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Activity of C1 esterase inhibitor in patients with vascular leak syndrome after bone marrow transplantation*

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Summary. Vascular-leak syndrome (VLS) is a common complication in the first 3 weeks after bone marrow transplantation (BMT). The patients present with weight gain, generalized edema, ascites, pericardial or pleural effusions, tachycardia, arterial hypotonia, and/or pre-renal failure. The aim of our study was to investigate the role of the complement system in VLS. The protein concentrations of C3 and C4 were studied by immunodiffusion, and total hemolytic complement activity was studied by assessment of CH50. C1 esterase inhibitor (C1 Inh), the major inhibitor of the classical pathway of complement, was assessed by a functional test. Activation of complement was assessed by C4d (a C4 activation product). Twelve patients were followed prospectively from start of conditioning therapy to day +21 after bone marrow transplantation. Eight of 12 patients did not develop VLS. These patients had an increase of C3 between day +9 and day +13 (range: 1.3- to 1.5-fold, median: 1.4-fold), C4 (range: 1.3- to 1.9-fold, median: 1.4-fold), CH50 (range: 1.3- to 1.6-fold, median: 1.4-fold), and C1 Inh (range: 1.2- to 1.5-fold, median: 1.3-fold). Four of 12 patients developed VLS. C1 Inh activity was decreased to 0.60- to 0.80-fold. This decrease began 2–6 days prior to clinical diagnosis of VLS ($n=3$), or at onset of VLS ($n=1$). Patients with VLS showed elevated C4d concentrations (up to 2.4 mg/dl, upper normal treshold value: 0.9 mg/dl). Patients with VLS reveal an activated state of the complement system which is accompanied by a reduced activity of C1 Inh. Insufficient control of complement activation may contribute to VLS in patients after BMT.

Key words: Vascular leak syndrome – Bone marrow transplantation – Complement activation – Contact system activation – C1 esterase inhibitor

Introduction

Vascular leak syndrome (VLS) is a frequent complication following bone marrow transplantation (BMT). The symptoms represent consequences of the loss of vascular fluids to third spaces: the patients present with generalized edema, pleural or pericardial effusion, ascites, weight gain, tachycardia, hypotension, and/or pre-renal failure. Typical time of onset is day +7–+14 after BMT. At this stage, the patients have several overlapping conditions: (a) infections and aplasia, (b) nonhematopoietic toxicity of the conditioning regimen, and (c) the beginning of engraftment. Therefore, it is difficult to causally relate VLS to one of these complications, and there is no specific treatment for this condition. An early diagnosis of this syndrome would increase our ability to initiate unspecific treatment (such as restriction of sodium and fluid administration and/or restriction of nephrotoxic medications, e.g., cyclosporin A) in order to positively influence the course of VLS.

VLS is also described as a complication of systemic IL-2 therapy, especially when high doses of IL-2 are administered within a relatively short infusion time [17]. This form of VLS shows systemic activation of complement, similar to that in sepsis syndrome [22]. Activated complement components such as C3b may bind covalently to circulating cells and induce cytotoxicity [23]. Assuming that the target cell carries receptors for complement components, it seems reasonable that cytotoxity of activated lymphocytes might increase. Based on these findings, we prospectively analyzed the complement system in patients after BMT to

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Abbreviations: BMT, bone marrow transplantation; VLS, vascular leak syndrome; BW, body weight; C1 Inh, C1 esterase inhibitor; CH50, total hemolytic complement; C3, third component of complement; C4, fourth component of complement; C4d, C4 activation product

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test the possibility of an early, laboratory diagnosis of VLS.

