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Regular whole blood donation and gastrointestinal, breast, colorectal and haematological cancer risk among blood donors in Australia

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Abstract

Background and Objectives: Several studies have suggested that blood donors have lower risk of gastrointestinal and breast cancers, whereas some have indicated an increased risk of haematological cancers. We examined these associations by appropriately adjusting the 'healthy donor effect' (HDE).

Materials and Methods: We examined the risk of gastrointestinal/colorectal, breast and haematological cancers in regular high-frequency whole blood (WB) donors using the Sax Institute's 45 and Up Study data linked with blood donation and other health-related data. We calculated 5-year cancer risks, risk differences and risk ratios. To mitigate HDE, we used 5-year qualification period to select the exposure groups, and applied statistical adjustments using inverse probability weighting, along with other advanced doubly robust g-methods.

Results: We identified 2867 (42.4%) as regular high-frequency and 3888 (57.6%) as low-frequency donors. The inverse probability weighted 5-year risk difference between high and low-frequency donors for gastrointestinal/colorectal cancer was 0.2% (95% CI, −0.1% to 0.5%) with a risk ratio of 1.25 (0.83–1.68). For breast cancer, the risk difference was −0.2% (−0.9% to 0.4%), with a risk ratio of 0.87 (0.48–1.26). Regarding haematological cancers, the risk difference was 0.0% (−0.3% to 0.5%) with a risk ratio of 0.97 (0.55–1.40). Our doubly robust estimators targeted minimum loss-based estimator (TMLE) and sequentially doubly robust (SDR) estimator, yielded similar results, but none of the findings were statistically significant.

Conclusion: After applying methods to mitigate the HDE, we did not find any statistically significant differences in the risk of gastrointestinal/colorectal, breast and haematological cancers between regular high-frequency and low-frequency WB donors.

Keywords
blood donor, cancer, HDE, healthy donor effect, malignancy, whole blood

Highlights
- We used the 'qualification period' method along with advanced statistical methods, such as inverse probability weighting, and doubly robust g-methods with ensemble machine learning algorithms, to mitigate the impact of the 'healthy donor effect'.
- We found that regular high-frequency whole blood donation does not significantly alter the cancer risk.
- Studies with relevant data on ongoing health of donors are required to produce unbiased results when examining the effect of blood donation on long-term health outcomes.

INTRODUCTION

Studies have suggested that the level of iron in the human body may affect the occurrence of cancers [1–7]. Due to loss of iron from the body after each whole blood (WB) donation, it has been hypothesised that frequent WB blood donors may have different risk of cancers compared to less-frequent donors or non-donors [6]. Studies have also indicated that temporary immune system alterations such as lowering of the level and activity of natural killer cells and enhanced cell proliferation after each blood donation, could affect the risk of haematological cancers [8, 9]. In relation to the level of iron in the body, it has been observed that in iron overload diseases like hereditary hemochromatosis there is increased risk of hepatocellular carcinoma, particularly in patients with liver cirrhosis [10], and potentially other type of cancers such as colorectal cancer [5, 11].

Studies conducted in blood donors have reported contraindicatory findings in relation to the risk of cancers. Several studies have reported that the risk of cancers is lower or not different in donors compared to general population or less-frequent donors [6, 12–15]. However, some have also reported a higher incidence of overall cancers or some particular cancers among blood donors compared to the general population [6, 13, 14, 16].

The results from many of the above studies may have been impacted by a bias called the 'healthy donor effect (HDE)'. This bias arises when healthier people self-select to donate blood. Further health screening by blood collection agencies to ensure that donors are eligible to give blood compounds this effect. Comparison of this relatively healthier group without adequate adjustments for health differences from the non-donor population (or with low-frequency donors) usually suggests that blood donors have a lower risk of almost any health outcome measured [17].

In this study, we examined the possible association between regular high-frequency WB donation and the risk of gastrointestinal/colorectal, breast and haematological cancers among blood donors in Australia. To mitigate the HDE, we utilized a 5-year qualification period method, similar to the 'qualification period' method described by Peffer et al. and applied several statistical adjustments in the analyses [18]. The 'qualification period' refers to the time period during which the donor must be actively donating blood and must fulfil other qualifying criteria. This method identifies active donors (enabling the within donor comparison) within a defined time period and also separates the exposure period and follow-up period, which further reduces the reverse causation bias as the exposure and outcome cannot influence each other.

METHODS

Data sources and linkage

In this study, we used the Sax Institute's 45 and Up Study data, linked to other electronic health datasets—the Australian Red Cross Lifeblood Donor data, Registry of Birth, Deaths and Marriages-Deaths Registrations (RBDM), New South Wales Cancer Registry (NSWCR) and Medicare Benefit Schedule (MBS) data.

The Sax Institute's 45 and Up Study enrolled 267,357 individuals aged 45 years or above in New South Wales, Australia, between 2005 and 2009 [19]. The study recruited prospective participants through random selection from the Services Australia Medicare enrolment database, which includes all Australian and New Zealand citizens and Australian permanent residents, resulting in a participation rate of 19.2% [20]. People aged 80 years and above and people living in rural and remote areas were oversampled [19]. Participants completed an initial questionnaire that covered a wide range of topics, including socio-demographic information, health status, lifestyle choice and behaviours. Additionally, they provided consent for their data to be linked with various administrative datasets, allowing for long-term follow-up analysis.

