Study of possible clinical and laboratory predictors of alloimmunization against red blood cell antigens in cancer patients

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Background: *The inflammatory background of patients influences the process of alloimmunization against red blood cell antigens. Proof of this statement to clinical practice is still lacking.*

Objective: *The aim of this study was to verify whether factors related to disease severity and inflammatory status of cancer patients can predict alloimmunization.*

Methods: *This was a case-control study in which alloimmunized oncologic patients treated between 2009 and 2012 were compared with a non-alloimmunized control group regarding the severity of the disease (metastasis/performance status/body mass index) and C-reactive protein levels.*

Results: *The groups did not differ significantly in terms of C-reactive protein, Eastern Cooperative Oncology Group (ECOG)/Karnofsky performance status, presence of metastasis and body mass index.*

Conclusion: *It is not possible to predict alloimmunization in cancer patients based on severity of illness and inflammatory markers. Strategies of screening patients by phenotyping blood based on these criteria are not justified.*

Keywords: *Erythrocyte transfusion; Blood transfusion, autologous; Immune system processes; Erythrocytes/immunology; Isoantibodies/blood; C-reactive protein; Neoplasms*

Introduction

Alloimmunization against red blood cell (RBC) antigens is a late complication of blood transfusions that affects 8% to 12% of all recipients[1]. This percentage increases significantly if patients on chronic transfusion regimens, such as those with sickle cell anemia and myelodysplastic syndrome, are taken into account. In this scenario, approximately 15% of patients develop antibodies against one or more blood group antigen[1-3]. The alloantibodies in recipient's sera are associated with transfusion delays due to the complexity of pre-transfusion tests, difficulty in finding compatible RBC units and late hemolytic transfusion reactions.

A classic stochastic modeling of the RBC alloimmunization process suggested that less than 15% of all blood recipients are prone to developing antibodies after an antigen-mismatched transfusion stimulus (immunologic responders)[4]. The number of transfusions and the presence of an hemoglobinopathy are associated with a higher risk of alloimmunization[5]. However, other clinical factors capable of accurately identifying those individuals before the appearance of the first alloantibody are still lacking. Recent evidence suggests that patients with solid cancer may be at higher risk for developing alloantibodies against RBC antigens[6].

Development of an alloantibody after exposure to an external RBC antigen is influenced by the recipients T cell function[7] and underlying disease status[8]. In cancer patients, poor performance status and a poor quality of life is associated with the patient's systemic inflammatory background and with shorter survival[9]. Moreover, cancer aggressiveness, represented by the presence of metastasis and by undifferentiated histology, is associated with higher levels of inflammatory markers. This leads to worse performance in inflammation-based prognostic scores[10].

Providing phenotyped packed RBCs (comprising mostly of immunogenic antigens) to all solid cancer patients may be a useful strategy to prevent alloimmunization. On the other hand, it has a negative economic impact, since the price paid for a phenotyped red cell pack is higher than that paid for a regular unit. It would be useful to determine whether there are any clinical features capable of predicting alloimmunization in oncologic patients to justify the prescription of phenotyped RBC units.

The aim of this study was to evaluate whether factors related to disease severity (performance status/presence of metastasis/body mass index) and inflammatory background (C-reactive protein - CRP) can predict the risk of RBC alloimmunization in cancer patients.

Methods

All patients known to have become alloimmunized in a tertiary oncology service between 2009 and 2011 (Group 1) were selected for this case-control study. Patients were selected if they developed antibodies against any RBC antigen and if they had at least one negative

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antibody result. Patients with hematologic malignancies were excluded as they present a higher rate of transfusion and different clinical behavior from solid tumors.

Every time an alloimmunized patient was included in the study, two control patients (Group 2) were selected amongst all patients that had been transfused in the hospital on the same day as long as they met the following criteria: 1) negative antibody screen, 2) same number of transfusions as the alloimmunized patient, 3) confirmed diagnosis of solid cancer and 4) same hospital floor or ambulatory as the case. All patients received bed side, leukodepleted RBC units and none received phenotyped units before the development of the first alloantibody.

Groups were compared in terms of the Eastern Cooperative Oncology Group (ECOG) performance status scale, Karnofsky performance status scale, CRP, presence of liver, lung or bone metastasis and body mass index (BMI). The ECOG performance scale ranges from 0 to 5, with 0 denoting perfect execution of daily activities and 5, death. Similarly, the Karnofsky scale ranges from 100 to 0, where 100 represents perfect functional status and 0, death. Antibody screening and identification were performed using Biorad®, Brazil RBC panels. CRP was dosed using an immunoturbidimetric method.

All scale variables were first analyzed in terms of normality using the Kolmogorov-Smirnov test. The student t-test was used for variables with normal distribution, and the Mann-Whitney test for data with non-normal distribution. Categorical variables were compared using the chi-square test. A p-value < 0.05 was considered significant.

Results

Twenty-two alloimmunized patients were allocated to Group 1 and 44 control patients to Group 2. Demographic characteristics of Group 1 and Group 2 are listed in Table 1. The mean age of patients in Group 2 was higher than those in Group 1. Both were homogeneous in respect to the remaining criteria. The number of transfusions was similar for both groups (a mean of 4.1 transfusions per patient). The antibodies identified in the patients of Group 1 are shown in Figure 1. No patient with anti-D was included because all patients were previously sensitized to this antigen.

