR

The role of rituximab in a case of EBV-related lymphoproliferative disease presenting with haemophagocytosis

Dear Editor,

With interest we read the case report by Wijsman, *et al.*[1] In addition to their patients, we describe a 21-year-old woman with a systemic inflammatory response syndrome (SIRS) and pancytopenia after a primary Epstein-Barr virus (EBV) infection. Three weeks earlier she had been admitted elsewhere with fever, anorexia, cervical lymphadenopathy, jaundice, rash and myalgias. Her medical history consisted of ulcerative colitis (UC), for which she received treatment with azathioprine (150 mg daily). EBV IgM and monospot were positive and EBV load determined by PCR was markedly elevated, consistent with the diagnosis of a primary EBV infection. Laboratory investigation now revealed pancytopenia, haemoglobin level 5.5 mmol/l, leucocyte count 0.9 x 10^9/l, thrombocyte count 58 x 10^9/l, elevated CRP level 194 mg/l, bilirubin 309 µmol/l, elevated ALAT 91 U/l and ASAT 180 U/l, elevated LDH 1279 U/l, hyperferritinaemia >15,000 µg/l, and a normal level of triglycerides. A CT scan confirmed the slightly enlarged lymph nodes in neck and both axillae, and showed only slight hepatosplenomegaly. Quantitative PCR for EBV was positive with a viral load of 200,000 c/ml. At first a diagnosis of EBV-related lymphoproliferative disease (LPD) was considered, but bone marrow biopsy showed an increased number of macrophages with phagocytosed red blood cells (= haemophagocytosis) and no signs of a LPD. Therefore, and because she met the clinical and laboratory criteria for secondary haemophagocytic (HLH),[2] a primary EBV infection complicated by HLH was diagnosed.

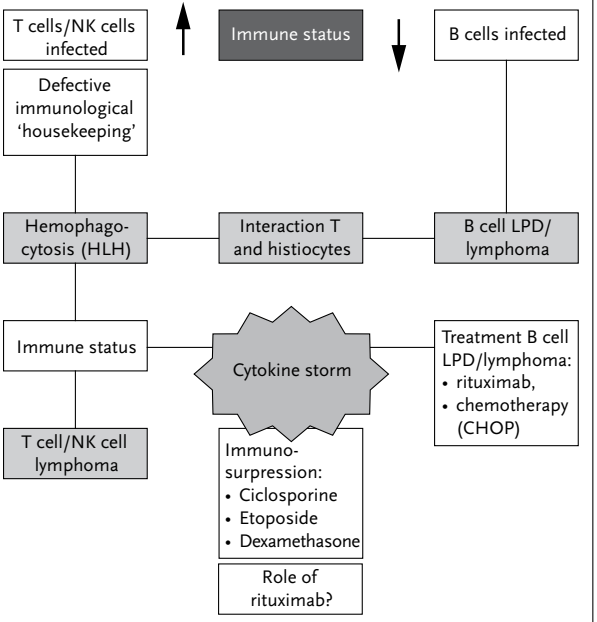
She needed haemodynamic and respiratory support, and was treated in the ICU according to the treatment protocol of the second international HLH study with cyclosporine and dexamethasone (but without etoposide and methotrexate).[2] She improved with resolution of the pancytopenia, although the EBV viral load did not decrease. Also, subset analysis of the patient's lymphocyte population showed that the cytotoxic T cells and NK cells were not infected with EBV, as has been reported in case of EBV-related HLH.[3,4] This urged us to re-evaluate the possibility of an underlying EBV-related LPD. Meanwhile, she developed a bacterial peritonitis caused by perforation of the large intestine, and a partial resection of the colon was performed. Pathological examination revealed monomorphous EBV-related B-LPD, histologically a diffuse large B cell lymphoma. A repeated CT scan now showed multiple circumscribed lesions in the lungs, liver, spleen, kidneys and pancreas.

We concluded that she was suffering from an EBV-related LPD following a primary EBV infection whilst on azathioprine. Cyclosporine was immediately discontinued and the patient was treated with rituximab and CHOP chemotherapy (cyclophosphamide, adriamycine, vincristine and prednisone), after which she improved. After eight courses of R-CHOP she is now in a complete remission with a good clinical condition and with EBV viral transcript that is no longer detectable.

Our case demonstrates that clinical pictures overlap and that differentiation is essential for immediate appropriate treatment. Although our patient was suffering from an EBV-related B-LPD, resulting from a primary EBV infection during immunosuppressive therapy because of UC, on admission her clinical picture was dominated by SIRS, ARDS and haemophagocytosis, consistent with secondary HLH. The haemophagocytosis on presentation was probably caused by the cytokine storm or indirect functional impairment of T cells/histiocytes resulting from the rapid B cell proliferation in combination with immunosuppressive UC therapy.

Haemophagocytosis due to EBV-related B-LPD and EBV-related HLH are two different but overlapping pathophysiological entities. In fact, the most important difference is the primary cell type infected by EBV. In HLH mainly cytotoxic T cells and NK cells, both essential for immune regulation, are infected.[4] In EBV-related B-LPD B lymphocytes are infected and ultimately transformed to lymphoma. This process can be accompanied or facilitated by functional impairment of histiocytes/T cells and cytokine release with subsequent haemophagocytosis. In patients, detecting the infected cell type could therefore be useful for differentiating these two entities. In our patient, the cytotoxic T cells and NK cells were not infected with EBV, therefore the clinical picture could not be classified

Figure 1. *EBV-related haemophagocytosis*



as 'classic' secondary HLH. This knowledge, if known earlier in the course of her illness, could have prevented the occurrence of the rapidly progressive EBV-related LPD.

The differences between EBV-related HLH and LPD have important consequences for the initiation of appropriate treatment.[5] The common feature of EBV-related LPD and HLH is the so-called 'cytokine storm', caused by a deregulated immune response. Although immunosuppressive therapy is necessary to prevent SIRS and even death, not all patients benefit from immunesuppression alone, especially those with mainly infected B cells. Our patient was treated with rituximab to prevent the originally polymorphic EBV-related LPD from evolving monomorphic lymphoma during immunosuppressive therapy. Rituximab, by destroying infected B cells, decreases the EBV-induced hyperactive immune response by decreasing the load of the causative pathogen EBV as well as the chance of malignant transformation of these B cells.[6] On the other hand, secondary HLH, a problem of T cell and NK cell dysfunction, can safely be treated with immunosuppression alone, as it is rarely complicated by lymphoma. Since it involves predominantly T cells and NK cells not expressing CD20, addition of rituximab seems of little benefit.

Concluding, EBV-related LPD and HLH are life-threatening disorders. In case of EBV-related haemophagocytosis an underlying malignant lymphoproliferative process should be considered and excluded, especially with EBV infection/reactivation in the immune-compromised host. EBV viral load should be carefully monitored during treatment and if possible the infected cell type determined. Early treatment with rituximab results both in the reduction of the viral load and the elimination of transformed B cells, reducing the risk of LPD. The role of rituximab in 'classic' EBV-related HLH is however not defined. Therefore treatment with rituximab should be considered in EBV-related haemophagocytosis, especially when fast differentiation between LPD and HLH is not possible.

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2. Kaplan NM. Clinical Hypertension. 7th ed. Baltimore: Williams & Wilkins; 1998.
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