Australian Red Cross Lifeblood is the sole agency responsible for collecting, processing and distributing blood and blood products in

Australia. It also keeps track of donor data in a central system called the National Blood Management System (NBMS). Before 2007, the methods used by Lifeblood to store donor data varied. However, after a national merger in 2007 of what was to that time separate, state-based sets of donor data, all donor information was consolidated within the NBMS. However, for New South Wales (NSW) complete records for blood donations were available from 1 June 2002. Therefore, for the purpose of data linkage, the dataset used included blood donation information spanning from 1 June 2002, to 31 December 2018.

The NSWCR keeps track of individuals diagnosed with cancer in NSW. Since 1972, the NSWCR has maintained comprehensive records that include demographic information, incidence data and death details for individuals who have been diagnosed with cancer. In our study, we used this dataset to ascertain the occurrence and date of cancer diagnosis. The data were complete up to December 2015.

The details of other datasets and the linkage process is presented in the Supporting Information: Data S1 and also described elsewhere [21].

Study population, qualification period

We employed a 5-year qualification period to select the participants and determine exposure status inspired by the method used by Peffer and colleagues [22] (Figure 1). The qualification period refers to the time in which the donor is needed to actively give blood while satisfying other requirements for eligibility to donate. In our analysis, this qualification period includes the time period 3 years before the enrolment into the 45 and Up Study data and 2 years thereafter. For our analysis, donors must have made at least one WB donation on the first and fifth years of the qualification period and be alive and cancer-free for the full 5-year period. The qualification period method can also be described as 'exposure window' method, as described and used by Edgren et al.; however, the qualification period method implemented in this study includes specific qualification criteria that ensure donors are active donors during the time period of exposure assessment as well as are free of the study outcome being measured [6]. We excluded donors who performed any plasma or platelet donation during the 5-year window to keep only WB donors for the analysis. Donors who had cancers before the start of qualification period were also excluded.

Exposure variable

We considered several exposure scenarios to measure the frequency and regularity of blood donations made by participants during each year of qualification period (i) at least one WB donation during each year of qualification period versus others, (ii) at least two WB donation during each year of qualification period versus others and (iii) at least three WB donation during each year of qualification period versus others.

Ascertainment of WB donation

Utilizing linked Lifeblood donation history data, instances of a WB donations were determined. If a person successfully donated a unit of WB, the individual was regarded as a WB donor.

Ascertainment of cancer

The primary outcomes of this study were gastrointestinal, colorectal, breast and haematological cancers. All the cancer information was ascertained from the linked NSWCR dataset. By using the international classification of disease 10th revision (ICD10) codes, an individual was confirmed to have experienced either gastrointestinal or colorectal cancer if the cancer diagnosis codes were C15 (oesophageal) or C16 (stomach) or C17 (small intestinal) or C22 (liver) or C23-C24 (gallbladder) or C25 (pancreatic) or C18 (colon) or C19-C21 (rectal). Moreover, an individual was confirmed to have experienced breast cancer if the diagnosis code was C50 (Breast). Furthermore, an individual was confirmed to have experienced haematological malignancy if the diagnosis codes were C920 (acute myeloid leukaemia) or C910 (acute lymphoblastic leukaemia) or C81 (Hodgkin lymphoma) or C8890 (multiple myeloma) or C82 (non-Hodgkin lymphoma) or C919 (other lymphoid leukaemia) or C929 (other myeloid leukaemia) or C94 (other specified leukaemia) (Table 1). We only considered the first diagnosed cancer for this analysis if a person had multiple malignancies over the follow-up period.

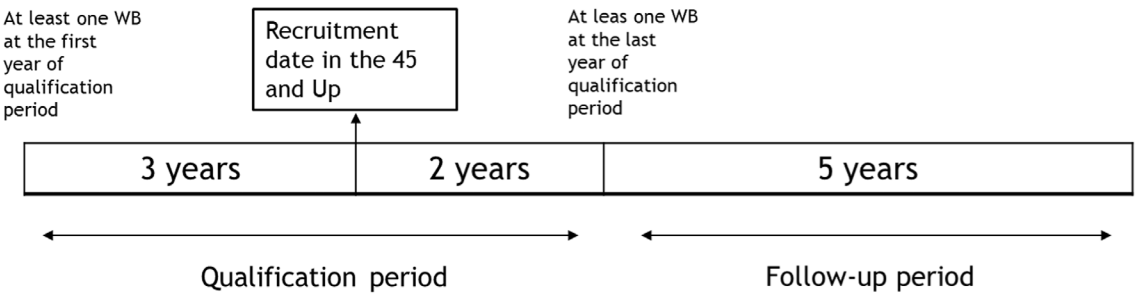

FIGURE 1 The 5-year qualification period and follow-up period.

TABLE 1 International classification of diseases 10th revision (ICD10) codes used to ascertain cancer cases.

