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Short communication

Ascorbic acid serum levels are reduced in patients with hematological malignancies

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ABSTRACT

In this paper we demonstrate that patients treated with chemotherapy and/or hematopoietic stem cell transplantation (HSCT) have highly significant reduced serum ascorbic acid (AA) levels compared to healthy controls. We recently observed in *in vitro* experiments that growth of both T and NK cells from hematopoietic stem cells is positively influenced by AA. It might be of clinical relevance to study the function and recovery of immune cells after intensive treatment, its correlation to AA serum levels and the possible effect of AA supplementation.

1. Introduction

Vitamin C or ascorbic acid (AA), an essential water-soluble vitamin with many functions [1,2], has a crucial role in cellular immune responses [3]. Patients treated with intensive chemotherapy and/or hematopoietic stem cell transplantation (HSCT) have low immune cell counts for weeks to months [4]. Meanwhile, patients are highly susceptible to infections resulting in morbidity and mortality. We recently observed that in the presence of AA, early hematopoietic progenitors commit and mature into T cells and proliferate faster [5]. Moreover, we showed that AA enhances proliferation and maturation of NK cells [6]. As AA has a major influence on (re)generation of immune cells *in vitro*, we executed an observational study in which AA serum values of patients with hematological malignancies treated with and without HSCT were compared with those of healthy volunteers to see if low AA levels should be considered of importance regarding immune recovery of these patients.

2. Materials and methods

2.1. Patients and controls

Basic patient characteristics are shown in Table 1.

AA levels were measured in 42 non-selected hemato-oncology patients admitted to the hospital because of treatment or complications. Samples were taken of all patients admitted on the day routine blood samples are taken. Therefore, the patient population is a mixture of all type of patients as well as stages of disease and treatment. As control group, healthy volunteers ($N=79$, mean age=36.87 (range 20–63) working in the hospital donated blood samples for clotting parameter reference values of our laboratory. This study was performed in accordance with the Declaration of Helsinki and according to Dutch Code for Proper Secondary Use of Human Tissue [7].

Patients receiving autologous HSCT had the following diseases and treatment: MM (6; conditioning with high dose Melfalan); NHL (3; condition with BEAM) and AML (1, conditioning with Busulphan and Cyclophosfamide). Patients receiving an allogeneic HSCT had the following diseases and treatment: AML (7); ALL (2); NHL (4); CLL (1); CML (1); Myelofibrosis (1). All were treated with a non-myeloablative conditioning regimen (Fludarabine with low dose total body irradiation with or without anti-thymocyte globulin (Rabbit ATG, 2 mg/kg)

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Table 1
Characteristics of the patients.

Patient characteristics	HSCT	Non-HSCT	Total
n	26	16	42
Sex; male/female	15/11	7/9	22/20
Age; median (range), year	56.5 (39–72)	62.5 (40–71)	59 (39–72)
Disease			
AML	8	8	16
ALL	2	2	4
CML	1		1
CLL	1	2	3
MM	6		6
Myelofibrosis	1		1
NHL	7	4	11
HSCT			
Autologous	10		
Allogeneic	16		
GVHD	7		

HSCT: Hematopoietic stem cell transplantation; AML: Acute Myeloid Leukemia; ALL: Acute Lymphatic Leukemia; CML: Chronic Myeloid Leukemia; CLL: Chronic Lymphoid Leukemia; MM: Multiple Myeloma; NHL: non-Hodgkin Lymphoma; GVHD: Graft versus host disease. The non-transplanted patients were all admitted for chemotherapy treatment, except for one patient with CLL that was treated with prednisone only. The patients with MM and autologous transplantation were conditioned with high dose Melphalan and the patient with NHL undergoing autologous transplantation received BEAM conditioning. All patients undergoing donor transplantation were treated with Fludarabine and low dose total body irradiation, with or without anti-thymocyte globulin, depending on the Human Leukocyte Antigen mismatch. Only the one patient < 40 years of age was treated with intensive chemotherapy regimen (Busulfan and Cyclophosphamide).

depending on the Human Leukocyte Antigen mismatch), except for one patient with AML who was treated with intensive chemotherapy (Busulfan and Cyclophosfamide).

