Blood donors with positive direct antiglobulin tests are at increased risk for cancer

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BACKGROUND: Positive direct antiglobulin tests (DATs) have been associated with both autoimmunity and lymphoproliferative disorders. However, it is unknown whether DAT+ in healthy blood donors is associated with an increased risk of malignancies.

STUDY DESIGN AND METHODS: In the current study, all blood donors with DAT+ samples were identified during the years 1999 through 2003 through the Magen David Adom National Blood Services in Israel. This study compared the risk of cancer among 586 DAT+ and 2344 DAT– donors who were matched according to sex, age, and year of donation. The risk of cancer in DAT+ donors was also compared to expected rates in the general Israeli population. Cancer was ascertained through the Israel Cancer Registry.

RESULTS: Malignancies occurred among 17 (2.9%) of the DAT+ and 27 (1.2%) of the DAT– blood donors; of these, 3 donors in the DAT+ group were diagnosed with hematopoietic malignancies within 12 months of their donation. Even after excluding these early cases, the relative risk of developing cancer was 2.14 (95% confidence interval [CI], 1.13-4.10) comparing DAT+ with DAT– donors, while the relative risk for hematopoietic cancer was 8.3 (95% CI, 1.5-43.2). Comparing DAT+ blood donors with the general population, the standardized incidence ratios (observed/expected cases) were elevated at 2.11 (95% CI, 1.15-3.54; $p = 0.16$) for all malignancies and 8.03 (95% CI, 2.2-20.6; $p = 0.003$) for hematologic malignancies.

CONCLUSION: There is evidence of a significantly increased risk of cancer, especially hematologic malignancies, among blood donors with a positive DAT even within a short follow-up period.

A positive direct antiglobulin test (DAT) is observed due to coating of red blood cells (RBCs) in vivo by antibodies of different immunoglobulin isotypes or complement.[1,2] The proportion of positive DATs among blood donors ranges from 1:1000 to 1:36,000[3-8] and is higher in older donors than in younger ones.[6] Several risk factors have been associated with a positive DAT result, including cardiolipin antibodies,[8,9] acquired immunodeficiency syndrome,[10] and drugs.[7,11,12] In addition, elevated serum immunoglobulin G (IgG) levels and nonreactive eluates have been found in 33 percent of patients with a positive DAT. The higher the elevated serum IgG, the more frequently a DAT+ result is observed.[5,7] An increased prevalence of gammopathies is found in lymphoma, hairy cell leukemia, gastric cancer, ovarian cancer, lung cancer, chondrosarcoma, and hepatocellular carcinoma, as well as liver disease and connective tissue diseases.[13] Furthermore, a positive DAT is frequently encountered in patients with multiple myeloma.[14] The reaction is due to passive absorption of the monoclonal protein onto the RBCs and this usually does not produce hemolysis.[14]

It is not known whether the risk of malignancy is higher among healthy individuals with a positive DAT. One study reported that no cancers developed over a follow-up period of 14 years among 32 blood donors who were DAT+.[15] However, this observation focused on a small

ABBREVIATION: SIR(s) = standardized incidence ratio(s).

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group without a control group. To evaluate whether a positive DAT result among healthy blood donors is associated with an increased risk of cancer we conducted a historical prospective study based on a large number of blood donors at the National Blood Services in Israel.

MATERIALS AND METHODS

Data were obtained from Magen David Adom National Blood Services in Israel. All blood donations at the National Blood Services undergo autocontrol testing using automated blood grouping equipment (Autogrouper [Siemens Medical Solutions Diagnostics, Tarrytown, NY], 1999-2000; and Olympus PK7200 [Olympus, Melville, NY], 2000-present), as part of the routine blood type determination of the donated unit. All the autocontrol-positive donations and autocontrol-negative blood units that were subsequently detected as DAT+ at peripheral hospital blood banks were further tested for DAT using the tube method (AABB *Technical Manual,* 15th ed., pp. 760-61) and/or gel cards (DiaMed, Cressier sur Morat, Switzerland) with anti-IgG, anti-C3, and anti-IgG/C3.

DAT+ was defined as a reaction strength of +2 or greater in any single donation or repetitive DAT+ in more than a single donation regardless of the strength of the reaction. A notification letter is sent to DAT+ donors, referring them to their family physician for further medical counseling and follow-up. We identified consecutive DAT+ donors during 1999 through 2003 and frequency matched them with DAT– donors in a 1:4 ratio for sex, year of donation, and age (by 5-year categories).