Patients

Characteristics

The courses of 12 patients (aged 2–24 years; 9 years) were analyzed. Six patients were female and six male. Underlying diseases were ALL in second or later remission ($n=4$), severe aplastic anemia ($n=1$), Fanconi's anemia ($n=1$), neuroblastoma IV ($n=2$), multifocal or early-relapsed Ewing's sarcoma ($n=3$), and early-relapsed lymphoepitheleal carcinoma (nasopharynx carcinoma) with peripheral bone metastases ($n=1$). Preparative regimens prior to BMT were dependent on the underlying diseases:

Ewing's sarcoma, neuroblastoma, lymphoepitheleal carcinoma: hyperfractionated total body irradiation (4 days each 2×1.5 Gy, total dose: 12 Gy); 4×35 mg/kg body weight (BW) melphalan between the irradiation sessions, followed by 1200 mg/m^2 etoposide and up to 3× 500 mg/m^2 carboplatinum (Hyper-Carbo-VAMP-Protokoll [7]
Aplastic anemia: 4×50 mg/kg BW cyclophosphamide and 7.5 Gy total lymph node irradiation [9]
Fanconi's anemia: 4×5 mg/kg BW cyclophosphamide and 5 Gy total lymph node irradiation [9]
ALL: hyperfractionated total body irradiation (3 days each 2×2 Gy, total dose: 12 Gy) combined with 1×60 mg/kg BW etoposide for related BMT (ALL-BFM) or with 1×40 mg/kg BW etoposide plus 2×60 mg/kg BW cyclophosphamide for unrelated BMT [3, 21]

Six patients were grafted with allogenous stem cells (four ALL – two of these unrelated transplants, one plastic anemia, one Fanconi's anemia) and six with autologous stem cells (three Ewing's sarcoma, one lymphoepitheleal carcinoma, two neuroblastoma). Autologous BMT was followed by hematopoietic rescue using GM-CSF (study preparation) or G-CSF (Neupogen) [7]. Total daily fluid administration (including antibiotics, parenteral nutrition, etc.) was 2000 cc/m^2 in the nonfebrile patient.

Definition of the diagnosis "VLS"

The criteria for diagnosis of "VLS" are intravasal volume depletion, lowered central venous pressure, peripheral edema, ascites, and pleural or pericardial effusions [2]. Based on previous experience with patients after BMT, diagnosis of VLS for this study was defined as follows: more than 3% increase of BW within 24 h, but at least 500 cc, combined with generalized edema.

Materials and methods

Serum processing and controls

All blood samples from patients were drawn from Hickmann lines. As controls served 30 blood donors, from whom native blood (for serum) and EDTA blood was collected via venopuncture. Serum and EDTA plasma samples were immediately portioned, frozen at −70° C, and thawed only once. As control serum or EDTA pools we used pools from equal amounts of serum or EDTA plasma of the blood donors.

Determinations of the complement system

The classical pathway was assessed by total hemolytic complement (CH50) and serum-C3 and -C4 concentrations. Activation of the classical pathway was assessed by the C4 activation product (C4d). Regulation of the classical pathway was assessed by determination of C1 esterase inhibitor (C1 Inh) activity. All data were corrected for serum protein concentration.

CH50. This functional test is based on the kinetics of hemolysis of antibody-sensitized sheep erythrocytes [14], using the Chromotimer (Behringwerke AG, Marburg, FRG). The system was modified as follows: (a) Serum was applied (instead of citrate plasma) to avoid interference of the anticoagulins; (b) the samples were more diluted (1 in 30, instead of 1 in 20) and the difference of extinction of the Chromotimer was increased (0.2 instead of 0.1) to reveal a standard curve, not too steep, in the range of 50%–120% of control serum pool. The reliability of this method was significantly increased by these measures. The individual serum samples of 30 blood donors ranged from 80% to 120% of the control serum pool.

C3 and C4. The third and fourth complement components were determined using immunodiffusion plates (Behringwerke AG, Marburg, FRG). The individual serum samples of 30 blood donors ranged from 65 to 120 mg/dl (for C3) and from 20 to 50 mg/dl (for C4) of the control serum pool.

C4d. After it was seen that C1 Inh decreased in some patients after BMT, C4d was determined in the subsequent patients to assess activation of the classical pathway of complement. C4d was determined in EDTA plasma using an ELISA based on neoantigen-specific monoclonal antibodies against C4d (Quidel-Cytotech, San Diego, CA, USA). Specificity of the system was checked by C4d determination in factor-I-deficient plasma [16]. Range for 30 blood donors: 0.2–0.9 mg/dl median: 0.6 mg/dl.

