To the editor:

Lack of association between blood donor age and survival of transfused patients

Senthil K. Vasan,[1] Flaminia Chiesa,[1] Klaus Rostgaard,[2] Patrik K. E. Magnusson,[1] Märit Halmin,[1] Kaspar René Nielsen,[3] Kjell Einar Titlestad,[4] Henrik Hjalgrim,[2,5] and Gustaf Edgren[1,6]

[1]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [2]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark; [3]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark; [4]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark; [5]Department of Hematology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; and [6]Hematology Centre, Karolinska University Hospital, Stockholm, Sweden

The possible rejuvenating effects of transfusions from young donors to older patients have generated considerable interest recently, following publication of a study showing improvements in muscle regeneration when older mice were transfused with blood from younger mice.[1] Subsequent studies where the circulatory systems of young and old mice have been connected have provided evidence of beneficial effects of blood from young mice on several age-related functions such as cognitive decline,[2] hepatocyte proliferation,[1] reversal of cardiac hypertrophy,[3] and improved remodeling of aged bone.[4] More recently, the plasma protein growth differentiation factor 11 (GDF-11), which is found in plasma of younger mice, has been implicated in driving some of the beneficial effects of transfusions from young donors.[5,6]

To date, there are no studies investigating the rejuvenating effects of transfusions from young donors in humans. A number of possible therapeutic implications, particularly the functional restoration of age-related damaged tissues, have been theorized and, consequently, 2 trials (www.clinicaltrials.gov, #NCT01561053 and #NCT02256306) have been initiated to study whether Alzheimer disease patients experience cognitive improvements when transfused with plasma from healthy younger donors.[7]

To test the hypothesis that transfusion of blood from young donors improves survival of patients, we performed a cohort study with data on blood donors and transfused patients extracted from the Scandinavian Donations and Transfusions database (SCANDAT2), which includes information on blood donors, donations, transfusions, and patients from Sweden and Denmark dating back to the 1960s and 1980s, respectively.[8] SCANDAT2 was linked with nationwide population and health data registers using unique national registration numbers assigned to all individuals in both countries, thus allowing for unbiased long-term follow-up for a range of health outcomes including death, hospital care, and cancer.[9,10]

We established 2 separate but partly overlapping patient cohorts transfused between 1995 and 2012, 1 of plasma recipients and 1 of red cell recipients. Patients in the plasma cohort were thus allowed to receive red cell units and vice versa. Because platelet components are often composed of units pooled from 4 to 6 donations where contributing donors would often be of different age, we did not include a platelet cohort. For analytical simplicity, the assessment of transfusion exposure was restricted to the first 7 days of transfusions for each person, referred to as a "transfusion episode." Previously transfused patients and recipients of autologous transfusions were excluded. In both cohorts, patients were grouped according to the ages of all of their contributing donor(s). We established 3 groups with patients who had exclusively received products from donors younger than 25 years, donors aged 25 to 50 years, or donors older than 50 years. Patients who received blood from donors of >1 age category were excluded from the analyses. Analyses were conducted as a matched cohort study. We first identified all patients who exclusively received blood from donors younger than 25 years. For these index patients, 3 control patients were then selected from each of the other 2 exposure groups, matched to have received the exact same number of transfusions at the same hospital at approximately the same time (±1 year).

Mantel-Haenszel tests were used to assess differences between the groups for categorical variables and conditional linear regression for continuous variables.[11] Patients were followed from the last transfusion in the transfusion episode until the first of death, emigration, December 31, 2012, or end of follow-up (30 days or 1 year after the start of the transfusion episode). Hazard ratios (HRs) for death were estimated using a stratified Cox model accounting for the matched sets. Analyses were further adjusted for sex, age, and calendar period. Ninety-five percent confidence intervals (95% CIs) were constructed using robust standard errors. A priori subanalyses were done, restricted to patients with a prior diagnosis of dementia, cardiovascular disease, or cerebrovascular disease. We also performed sensitivity analyses with donor age categorized as <20, 20 to 50, or >50 years old.