To determine cancer incidence, the two groups were linked via their unique identity number assigned to all Israeli residents to the Israel Cancer Registry. The Registry receives compulsory notification from numerous data sources, including pathology reports, discharge summaries, and death certificates. Completeness of the registry was found to be about 95 percent for solid tumors.[16] Demographic data were retrieved from the Central Population Registry. Persons who had been diagnosed with cancer before the blood donation were excluded. Cancer incidence was ascertained through July 30, 2006.

We used two methods to determine whether DAT+ donors were at increased risk of developing cancer. First, we compared observed rates of cancer, with expected rates in the general Israeli population, after controlling for the age and sex of cohort members. The number of expected cancer cases in each cohort was calculated using multiplication of person-years under observation by the sex- and age-specific incidence. Standardized incidence ratios (SIRs) were calculated by dividing the number of observed over expected cases to compare cancer incidence in the DAT+ group as well as donors in the DAT– group with that of the general population. Confidence intervals (CIs) for the SIRs were calculated using the Poisson distribution.

We then directly compared the risk of cancer among DAT+ and DAT– donors by performing an age-adjusted Cox regression analysis and country of birth, taking into account the time from donation to the time of cancer diagnosis. Follow-up time was from the blood donation date at which the DAT test was recorded until the date of diagnosis, death, or July 30, 2006. For the initial analyses we used all cancer cases diagnosed after the blood donation. Since some cancers may be undiagnosed but present at the time of the donation we performed a further analysis using a 1-year latency period, excluding all cancers that were diagnosed within 1 year of the donation. In this analysis, age-adjusted Cox regression models were constructed measuring follow-up time starting 1 year after the donation instead of the donation date. For analysis of the study population's characteristics, continuous variables were compared by t test and categorical variables were compared with Fisher's exact test. A p value of less than 0.05 was considered significant. Computer software (SPSS, Version 14, SPSS, Inc., Chicago, IL) was used for statistical analysis. Strict confidentiality was maintained. The study was approved by the legal department of Magen David Adom Blood Services and requirement for individual informed consent was waived by the Hadassah institutional review board.

RESULTS

A total of 586 blood donors with a positive DAT were identified between the years 1999 and 2003 and frequency matched to 2344 donors who were DAT–. In both groups 62.6 percent were males. The mean age was 34.5 years among the DAT+ population (range, 17-67 years) and 32.0 years among DAT– donors (range, 18-71 years). More than three-quarters of the donors were born in Israel (77.2%), followed by 12.0 percent in Europe, 4.4 percent in America, 4.1 percent in Africa, and 2.1 percent in Asia (Table 1). The mean age among the donors who were born in Israel was lower (30.4 years vs. 39.4 years in the other groups; $p < 0.001$).

After a mean follow-up of 66.0 months (range, 14-91 months), 17 malignancies were diagnosed among DAT+ and 27 cases among the DAT– blood donors The most common malignancies in the DAT+ group were lymphomas (4 cases), followed by multiple myeloma (3 cases), accounting for 41.1 percent of all the malignancies in this group. Three patients among the DAT+ group developed cancer within 12 months of the donation (Hodgkin's lymphoma within 2 months, non-Hodgkin's lymphoma within 4 months, and multiple myeloma within 8 months). Among donors with a negative DAT, breast cancer was the most common cancer (5 cases; 3 patients had Stage I, 1 patient had Stage II, and the stage of the last

patient was unknown), while lymphomas accounted for only 7.2 percent (2 cases) of all the malignancies in this group. No cases of multiple myeloma were ascertained among the DAT– group. The incidence of gastrointestinal malignancies and prostate cancer was similar in both groups (Table 2). Among DAT– donors none developed cancer within 12 months of their blood donation.

In the analysis including all incident cancer cases, the hazard ratio comparing DAT+ to DAT– donors was 2.06 (95% CI, 1.10-3.83; p = 0.02) after adjusting for age and place of birth (Table 3). Persons who were born in Africa had a lower risk of developing cancer after controlling for age and DAT compared to those born in Israel (hazard ratio, 0.30; 95% CI, 0.10-0.89; p = 0.03). Comparing the risk of cancer in DAT+ donors to that of the general population revealed a significantly elevated SIR for all malignancies of 2.54 (95% CI, 1.48-4.07; p = 0.001).

All further analyses were performed using a 1-year latency, that is, excluding cases that occurred within 12 months of the donation. An elevated SIR of 2.28 (95% CI, 1.24-3.82; p = 0.009) among DAT+ donors, compared to expected rates in the general population was observed for cancer at all sites (Table 4) and a remarkably elevated SIR of 11.30 (95% CI, 3.80-28.93; p = 0.001) was observed for hematologic malignancies. On the other hand, among DAT– donors a non-significant elevation SIR for developing cancer at all sites of 1.50 (95% CI, 0.99-2.18; p = 0.05) and 1.51 (95% CI, 0.97-2.23; p = 0.06) for solid tumors (Table 2) was observed.