2.2. Ascorbic acid measurements

Serum AA was indirectly determined by measuring ferrous ion and 2,4,6-tris(2-pyridyl)-s-triazine (Fe^{2+}-TPTZ, Sigma-Aldrich, Zwijndrecht, the Netherlands). This reaction product is formed by nonspecific reduction of the corresponding ferric ion complex (Fe^{3+}-TPTZ) by biological reducing agents such as AA at pH 3.6. AA was specifically quantified by pretreating one of a pair of replicate samples with the enzyme Ascorbate oxidase (Sigma-Aldrich), oxidizing AA to dehydroascorbic acid, then reacting both samples with Fe^{3+}-TPTZ and measuring the difference in absorbances at 600 nm on the Cobas Mira Plus (Roche, Basel, Switzerland). The AA concentration was calculated from a standard addition curve with a 10 μMol/L detection limit.

2.3. Statistical analysis

Data are represented as median with corresponding interquartile range and compared with the Mann–Whitney *U* test; a $p < 0.05$ was considered statistically significant. Determinants of AA serum levels were corrected with multivariable regression analysis. Analyses were performed with Prism (GraphPad Software Inc) and IBM SPSS (SPSS).

3. Results

Healthy volunteers had serum AA levels of 65μMol/L (median, 95%CI 61.56-69.46), while a significant decrease was observed in patients with hematological malignancies who had AA serum levels of 20.5 μMol/L (median, 95%CI 21.27–32.68, Fig. 1A). Eight patients (19% of total patients) had AA serum values < 11.4 μMol