Cancer group	ICD10 codes
Gastrointestinal or colorectal cancer	C15 (oesophageal) C16 (stomach) C17 (small intestinal) C22 (liver) C23-C24 (gallbladder) C25 (pancreatic) C18 (colon) C19–C21 (rectal)
Brest cancer	C50 (Breast)
Haematological malignancies	C920 (acute myeloid leukaemia) C910 (acute lymphoblastic leukaemia) C81 (Hodgkin lymphoma) C8890 (multiple myeloma) C82 (non-Hodgkin lymphoma) C919 (other lymphoid leukaemia) C929 (other myeloid leukaemia) C94 (other specified leukaemia)

Follow-up period

The follow-up period commenced from the last day of the qualification period and ended at the conclusion of either 5 years from the start of the follow-up, the death date or the cancer diagnosis date, whichever occurred first. This end date of follow-up was chosen to enable us to study the 5-year risk of cancer. For sensitivity analyses, we also considered an administrative end date of the follow-up, so that the study started from the last day of the qualification period and ended on 30 December 2015 (corresponding to available cancer registry data), death date or cancer diagnosis death, whichever occurred first.

Potential confounding factors

A number of demographic/socioeconomic, health status and blood donation-related variables were considered as potential confounding factors. The demographic/socioeconomic variables were age, sex (male, female), geographical location (metro, regional/remote), education (no formal education, school to diploma, university) and gross annual household income (<20 k, 20–39 k, 40–69 k, 70 k+ Australian dollars). The health status-related variables were body mass index (body mass index [BMI]—underweight, normal, overweight, obese), self-reported general health (excellent, very good, good, fair/poor), smoking status (never, former, regular), daily alcohol intake (≤1/day, >1/day), weekly physical activity (<1, ≥1), daily fruit or raw vegetable consumption (0–2, 3–4, 5+), intake of multivitamins and minerals (no, yes), consumption of red meat (<5/week, ≥5/week), consumption of processed meat (<3/week, ≥3/week), number of general practice (GP) visits in the last 1 year, number of specialist consultations and pathology test referrals in the last 1 year, family history of cancers (no, yes) and any cancer screening (no, yes). Blood donation-related variables were average blood pressure levels during the qualification period, average haemoglobin level during the qualification period and blood group. The detailed derivation of the variables is given in Table S1.

Ethics approval

The 45 and Up Study received approval from the Human Research Ethics Committee (HREC) at the University of NSW. Additionally, this specific study was approved by the NSW Population Health Services Research Ethics Committee and Lifeblood Ethics Committee.

Statistical methods

We calculated 5-year cancer risk, risk difference and risk ratio (RR) by inverse probability weighting (IPW) of a marginal structural model for gastrointestinal and colorectal cancers together and for breast and haematological cancers separately. We fitted a pooled logistic regression model by adding a constant plus linear and quadratic terms of time and also linear and quadratic product terms of donation status and time. The baseline covariates were adjusted by calculating the inverse probability weights and then using the weights in the outcome regression model. The IPW was truncated at the 99th percentile to remove any extreme weights from outliers. Finally, we used non-parametric bootstrapping with 500 samples to calculate all the 95% CIs. Inverse probability weighted Kaplan–Meier survival curves were also plotted for the cancer outcomes with three different exposure definitions.

We also utilized two alternative g-methods, namely the targeted minimum loss-based estimator (TMLE) and the sequentially doubly robust (SDR) estimators, to compute 5-year cancer risk, risk difference and RRs [23, 24]. These estimators, including IPW, rely on two mathematical models: the treatment model and the outcome model, both of which are functions of the confounding variables. The IPW is a singly robust estimator, as its accuracy depends on correctly specifying the treatment model. On the other hand, TMLE and SDR are doubly robust estimators, meaning that their estimates remain unbiased even if one of the treatment or outcome models is misspecified.

Additionally, the inverse probability weighted marginal structural models can produce a biased estimate if affected by violations of the positivity assumption. In contrast, doubly robust estimators often produce less biased results than IPW estimators, even if the positivity assumption is extremely violated [25, 26]. Moreover, these doubly robust estimators have the advantage of being able to utilize machine learning algorithms to fit the treatment and outcome models, allowing them to capture complex associations that may not be possible with simple regression-based approaches [24, 27]. As blood donation behaviour is assumed to be time-varying in nature, we also estimated time-varying TMLE and SDR estimators in one of the sensitivity analyses. We used the R package 'SuperLearner' version 2.0–29 and 'lmtp' version 1.4.0 to implement this analysis [28].

A few variables had missing values (maximum of approximately 16%). Although we assumed that the data were missing at random, we still did multiple imputations to calculate missing values, as

TABLE 2 Characteristics of the study participants.