TABLE 1. Baseline characteristics of DAT+ donors compared to DAT– donors

Characteristic	DAT+ (n = 583)	DAT– (n = 2344)	p Value
Females	218 (37.4%)	876 (37.4%)	0.99
Age (years)			
Range	17-67	17-74	
Mean	32.0 ± 12.4	34.5 ± 13.3	<0.001
Country of birth Israel*			
Donor	420 (72.2%)	1842 (78.6%)	0.001
Donor's mother	177 (30.4%)	913 (39.0%)	<0.001
Donor's father	156 (26.7%)	793 (33.9%)	0.001

* Compared to born elsewhere.

TABLE 2. Distribution of cancer sites among DAT+ donors compared to DAT– donors

	DAT+ blood donors (n = 17)		DAT– blood donors (n = 27)		
Cancer sites	Male/female	Number (%)	Male/female	Number (%)	p Value
Lymphoma	1/1	2 (14.3%)	1/1	2 (7.4%)	0.132
Multiple myeloma	2/0	2 (14.3%)		0	0.005
Gastrointestinal	1/1	2 (14.3%)	4/2	6 (22.2%)	0.717
Thyroid gland	1/1	2 (14.3%)		0	0.005
Prostate gland	2/0	2 (14.3%)	4/0	4 (14.8%)	0.410
Bladder		0	2/0	2 (7.4%)	0.481
Breast		0	1/4	5 (18.5%)	0.265
Skin		0	3/1	4 (14.8%)	0.319
Others	3/1	4 (23.5%)	1/3	4 (14.8%)	0.033

TABLE 3. Cox regression analysis comparing hazards of cancer in DAT+ and DAT– donors, adjusted for age and country of birth

Model	Hazard ratio: DAT+ compared to DAT– donors (reference group)	95% CI	p Value
1. All incident cancers			
a. All sites	2.06	1.10-3.83	0.02
b. Hematopoietic malignancies	10.06	2.65-61.98	0.002
c. Solid tumors	1.25	0.59-2.67	0.55
2. Excluding cases diagnosed within 1 year after donation			
a. All sites	1.70	0.87-3.29	0.11
b. Hematopoietic malignancies	7.17	1.29-39.76	0.02
c. Solid tumors	1.31	0.61-2.80	0.49

TABLE 4. Observed and expected number of cancer cases and SIRs compared to general population

	DAT+				DAT–			
Variable	Observed	Expected	SIR	p Value	Observed	Expected	SIR	p Value
All sites	17	6.68	2.54 (1.48-4.07)	0.001	27	17.94	1.50 (0.99-2.18)	0.055
Hematologic malignancies	7	0.53	13.21 (5.30-27.22)	0.001	2	1.43	1.39 (0.16-5.05)	0.83
Solid tumors	10	5.78	1.73 (0.83-3.18)	0.13	25	16.51	1.51 (0.97-2.23)	0.06

After excluding malignancies diagnosed within 1 year of follow-up, a hazard ratio for all cancers of 1.70 (95% CI, 0.87 to 3.29; p = 0.11) and an elevated hazard ratio for hematologic malignancies of 7.17 (95% CI, 1.29 to 39.76; p = 0.02) were found (Table 3). There was no difference in the age at cancer diagnosis between the two groups (52.1 among the DAT+ compared to 49.3 among the donors with DAT–; p = 0.587) or gender of the cancer cases (71.4% females compared to 59.3%; p = 0.44, respectively). The median time until diagnosis, after excluding cases occurring in the first 12 months was shorter among DAT+ donors for all cancers (25.5 months compared to 47.0 months; p = 0.004) for solid tumors (25.5 months compared to 49.0 months; p = 0.004), but not for hematologic malignancies (29.0 months compared to 34.5 months; p = 0.96).

DISCUSSION

To the best of our knowledge, this is the first cohort study examining the risk of cancer among healthy blood donors with a DAT+. The findings support an increased incidence of hematologic malignancies in the follow-up of DAT+ donors. The opposite finding, that is, a high prevalence of DAT+ in patients with mainly hematological malignancies has been reported by Dalal and colleagues[14] among others. The observed increased risk of cancer in this group may be due to undiagnosed prevalent cancers, implying that DAT positivity may represent a risk marker rather than a risk factor. This is especially plausible given the relatively high number of hematologic malignancies diagnosed within 1 year of the DAT+ donation. However, even after exclusion of these donors, the hazard ratio for hematologic malignancies was significantly elevated in the DAT+ group compared to donors with DAT–.