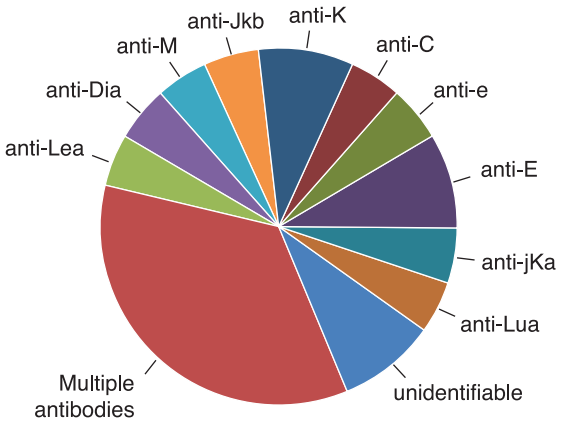

Figure 1 - Antibodies identified amongst alloimmunized cancer patients (Group 1)

Most of our patients developed more than two alloantibodies (36%) and, in 9% of cases, an unidentifiable antibody directed to a possible low-frequency antigen was present.

Table 1 - Demographic characteristics of alloimmunized (Group 1) and non-alloimmunized (Group 2) cancer patients

	Group 1	Group 2	p-value
Number	22	44	
Female/Male	14/8	27/17	0.858
Age (years - mean ± SD)	49.4 ± 13.65	57.9 ± 16.12	0.038
Diagnosis (n)			0.399
Esophagus cancer	1	0	
Stomach cancer	1	5	
Head and neck cancer	1	4	
Lung cancer	0	1	
Breast cancer	2	3	
Prostate cancer	1	1	
Bone cancer	0	1	
Sarcoma	0	1	
Colon cancer	3	11	
Uterus cancer	5	2	
Others	8	15	
Smoking (n)			0.535
Yes	12	19	
No	10	22	
Alcohol consumption (n)			0.689
Yes	4	9	
No	18	31	
Need for Hospitalization (n)			0.705
Yes	16	30	
No	6	14	
Need for ICU (n)			0.848
Yes	6	13	
No	16	31	
Chemotherapy (n)			0.619
Yes	6	14	
No	16	28	

For Group 2 patients, some information is missing regarding smoking (3), alcohol consumption (4) and chemotherapy (2).

Table 2 - Comparison between alloimmunized (Group 1) and non-alloimmunized (Group 2) cancer patients in terms of possible clinical predictors of red blood cell alloimmunization

Variable	Group 1	Group 2	p-value
Mean BMI [kg/m² - mean ± SD (n)]	23.4 ± 5.1 (20)	21.5 ± 4.1 (42)	0.608
CRP [mg/L - mean ± SD (n)]	115 ± 115.3 (11)	157.3 ± 110.6 (24)	0.320
Presence of metastasis			0.741
Yes	11	19	
No	11	18	
Karnofsky score			0.964
≤ 70%	8	15	
>70%	13	25	
ECOG score			0.730
≥ 2	5	8	
< 2	16	32	

BMI: Body mass index; CRP: C-reactive protein; ECOG: Eastern Cooperative Oncology Group performance status scale

No statistically significant differences were found between Groups 1 and 2 in terms of CRP (p-value = 0.32), the ECOG performance status (p-value = 0.73) and Karnofsky performance status (p-value = 0.964). The presence of liver, lung or bone metastasis and the mean BMI also did not differ significantly between the two groups (p-value = 0.741 and p-value = 0.608, respectively). Table 2 summarizes data obtained from both groups.

Discussion

Our results show that neither the severity of the illness (ECOG performance scale, Karnofsky scale, presence of metastasis and BMI) nor inflammatory background (CRP) can predict the risk for RBC alloimmunization in solid cancer patients. To our knowledge, this is the first study to assess alloimmunization phenomena in patients with non-hematologic malignancies.

Much of the current knowledge about alloimmunization is based on experiments performed on murine models. Those experiments demonstrated that the generation of an alloantibody against a RBC antigen after a transfusion stimulus relies on the regulatory function of Treg cells, which is declined in responders[7] and also depends on the way a foreign RBC antigen is presented to T cells by spleen antigen presenting cells (APC)[8,11]. In regards to this last statement, recent evidence suggests that a viral-like inflammation stimulus leads to a higher rate of consumption of transfused RBCs by splenic dendritic cells and, consequently, higher rates of alloimmunization[12].

In humans, observational studies have been performed to search for an association between HLA molecules and risk for alloimmunization. An HLA restriction has been observed for the Fya and Jka antigens, but not for more promiscuous antigens such as K[13]. In respect of a higher inflammatory background as a predisposing factor for alloimmunization, a few case reports of patients with autoimmune diseases have shown that they are prone to alloimmunization[14]. An association was found between the development of RBC alloantibodies and acute febrile reactions during transfusion. The febrile reaction may act as an inflammatory stimulus[15].

Our results do not go against the inflammatory hypothesis presented above, but also do not reinforce it. Our findings were based on a small sample that may not have enough power to detect predictors. As in cancer patients, the aggressiveness of the disease is associated with its inflammatory background[10], it would be plausible to find an association between the presence of metastasis, poor performance status, CRP or BMI and alloimmunization. However, the lack of a clinical predictor between factors related to the severity of cancer and the inflammatory background of the recipient probably highlights that RBC alloimmunization depends on a combination of variables rather than on one isolated feature. Dosing suppressive cytokines (interleukin-10 and transforming growth factor-beta) in recipients may be a better parameter of Treg cell function compared to CRP however those assays are definitely less widely available. Based on the findings of this study, neither the severity of cancer nor higher CRP levels clearly define a group of cancer patients as 'responders'.

Conclusions

The severity of illness and inflammatory background do not predict RBC alloimmunization in oncologic patients. Strategies to provide phenotyped blood to recipients with cancer based on these variables should not be encouraged. Further studies assessing molecular immunological behavior of this specific population may be helpful in understanding and preventing alloimmunization events.

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