Characteristics	At least 2 whole blood donations in each year of the qualification period	
	No (low frequency)	Yes (regular high frequency)
Participants, *n* (%)	3888 (57.6)	2867 (42.4)
Sex, *n* (%)		
Male	1717 (44.2)	1585 (55.3)
Female	2171 (55.8)	1282 (44.7)
Age at baseline, mean (SD)	57.72 (6.68)	60.3 (6.9)
Haemoglobin, g/dL, mean (SD)	140.99 (10.36)	143.38 (9.86)
Systolic blood pressure, mean (SD)	127.39 (12.05)	128.66 (11.17)
Diastolic blood pressure, mean (SD)	76.95 (6.84)	77.26 (6.25)
Total no. of WB donation in qualification period, mean(SD)	9.78 (3.56)	16.85 (2.65)
Blood group, *n* (%)		
Non-O	1976 (50.8)	1401 (48.9)
O	1912 (49.2)	1466 (51.1)
Body mass index, kg/m^2, *n* (%)		
Underweight	10 (0.3)	8 (0.3)
Normal	1306 (33.6)	897 (31.3)
Overweight	1527 (39.3)	1231 (42.9)
Obese	793 (20.4)	577 (20.1)
Missing	252 (6.5)	154 (5.4)
Body mass index, kg/m^2, mean (SD)	26.92 (4.35)	27.08 (4.21)
Smoking status, *n* (%)		
Never	2435 (62.6)	1884 (64.3)
Former	1282 (33.0)	921 (32.1)
Regular	157 (4.0)	90 (3.1)
Missing	14 (0.4)	12 (0.4)
Self-rated health, *n* (%)		
Excellent	1040 (26.8)	850 (29.7)
Very good	1791 (46.1)	1361 (47.5)
Good	854 (22.0)	564 (19.7)
Fair/poor	130 (3.3)	57 (2.0)
Missing	73 (1.9)	35 (1.2)
Alcohol consumption/day, *n* (%)		
None	877 (22.6)	600 (20.9)
≤1/day	1521 (39.1)	1100 (38.4)
>1/day	1461 (37.6)	1148 (40.0)
Missing	29 (0.8)	19 (0.7)
Vigorous physical activity in the last week, *n* (%)		
<1	1415 (36.4)	964 (33.6)
1–3	1331 (34.2)	967 (33.7)
4+	660 (17.0)	603 (21.1)
Missing	482 (12.4)	333 (11.6)
Education level, *n* (%)		
No formal education	215 (5.5)	175 (6.1)
School to Diploma	2432 (62.6)	1927 (67.2)
University	1213 (31.2)	747 (26.1)
Missing	28 (0.7)	18 (0.6)

(Continues)

TABLE 2 (Continued)

Characteristics	At least 2 whole blood donations in each year of the qualification period	
	No (low frequency)	Yes (regular high frequency)
Annual household income, *n* (%)		
<20 k	313 (8.1)	257 (9.0)
20–39 k	521 (13.4)	503 (17.5)
40–69 k	954 (25.5)	762 (26.6)
70 k+	1484 (38.2)	901 (31.4)
Missing	616 (15.8)	444 (15.5)
Location, *n* (%)		
Major city	1909 (49.1)	1161 (40.5)
Regional/Remote	1888 (48.6)	1646 (57.4)
Missing	91 (2.3)	60 (2.1)
Daily fruits/vegetable consumed, *n* (%)		
0–2	229 (5.9)	160 (5.6)
3–4	928 (23.9)	688 (24.0)
5+	2259 (58.1)	1685 (58.8)
Missing	472 (12.1)	334 (11.7)
Taking any vitamin or mineral supplement, *n* (%)		
No	2975 (76.5)	2236 (78.0)
Yes	912 (23.5)	631 (22.0)
Missing	<5 (<0.0)	<5 (<0.0)
Consumption of red meat, *n* (%)		
<5/week	2954 (76.0)	2134 (74.4)
≥5/week	865 (22.3)	697 (24.3)
Missing	68 (1.8)	36 (1.3)
Consumption of processed meat, *n* (%)		
<3/week	2869 (73.8)	2097 (73.1)
≥3/week	577 (14.8)	459 (16.0)
Missing	442 (11.4)	311 (10.9)
Family history of cancer, *n* (%)		
No	2058 (52.9)	1517 (52.9)
Yes	1830 (47.1)	1350 (47.1)
Cancer screening, *n* (%)		
No	421 (10.8)	301 (10.5)
Yes	3428 (88.2)	2545 (88.8)
Missing	39 (1.0)	21 (0.7)
No. of GP visits in the past 1 year, mean (SD)	4.68 (4.15)	4.15 (3.41)
No. of referrals in the past 1 year, mean (SD)	2.84 (2.69)	2.51 (2.35)
Outcomes		
Gastrointestinal/colorectal, *n* (%)	25 (0.6)	27 (0.9)
Breast[a], *n* (%)	40 (1.8)	21 (1.6)
Haematological, *n* (%)	23 (0.6)	20 (0.7)

Abbreviation: GP, general practice.
[a]Breast cancer cases are calculated only from female donors.

removing participants with missing values would lower the number of cases for analysis. The imputation was a fully conditional specification that used classification and regression trees and was implemented by the R package 'mice' version 3.16.0 (used method = 'cart' in the mice function) [29].

We used R version 4.2.2 to conduct all the statistical analyses.