Although DAT positivity was associated with hematologic malignancies[14] and not with solid tumors, a shorter duration between the donation and diagnosis of solid tumor was actually shown among the DAT+ donors (25.5 months compared to 49.0 months; p = 0.004) indicating that a DAT positivity may also be associated with solid tumors. A possible mechanism by which solid tumors could present with positive DATs includes an increased secretion of IgG and IgM within tumor cells as has been demonstrated in gastric carcinomas.[17] However, no correlation has been noted between immunoglobulin secretion in tumor cells and serum immunoglobulin values.[17]

The pathophysiology of an elevated cancer risk among DAT+ donors is uncertain. However, hypergammaglobulinemia is a common cause of DAT+,[1,7,10] and patients with polyclonal gammopathy or monoclonal gammopathy are at increased risk for cancer: After a median follow-up of 67 months for patients with polyclonal gammopathy, hematologic disorders developed in 5 percent and nonhematologic malignancies in 3 percent in a study from the Mayo Clinic.[13] Another possible explanation for increased risk of lymphoproliferative disorders in donors with positive DATs may be the effect of chronic antigenic stimulation on the immune system, leading to the eventual development of a clonal B-cell disorder.

Just as the presence of autoantibodies is frequently detected years before the onset of clinical autoimmune disease,[18] our study suggests that a DAT+ may predate the clinical detection of cancer by months to years. It is well known that monoclonal gammopathy of undetermined significance is associated with and predates the clinical onset of malignancies, mainly multiple myeloma, and less often other lymphoproliferative disorders.[19] Furthermore, sensitive polymerase chain reaction methods have detected the translocation t(14;18)(q32;q21) the hallmark of follicular lymphoma, in approximately 50 percent of circulating B cells in healthy blood donors.[20] These cells are long-lived due to the constitutive expression of the BCL-2 protein. The long-term consequence in donors carrying BCL-2 translocations is unknown. Malignant transformation may be mediated by several mechanisms, such as additional cytogenetic and molecular changes on the one hand and antigen stimulation and anti-idiotypic antibodies on the other hand.[20] Furthermore, monoclonal B-cell populations have also been observed in a small proportion of blood donors,[21] although their relationship with DAT has not been described. The clinical significance of autoantibodies among patients with hematologic malignancies is not clear.[22] IgG3 is the most meaningful autoantibody.[8] Detecting IgG3 on RBCs of a patient without hemolytic anemia is not common and may indicate reticuloendothelial dysfunction.[8] Low-grade and disseminated disease is more common among patients with non-Hodgkin's lymphoma and positive autoantibodies, and their presence may be associated with a shorter median survival in lymphomas.[22]

An interesting finding in our study was the borderline significantly increased SIR for solid tumors and cancer at all sites among DAT– donors. Breast cancer was the most common cancer in this group (five cases) with the majority presenting with early stage disease. This finding may reflect health behaviors such as increased use of screening mammography among blood donors, compared to the general population, leading to increased early detection of such cancers, or may be a chance finding.

The current study has several strengths. Since the majority of healthy blood donors are young (in Israel 75% of them are between the ages of 17 and 40 years) and cancer incidence in this age is very low, a large sample size is needed to establish a significant difference in the relative risk for cancer between DAT+ and DAT– donors. Using a large database of donors and follow-up of 66 months allowed the current study to reveal a significant elevation in the relative risk between the two groups. Despite the fact that completeness of the registry is lower for

hematologic malignancies than solid tumors, this bias does not reduce the validity of the results. This underregistration would decrease the power of study and, if anything, bias toward the null hypothesis, whereas in spite of this limitation, a significant association was detected between donors with DAT+ and hematopoietic cancer. In addition, the present cohort design is not associated with recall bias, which is common in case-control designs, and ascertainment bias was eliminated by linking the study populations with the Israel Cancer Registry, a data source with high validity and nationwide coverage.[16] Although the follow-up of this study is short, the current database can be used for subsequent analyses to confirm the current results.

In conclusion, an elevated risk of cancer, particularly hematologic malignancies, was demonstrated in healthy blood donors with DAT+. The results of our study support the current practice of notifying healthy blood donors of a positive test, as has been suggested by others,[8] and raises the question of informing them or their physicians regarding the possible presence of undiagnosed autoimmune disease or occult malignancies, especially lymphoproliferative disorders. Further studies are necessary to confirm this association, to determine the factors that influence the cancer risk in this group, and to assess the impact of notification on disease detection in these healthy populations.

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