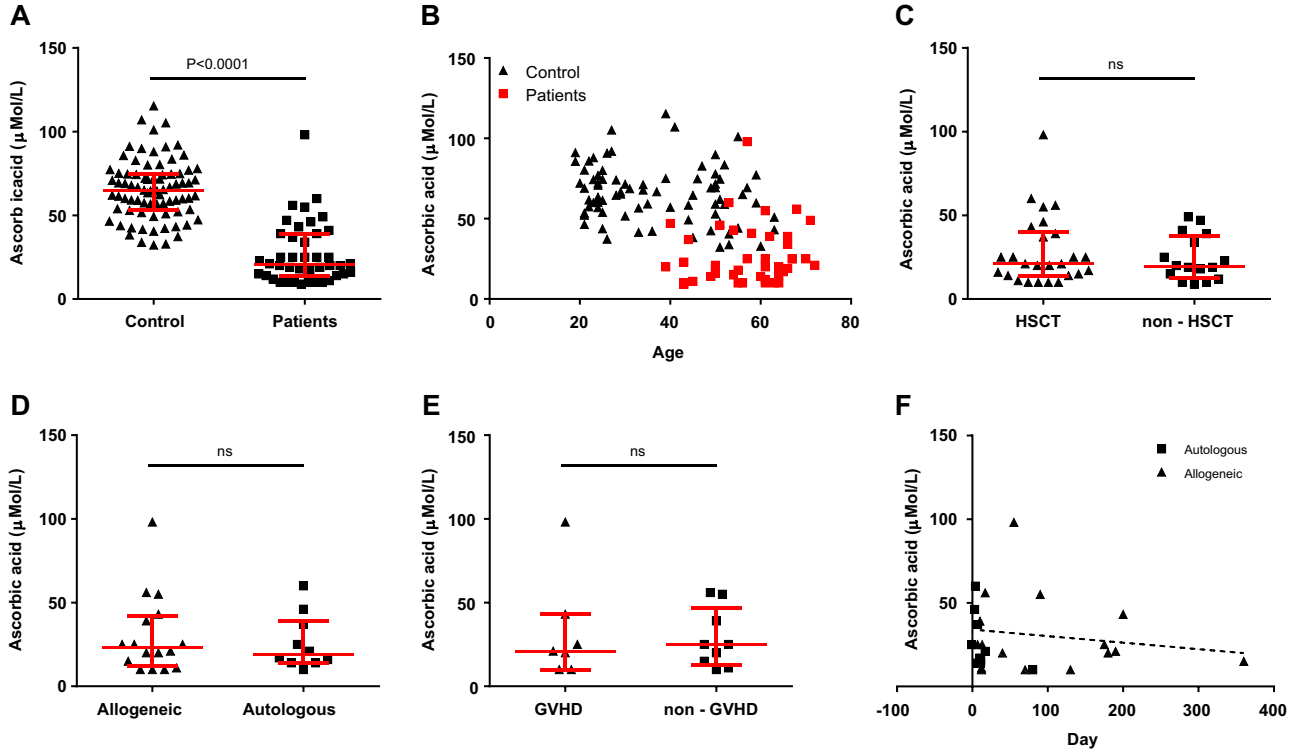

Fig. 1. Serum ascorbic acid levels. A: Serum ascorbic acid values of controls and patients are represented as μMol/L. Data were compared with Mann–Whitney *U* ($p < 0.0001$). For seven patients with undetectable AA levels (< 10 μMol/L), 10 μMol/L was appointed as AA value. B: Age (years) and serum AA values (μMol/L) of controls and patients. The regression coefficient of AA comparing healthy controls to patients with hematological malignancies is −38.5 μMol/L AA (95%CI −45.29 to −31.78). After correction for age and sex, being −34.4 μMol/L (95%CI −43.04 to −25.81) with $p < 0.0001$ comparing controls and patients. C: Serum AA values (μMol/L) of HSCT and non-HSCT patients ($p = 0.63$). D: Serum AA values of allogeneic versus autologous HSCT. Significance was tested with Mann Whitney *U* test and results in $p = 0.83$. E: Serum AA values (μMol/L) of allogeneic HSCT patients suffering from GVHD or not ($p = 0.87$). F: Serum ascorbic acid values of HSCT patients plotted to day of AA measurement after HSCT ($p = 0.58$).

and are considered to be AA deficient [8]. The patient group was significantly older than the control group (mean 57.2 vs. 36.87, respectively). After correction with multivariable regression analysis for age and sex the difference in serum AA was still significant ($p < 0.0001$, Fig. 1B).

Within the patient group (although subgroup sizes are small), patients treated with HSCT or chemotherapy/prednisone did not have significantly different serum AA values, nor was there a difference between patients after autologous transplantation with high dose chemotherapy or allogeneic transplantation with non-myeloablative conditioning for all except one patient (Fig. 1C and D). Furthermore, within the allogeneic HSCT group, no difference was observed in patients suffering from GVHD (Fig. 1E).

Additionally, low serum AA values were not only observed during the acute phase of disease but even up to 360 days after transplantation in a patient admitted because of GVHD (Fig. 1F).

4. Discussion

In this preliminary analysis we demonstrate that patients with a hematological malignancy, either treated with chemotherapy or with autologous or allogeneic HSCT might have highly significant reduced serum AA levels as compared to controls. Recently, it was shown that patients receiving allogeneic HSCT have low serum AA in the acute phase post-transplantation [9]. We show that low AA levels are also present in the chronic phase post-transplantation. Furthermore, our findings are not limited to HSCT patients, but are also convincing for non-transplanted patients.

Human AA levels depend on dietary intake; therefore limited food intake following treatment may explain the observed vitamin C deficiency. Moreover, impaired metabolism is observed in cancer patients [10]. In general patients with any illness might have lower AA levels. Of the 382 AA measurements performed in our hospital between 2010 and 2014, 30 patients (8%) had a low AA level, indicating that not only patients with hematological diseases (19%) might have low vitamin levels (data not shown). This could be related to the disease itself or the stage of the disease of the patients. It seems that cancer patients have lower AA levels than patients suffering from other diseases.

Although AA serum levels in the patients are low, it should be considered that intracellular levels of AA in leukocytes might not be reflected by serum levels since immune cells might accumulate AA [11]. However, serum measurements are the current gold standard and it is accepted that values < 11.4 μMol/L indicate vitamin C deficiency though reference values for normal vitamin C levels are scarce, and might partly depend on the methodology used. No separate reference values are available for our region [8,12]. According to these values, a vitamin C deficiency is present in a substantial proportion of our patient group. Serum AA values of patients with GVHD after allogeneic HSCT – all with the gastrointestinal tract involved – were not further decreased compared to patients without GVHD, with the limitation of small group sizes and therefore with a small power to detect differences.

Since AA might be crucial for immune function and for *in vitro* development and expansion of T and NK cells from stem cells [5,6], we are currently studying the function and recovery of immune cells while patients are on treatment for various malignancies, and determine the correlation of AA serum and leukocyte levels and the possible effect of vitamin C supplementation. Not only in patients with intensive chemotherapy regimens but also with less intensive regimens, where recovery of granulocytes is often a limiting factor for adequate dosing of chemotherapy regimens responsible for substantial morbidity [13,14].

Disclosures

The authors declare no conflict of interest.

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