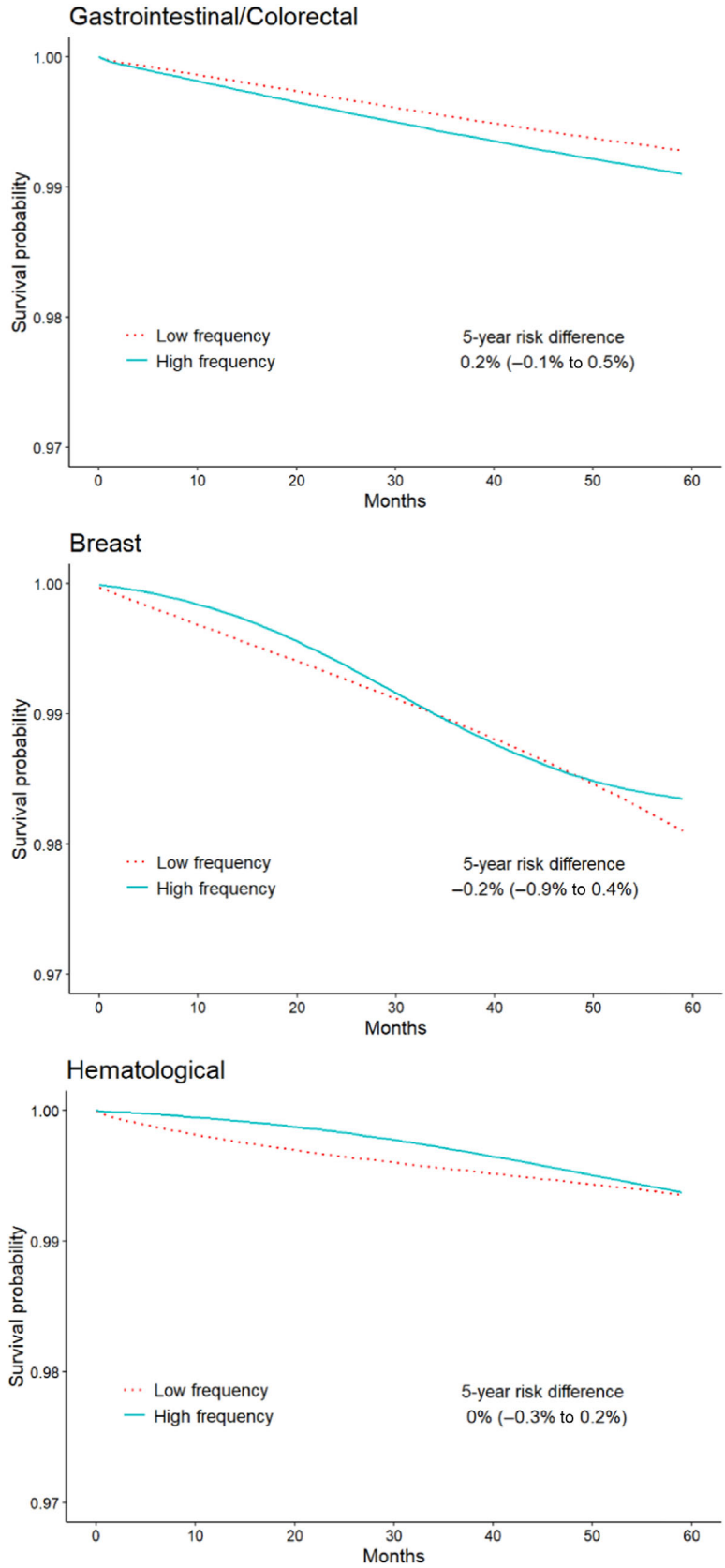

FIGURE 2 Weighted survival curves for a 5-year follow-up period for gastrointestinal/colorectal, breast and haematological cancers.

RESULTS

Table 2 shows the distribution of various characteristics of 6755 WB donors, of whom 2667 (42.4%) donated at least two WB units in each year of the qualification period (regular high-frequency donors), whereas 3888 (57.6%) donated less than two WB donations in any of the qualification year. Regular high-frequency donors were mostly male (55.3%) and also slightly older (average age 60.3 years) than low-frequency donors. Among the donors, 25/3888 (0.6%) from the low-frequency blood donor group were diagnosed with gastrointestinal/colorectal cancer during 5 years of follow-up, whereas 27/2867 (0.9%) were diagnosed with gastrointestinal/colorectal cancer in the high-frequency donor group. Among 3453 female donors, 40 (1.8%) breast cancer cases were identified from the low-frequency donor group and 21 (1.6%) from the high-frequency donor group during the 5-year follow-up period. For haematological cancer, we found 23 (0.6%) incident cases from the low-frequency donor group and 20 (0.7%) from the high-frequency donor group during the 5-year follow-up period. The detailed information about the variables in Table 2 can be found in the Table S1. Figure 2 shows no significant risk differences between low and high-frequency donors in the inverse probability weighted Kaplan Meyer survival curves for gastrointestinal/colorectal, breast and haematological cancers over a 5-year follow-up..