C1 Inh. The functional activity of C1 Inh was determined in EDTA plasma using the chromogenic substrate technique described by Heber et al. [12]. The individual plasma samples of 30 blood donors ranged from 75% to 125% of the control serum pool.

Results

Characteristics of patients with VLS

Four of 12 patients developed VLS according to the criteria used in this study (see above). These criteria were met by the individual patients on days +9, +13, +5, and +4 after BMT (see Table 1). The maximum increase of BW (in % of BW on admission) was in patient 1: 18% at day 15 (combined with ascites); in patient 2: 11% at day 20 (combined with pleural effusions); in patient 3: 3% at day 14 through day 18; in patient 4: 12% at day 6 (combined with renal failure). No patient developed veno-occlusive disease.

The underlying diseases in these four patients were: ALL ($n=1$), Ewing's sarcoma ($n=1$), and neuroblastoma ($n=2$). The preparative regimens were: ALL-BFM ($n=1$) and Hyper-Carbo-VAMP ($n=3$); three of four patients received hematopoietic growth factors (G-CSF or GM-CSF); one patient had allogenous, and three patients had autologous BMT. Three patients were male, one was female. Treatment of VLS was as usual: fluid and sodium restriction and restriction of

Table 1. Time relationship between decrease of C1 Inh, complement activation, and development of VLS

Patient no.	Decrease of C1 Inh levels (day after BMT)	Start of VLS (day after BMT)	Body weight		Complement activation (days after BMT)	Type of bone marrow	Diagnosis
			before BMT (kg)	maximum in VLS (kg)			
1	+ 9	+ 9	10.2	12.1	+ 9–+20	Autologous	Neuroblastoma
2	+11	+13	22.0	24.4	+11–+21[a]	Autologous	Neuroblastoma
3	+ 2	+ 5	73.1	75.3	+ 8–+20	Autologous	Ewing's sarcoma
4	− 2	+ 4	45.0	50.4	+ 5–+17	Allogenous	ALL

[a] Patient 2 decreased on day +21 after BMT

nephrotoxic medications. One patient underwent dialysis. Three of the four patients died of cardiovascular complications at days +21, +46, and +88. In contrast, none of the patients without VLS died of complications during the same observation period, or required intensive care measures otherwise, or developed veno-occlusive disease of the liver.

Complement parameters in patients without VLS

Patients without VLS ($n=8$) showed increasing values of CH50 (increase of median: 1.4-fold), C3 (increase of median: 1.4-fold), C4 (increase of median: 1.4-fold), and C1 Inh (increase of median: 1.2-fold) during the first days after BMT compared with values on admission (see Table 2). Highest values were reached at days +9–+13 (median). The increase of laboratory values was significant for all parameter ($p<0.05$, unpaired t-test). In the further course, the values decreased to the starting range by day +21 (end of observation period).

In the group that did not develop VLS, four of the eight patients showed elevated CH50 values (>120% control serum pool) prior to BMT. Underlying diseases of patients with elevated CH50 were: ALL in second remission (3/4 patients with ALL in this group) and lymphoepitheleal carcinoma ($n=1$). No increase of CH50 values were seen in patients with Fanconi's anemia ($n=1$). No increase of CH50 values were seen in patients with Fanconi's anemia ($n=1$), aplastic anemia ($n=1$), neuroblastoma IV ($n=1$), and multifocal Ewing's sarcoma ($n=1$).

Table 2. Complement parameters after BMT in patients without VLS

Parameter	Prior to BMT range (median)	Maximum after BMT range (median)	Reached at day (median)
CH50 (% NHS)	77–140 (117)	126–183 (160)	+13
C3 (mg/dl)	63–98 (92)	98–153 (132)	+ 9
C4 (mg/dl)	19–46 (35)	34–64 (50)	+10
C4d (mg/l)	1.8–5.5 (3.5)	1.9–6.3 (4.0)	+11
C1 Inh (% NPP)	81–111 (98)	107–127 (119)	+11

C1 Inh activity in patients with VLS

The courses of C1 Inh activity in patients without VLS (gray area) and in four patients with VLS (single drawn curves) are shown in Fig. 1. C1 Inh activity of three patients increased in the beginning of the transplant course, similar to the values of the patients without VLS. However, on defined days (patient 1: day +9, patient 2: day +11, patient 3: day +2), C1 Inh activity decreased significantly when compared intraindividually ($p=0.008$, paired t-test, Fig. 2). These values were, at defined time points, lower than the values of patients without VLS.