Table 3 presents the estimated 5-year cancer risk for gastrointestinal/colorectal, breast and haematological cancer, their risks, risk differences and RRs calculated by IPW, TMLE and SDR estimators. The IPW risk of gastrointestinal/colorectal cancer was 0.9% (95% confidence interval [CI], 0.6%–1.2%) for high-frequency donors and 0.7% (95% CI, 0.5%–0.9%) for low-frequency donors which resulted in the risk difference of 0.2% (95% CI, −0.1% to 0.5%) and RR of 1.25 (95% CI, 0.83–1.68). We found almost identical results from TMLE; the risk for high-frequency donors was 0.9% (95% CI, 0.7%–1.1%) and the risk for low-frequency donors was 0.7% (95% CI, 0.5–0.9), which resulted in risk difference of 0.2% (95% CI, −0.1% to 0.5%) and RR of 1.25 (95% CI, 0.86–1.81). The SDR estimator produced almost similar results (Table 3) to IPW and TMLE. The IPW risk of breast cancer was 1.6% (1.1%, 2.2%) for high-frequency donors and 1.9% (95% CI, 1.5%–2.3%) for low-frequency donors, which resulted in the risk difference of −0.2% (−0.9% to 0.4%) and the RR of 0.87 (0.48–1.26). Moreover, the IPW risk of haematological cancer was 0.6% (95% CI, 0.4%–0.8%) for high-frequency donors and 0.6% (95% CI, 0.5%–0.8%) for low-frequency donors, which produced a risk difference of 0.0% (95% CI, −0.3% to 0.2%) and RR of 0.97 (95% CI, 0.55–1.40). The TMLE produced almost similar results; risk of 0.6% (95% CI, 0.5%–0.8%) for high-frequency donors, risk of 0.6% (95% CI, 0.5%–0.8%) for low-frequency donors and risk difference of 0.0% (95% CI, −0.3% to 0.2%) and RR of 0.96 (0.66–1.40). The SDR estimator produced similar results to IPW and TMLE, except the RR was slightly higher than both estimators (RR, 1.01 [95% CI, 0.71–1.43]). None of the results for both gastrointestinal/colorectal and haematological cancer were statistically significant, indicating no increased/decreased risk of gastrointestinal/colorectal and haematological cancers among blood donors.

Sensitivity analysis

We found similar results to our main analysis when we ended the follow-up on 31 December 2015 instead of a fixed 5-year follow-up for each participant. The IPW RR for this analysis was 1.27 (95% CI, 0.74–1.80) for gastrointestinal/colorectal cancer, 0.99 (95% CI, 0.59–1.39) for breast cancer and 0.92 (95% CI, 0.53–1.30) for haematological cancer. For different exposure definitions, we also found similar

TABLE 3 Estimated 5-year cancer risk, risk difference and risk ratios for high- and low-frequency donors.

Outcomes	Models	Risk, % (95% CI)		Risk difference, % (95% CI)	Risk ratio (95% CI)
		Low frequency	High frequency		
Gastrointestinal/colorectal[a]	IPW	0.7 (0.5 to 0.9)	0.9 (0.6 to 1.2)	0.2 (−0.1 to 0.5)	1.25 (0.83 to 1.68)
	TMLE	0.7 (0.5 to 0.9)	0.9 (0.7 to 1.1)	0.2 (−0.1 to 0.5)	1.25 (0.86 to 1.81)
	SDR	0.8 (0.6 to 0.9)	1.0 (0.7 to 1.2)	0.2 (−0.1 to 0.5)	1.27 (0.89 to 1.80)
Breast[b]	IPW	1.9 (1.5 to 2.3)	1.6 (1.1 to 2.2)	−0.2 (−0.9 to 0.4)	0.87 (0.48 to 1.26)
	TMLE	1.9 (1.5 to 2.3)	1.7 (1.4 to 2.0)	−0.2 (−0.7 to 0.3)	0.89 (0.67 to 1.19)
	SDR	2.0 (1.6 to 2.4)	1.7 (1.4 to 2.0)	−0.3 (−0.8 to 0.3)	0.86 (0.65 to 1.14)
Haematological[a]	IPW	0.6 (0.5 to 0.8)	0.6 (0.4 to 0.8)	0.0 (−0.3 to 0.2)	0.97 (0.55 to 1.40)
	TMLE	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.8)	0.0 (−0.3 to 0.2)	0.96 (0.66 to 1.40)
	SDR	0.7 (0.5 to 0.9)	0.7 (0.6 to 0.9)	0.0 (−0.2 to 0.3)	1.01 (0.71 to 1.43)

Abbreviations: CI, confidence interval; IPW, inverse probability weighting; SDR, sequentially doubly robust; TMLE, targeted minimum loss-based estimator.
[a]Adjusted for sex, age, haemoglobin, systolic blood pressure, diastolic blood pressure, blood group, body mass index (BMI), smoking status, self-rated health, alcohol consumption, education, annual income, physical activity, daily consumption of fruits and vegetables, vitamin/mineral intake, red meat consumption, processed meat consumption, family history of cancer, cancer screening, location, no. of general practice (GP) visits in the past 1 year, no. of referrals in the past 1 year.
[b]Adjusted for all the variables in a except for sex.

results, except for breast cancer risk, where RR (0.5 [95% CI, 0.03–0.96]) was significantly lower for high-frequency donors when considered at least three donations per every qualification year vs other donation categories. For all other sensitivity analyses, we did not find any statistically significant association. The detail results and description of the sensitivity analyses can be found in Tables S2–S4.

DISCUSSION

In this study, we examined the association between regular high-frequency WB donation and the risk of gastrointestinal/colorectal, breast and haematological malignancies among Australian blood donors. We did not find a statistically significant relationship between regular high-frequency WB donations and risk of developing the various cancer outcomes studied.

We used the 5-year qualification period technique to ascertain the exposure (high-frequency donor) and control (low-frequency donor) groups, which is comparable to the qualification period method used by Peffer et al. [18]. It is likely that the HDE has a substantial impact on the studies that only used the lifetime number of donations to determine exposure status. Thus, Peffer et al., in their study, only included active donors and separated the exposure period from the follow-up period in their analysis, which can significantly reduce the HDE [18]. Similar to Peffer et al. our 5-year qualification period method likely has a comparable effect on lowering the HDE. In addition, we had access to several other health-related variables to adjust for the effect of HDE in our analysis. Although Peffer et al. have used a three-category exposure variable based on the tertiles of donations made during the 10-year qualification period and we have categorized the exposure variable that was based on the frequency and consistency of the donation pattern, these differences are likely to have only a minor impact while comparing the studies.

Several studies have examined the incidence of cancer among blood donors. Many of these studies have reported a lower risk of cancer occurrence and mortality among blood donors [13, 14, 30]. In a Scandinavian study, researchers utilized a nested case–control design to investigate the impact of iron depletion through blood donation on Swedish and Danish donors [6]. The study found a trend towards a reduced risk of liver, lung, colon, stomach and oesophageal cancers in males with a latency period of 3 to 7 years, comparing the lowest to highest estimated iron loss from donations. Nevertheless, the authors acknowledged their inability to account for several important confounding factors, such as smoking, alcohol consumption, nutrition, physical activity, anthropometric measures and occupational exposures, which might have influenced the observed results [6]. Another study from the United States reported there is no difference in the risk of colorectal cancer in regular male blood donors compared to non-donors [12]. Although they did not use any established method to reduce the impact of HDE, our findings of gastrointestinal and colon cancer are consistent with their findings.

Although none of our findings were statistically significant, our point estimates for gastrointestinal/colorectal cancer in the main analysis were slightly higher than the null value (IPW RR, 1.25 [95% CI, 0.83–1.68]). Increased cancer risk in high-frequency donors has been reported in prior studies, but none of them could conclusively report the association as causal [6, 15]. In one of our sensitivity analysis, we defined the high-frequency exposure group with at least one and three donations each year of the qualification period which ruled out the possibility of an increased risk that could not be detected by our sample. In addition to that, time-varying TMLE and SDR estimators also found almost zero risk differences among high and low-frequency donors. Moreover, because of blood donors' continuous screening during their donation career and comparatively higher health consciousness, it is not uncommon to have more cancer detection among frequent blood donors compared to casual donors [15, 16].

Our study has several strengths. First, the use of a qualification period method decreased the HDE by comparing cancer outcomes among active donor populations with a continuous donation career and presumably less variance in health status. Second, our data linkage allowed us to adjust for a variety of potential confounding variables, something that was lacking in the majority of previous studies. In addition, we utilized doubly robust statistical models, such as TMLE and SDR, which incorporated machine learning algorithms to determine the risk estimates. As the findings of our IPW model and our doubly robust models are nearly identical, our treatment and outcome models are less likely to have been misspecified.

Our study also has limitations. The majority of participants were older adults. As a result, the findings of this study may not be generalized to all blood donors. However, the representativeness of the 45 and Up Study (~19% response rate) is unlikely to be of importance as our study examined the relative risks [20, 31]. Due to the fact that our donation records are only available on or after June 2002, we were unable to analyse the duration since the first donation or the cumulative impact of the entire donation history. Moreover, compared to some previous studies, our sample size is somewhat small, and our follow-up period is also shorter (a maximum of 5 years), resulting in a smaller number of events. This may cause lower statistical power to detect clinically important small effect sizes. Because of the smaller number of events, we also did not conduct a sex-stratified analysis, which may be relevant for iron-induced outcomes. However, given the majority of the female participant in the study are older and likely reached menopause, the differences by sex should be minimal.

In conclusion, we did not find any convincing evidence of an altered risk of gastrointestinal/colorectal, breast and haematological malignancy among high-frequency WB donors donating regularly. Further exploration is needed with a longer follow-up time to better understand the relationship between these cancer outcomes and regular high-frequency WB donation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Available from the Sax Institute upon request but subject to approvals.