In the next step the changes in the course of C1 Inh were calculated in 7-day intervals. A significant difference between the non-VLS and the VLS group was seen between day 7 and day 14 after BMT (Table 3): The VLS group showed a stronger decrease of C1 Inh. This result corresponds to the finding that at least one patient already had VLS in this period and two others developed VLS in this period (see also Table 1).

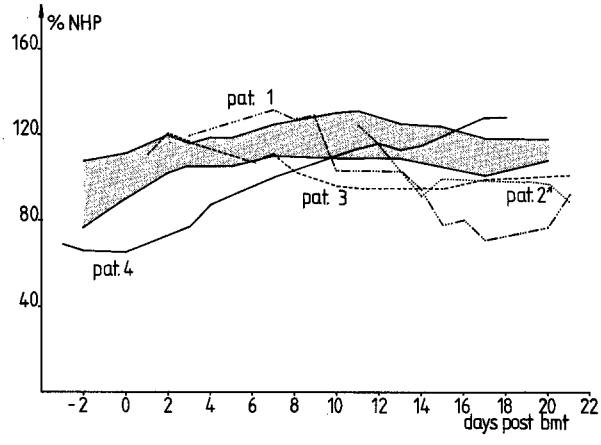

Fig. 1. C1 Inh during BMT. The *ordinate* shows the days prior/after BMT; transplant day is day 0. The *vertical axis* shows C1 Inh activity as percent of the control plasma pool (NPP). The range of C1 Inh values for blood donors was 75%–125% of NPP. C1 Inh activity values from patients after BMT without VLS lay inside the gray area (see Table 1). The courses of C1 Inh activity of four patients with VLS are shown by the *drawn* or *broken lines*: in patients 1–3 these values follow the gray area at the beginning, and then turn down on defined days (see also Table 2). The C1 Inh values in patient 4 were lowered prior to BMT

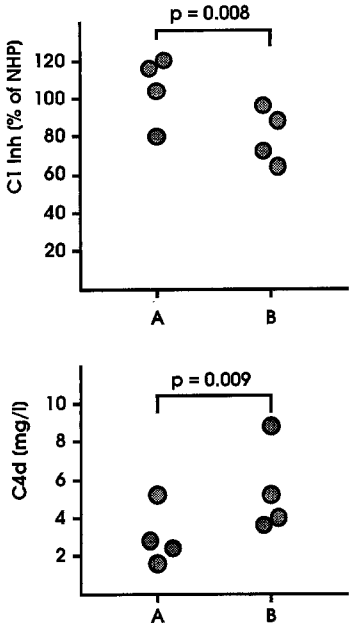

Fig. 2. Inverse relationship between C4d and C1 Inh. Demonstration of values for C1 Inh (O—O), and C4d (O—O) after BMT in patients with VLS. The *lower part* shows the C4d values 5 days prior to VLS (**A**) and at diagnosis of VLS (**B**), the *upper part* the corresponding C1 Inh values. The paired *t*-test was applied for statistical analysis, and the significance level is given in the figure

Table 3. Different changes of C1 Inh activity in patients with and without VLS[a]

Time	Non-VLS group	VLS group	Unpaired *t*-test
Prior to BMT	+ 3±1	− 1±9	n.s.
Day 0–7	+15±4	+17±9	n.s.
Day 7–14	− 2±2	−15±7	$p = <0.05$
Day 14–21	− 2±3	+ 4±8	n.s.

[a] The difference of C1 Inh (in % NHP) is given as mean ± S.D. for the time indicated on the left

Figure 2 shows the inverse course of C1 Inh decrease and C4d increase ($p = 0.009$, paired *t*-test). The highest C4d values of the VLS group were significantly increased compared with the highest C4d values found in the patients without VLS (Table 1; $p = 0.02$, unpaired *t*-test).