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REFERENCES

1. Huang X. Iron overload and its association with cancer risk in humans: evidence for iron as a carcinogenic metal. Mutat Res. 2003;533:153–71.
2. Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch Biochem Biophys. 1986;246:501–14.
3. Selby JV, Friedman GD. Epidemiologic evidence of an association between body iron stores and risk of cancer. Int J Cancer. 1988;41:677–82.
4. Knekt P, Reunanen A, Takkunen H, Aromaa A, Heliövaara M, Hakuunen T. Body iron stores and risk of cancer. Int J Cancer. 1994;56:379–82.
5. Nelson RL. Iron and colorectal cancer risk: human studies. Nutr Rev. 2001;59:140–8.
6. Edgren G, Reilly M, Hjalgrim H, Tran TN, Rostgaard K, Adami J, et al. Donation frequency, iron loss, and risk of cancer among blood donors. J Natl Cancer Inst. 2008;100:572–9.
7. Wen CP, Lee JH, Tai Y-P, Wen C, Wu SB, Tsai MK, et al. High serum iron is associated with increased cancer risk. Cancer Res. 2014;74:6589–97.
8. Marquet RL, Hoynck van Papendrecht MA, Busch OR, Jeekel J. Blood donation leads to a decrease in natural killer cell activity: a study in normal blood donors and cancer patients. Transfusion. 1993;33:368–73.
9. Ishii K, Birmann BM, Zhang X, Giovannucci E, Bertrand KA. A prospective analysis of blood donation history and risk of non-Hodgkin lymphoma. Leuk Lymphoma. 2016;57:1423–8.
10. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology. 2004;127:S79–86.
11. Agudo A, Bonet C, Sala N, Munoz X, Aranda N, Fonseca-Nunes A, et al. Hemochromatosis (HFE) gene mutations and risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Carcinogenesis. 2013;34:1244–50.
12. Zhang X, Ma J, Wu K, Chan AT, Fuchs CS, Giovannucci EL. Blood donation and colorectal cancer incidence and mortality in men. PLoS One. 2012;7:e39319.
13. Merk K, Mattsson B, Mattsson A, Holm G, Gullbring B, Björkholm M. The incidence of cancer among blood donors. Int J Epidemiol. 1990;19:505–9.
14. Edgren G, Tran TN, Hjalgrim H, Rostgaard K, Shanwell A, Titlestad K, et al. Improving health profile of blood donors as a consequence of transfusion safety efforts. Transfusion. 2007;47:2017–24.
15. Zhao J, Dahlén T, Brynolf A, Edgren G. Risk of hematological malignancy in blood donors: a nationwide cohort study. Transfusion. 2020;60:2591–6.
16. Vahidnia F, Hirschler NV, Agapova M, Chinn A, Busch MP, Custer B. Cancer incidence and mortality in a cohort of US blood donors: a 20-year study. J Cancer Epidemiol. 2013;2013:814842.
17. Atsma F, Veldhuizen I, Verbeek A, de Kort W, de Vegt F. Healthy donor effect: its magnitude in health research among blood donors. Transfusion. 2011;51:1820–8.
18. Peffer K, den Heijer M, de Kort WL, Verbeek AL, Atsma F. Cardiovascular risk in 159 934 frequent blood donors while addressing the healthy donor effect. Heart. 2019;105:1260–5.
19. 45 and Up Study Collaborators, Banks E, Redman S, Jorm L, Armstrong B, Bauman A, et al. Cohort profile: the 45 and up study. Int J Epidemiol. 2008;37:941–7.
20. Bleicher K, Summerhayes R, Baynes S, Swarbrick M, Navin Cristina T, Luc H, et al. Cohort profile update: the 45 and up study. Int J Epidemiol. 2023;52:e92–e101.
21. Karki S, Bell KJL, Hayen A, Liu B, Cust AE, Olynyk JK, et al. Regular high-frequency whole blood donation and risk of cardiovascular disease in middle-aged and older blood donors in Australia. Transfusion. 2023;63:1012–22.
22. Peffer K. Blood donation and cardiovascular disease. Addressing the healthy donor effect. (Doctoral dissertation, Radboud University Nijmegen) 2015. https://hdl.handle.net/2066/149279
23. Luedtke AR, Sofrygin O, van der Laan MJ, Carone M. Sequential double robustness in right-censored longitudinal models. *arXiv*. 2017. https://doi.org/10.48550/arXiv.1705.02459
24. van der Laan MJ, Rose S. Targeted learning in data science: causal inference for complex longitudinal studies. Cham, Switzerland: Springer; 2018.
25. Brooks JC, van der Laan MJ, Singer DE, Go AS. Targeted minimum loss-based estimation of causal effects in right-censored survival data with time-dependent covariates: warfarin, stroke, and death in atrial fibrillation. J Causal Inference. 2013;1:235–54.
26. Tran L, Yiannoutsos C, Wools-Kaloustian K, Siika A, Van Der Laan M, Petersen M. Double robust efficient estimators of longitudinal treatment effects: comparative performance in simulations and a case study. Int J Biostat. 2019;15:20170054.
27. Chernozhukov V, Chetverikov D, Demirer M, Duflo E, Hansen C, Newey W, et al. Double/debiased machine learning for treatment and structural parameters. Oxford: Oxford University Press; 2018.
28. Hoffman KL, Salazar-Barreto D, Rudolph KE, Díaz I. Introducing longitudinal modified treatment policies: a unified framework for

studying complex exposures. *arXiv*. 2023. https://doi.org/10.48550/arXiv.2304.09460
29. Van Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45:1–67.
30. Lasek W, Jakobisiak M, Stoklosa T. Decreased natural killer cell activity in whole-blood donors does not seem to result in increased cancer incidence. Transfusion. 1994;34:359–60.
31. Mealing NM, Banks E, Jorm LR, Steel DG, Clements MS, Rogers KD. Investigation of relative risk estimates from studies of the same population with contrasting response rates and designs. BMC Med Res Methodol. 2010;10:1–12.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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