Discussion

Physiological functions of C1 Inh and VLS

C1 Inh is a serine protease inhibitor (serpin) and is present in a relatively high concentration in plasma. C1 Inh is the only known inhibitor of complement C1r and C1s and the major inhibitor of activated Hagemann factor (=F XII of the contact activation system) [1] and of kallikrein [18].

Two types of inherited deficiency of C1 Inh are described: the reduced (or lacking) synthesis of C1 Inh and the synthesis of a dysfunctional protein. These patients suffer from acute, life-threatening edema of the larynx and epiglottis. During acute attacks of the hereditary angioedema, the patients show activation of complement, prekallikrein, and factor XII [19]. Substitution of C1 Inh concentrate inhibits activation, resolves the symptoms, and – when given prophylactically – prevents acute exacerbations [4].

The present study showed activation of the classical pathway of complement and decreasing activity of C1 Inh in patients who developed VLS after BMT. Activation of the classical pathway of complement results in the release of the anaphylatoxins C4a, C3a, and C5a. Anaphylatoxins increase vasopermeability, resulting in loss of fluids to the interstitium and, thus, development of edema [10].

However, other pathways might also contribute to VLS. Hack and colleagues [11] described VLS as a side effect of systemic IL-2 therapy-induced activation of complement, of factor XII (contact activation system), and of prekallikrein. Activation of the latter components initiates a positive feedback: activated factor XII activates prekallikrein to kallikrein, which itself activates factor XII, etc. [15]. Kallikrein activates the kinin-bradykinin system, inducing peripheral vasodilatation and hypotension. In a pathway parallel to the described feedback circle, activated factor XII activates fibrinolysis, thus initiating activation of the classical pathway of complement via plasmin [8].

Minor findings of the present study were elevated CH50 values in four of eight patients. Three of four patients had received treatment for ALL relapse (ALL-Rezidiv-BFM-90), with 4–6 weeks between the last chemotherapy and the start of the preparative regimen. However, a detailed analysis of complement parameters and the clinical situation prior to BMT (for malignant diseases: cytoreductive measures, consolidation strategies, cytokine administration; for aplastic anemia: immunsuppression, androgen administration) requires a study especially designed to answer these questions.

VLS after BMT

The patients described here developed VLS as a generalized form. However, localized forms of VLS may also occur; e.g., as pleural effusion or lung edema. Veno-occlusive disease can be understood as VLS of the liver as well.

Development of VLS after BMT could be influenced by the preparative regimen, cell lysis in relapse patients, and therapy with hematopoietic growth factors or other parameters. As far as can be concluded from a small patient group, the preparative regimen seems to influence VLS: three of six patients receiving the Hyper-Carbo-VAMP protocol developed VLS, compared with one of six who received other protocols. Whether or not VLS is influenced by the concomitant use of hematopoietic growth factors remains unknown. However, either G-CSF or GM-CSF is part of

the Hyper-Carbo-VAMP protocol [6], and at least GM-CSF has been implicated in the aggravation of VLS [7]. Cell lysis, which is warranted for patients in relapse or in partial remission, could result in increased release of proteases from malignant cells. These proteases could directly activate complement, or could interfere with the regulation of complement by enzymatic inactivation or degradation of C1 Inh. Proteolytic cleavage of C1 Inh by leukocyte elastases was described by Brower and Harpel [5].

Consumption (especially) of the complement components C1q and C2 and CH50 was described in two patients who developed fatal graft-versus-host disease (GVHD) [20]. The authors concluded activation of complement in these patients from severe decrease of functional and antigenic plasma levels (but they did not assess specific complement activation products). A theoretical link between GVHD, VLS, and complement activation was found in IL-2, a cytokine important for T-cell activation in GVHD and able to induce VLS, as mentioned above. GVHD was, however, not evaluated in the patients of the present study, since only six of the 12 received allografts.

The C1 Inh course in patients after BMT without VLS showed an increase of activity with a maximum at day +11 after BMT (Table 2). One of four patients had a decrease of C1 Inh on the day of clinical diagnosis of VLS, whereas in the other three C1 Inh activity decreased 2–6 days prior to clinical diagnosis of VLS (Table 2). This indicates consumption of C1 Inh, e.g., by reaction with target proteases. The role of C1 Inh in this context requires further investigation.

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