All data processing and statistical analyses were done using SAS statistical analysis software (version 9.4; SAS Institute, Inc, Cary, NC). P values < .05 were considered statistically significant. The study was approved by relevant authorities in both countries.

The current analysis included 45 664 plasma and 136 639 red blood cell transfusion recipients. There were only very small differences in baseline characteristics between the exposure groups (Table 1).

The 30-day and 1-year relative risks of death from any cause in relation to donor age are presented in Table 2. Donor age was not associated with overall 30-day and 1-year mortality following plasma or red cell transfusion. Similar results were seen in patients with known dementia or cardiovascular or cerebrovascular disease. The only exceptions to the overall inconspicuous pattern were a lower 30-day mortality for patients with known prior history of cerebrovascular disease who received plasma transfusions from donors older than 50 years (HR, 0.76; 95% CI, 0.59-0.97), and a contrastingly higher 1-year mortality among patients who received plasma units from older donors over 50 years of age (HR, 1.05; 95% CI, 1.01-1.09). Sensitivity analyses with a more extreme categorization of donor age (ie, as <20, 20-50, or >50 years old) yielded almost identical results, albeit with poorer statistical precision (data not

Table 1. Characteristics of the study population

	Donor age category			
	<25 y	25-50 y	51-70 y	*P*
Plasma cohort				
No. of patients (% of total)	5 334 (11.7)	20 723 (45.4)	19 607 (42.9)	n/a
Female sex, N (%)	2 404 (45.1)	9 773 (47.2)	9 151 (46.7)	.01
Median no. of transfusions (range)	1 (1-6)	1 (1-6)	1 (1-5)	1.00*
Patient mean age (SD), y	59.9 (25.4)	59.5 (25.5)	59.1 (25.7)	.06*
Country				
Denmark	1 374 (25.8)	5 329 (25.7)	5 020 (25.6)	1.00
Sweden	3 960 (74.2)	15 394 (74.3)	14 587 (74.4)	
Indication for transfusion				
Trauma	732 (13.7)	2 764 (13.3)	2 587 (13.2)	.32
Obstetric	149 (2.8)	586 (2.8)	575 (2.9)	
Cardiac/vascular surgery	1 028 (19.3)	4 085 (19.7)	3 959 (20.2)	
Malignancy surgery	559 (10.5)	2 187 (10.6)	2 064 (10.5)	
Other surgery	1 171 (22.0)	4 580 (22.1)	4 341 (22.1)	
Hematologic disease	344 (6.4)	1 331 (6.4)	1 258 (6.4)	
Other malignant disease	226 (4.2)	877 (4.2)	816 (4.2)	
All other indications	1 125 (21.1)	4 313 (20.8)	4 007 (20.4)	
Red cell cohort				
No. of patients (% of total)	19 828 (14.5)	58 978 (43.2)	57 833 (42.3)	n/a
Female sex, N (%)	11 365 (57.3)	34 007 (57.7)	33 141 (57.3)	.66
Median no. of transfusions (range)	1 (1-5)	1 (1-5)	1 (1-5)	1.00*
Patient mean age (SD), y	66.7 (19.6)	64.0 (24.8)	62.7 (25.7)	<.0001*
Country				
Denmark	7 022 (35.4)	20 894 (35.4)	20 526 (35.5)	1.00
Sweden	12 806 (64.6)	38 084 (64.6)	37 307 (64.5)	
Indication for transfusion				
Trauma	3 548 (17.9)	10 327 (17.5)	9 663 (16.7)	<.001
Obstetric	472 (2.4)	1 631 (2.8)	1 751 (3.0)	
Cardiac/vascular surgery	2 545 (12.8)	7 456 (12.6)	7 229 (12.5)	
Malignancy surgery	1 765 (8.9)	5 117 (8.7)	5 119 (8.9)	
Other surgery	4 434 (22.4)	12 945 (21.9)	12 846 (22.2)	
Hematologic disease	1 390 (7.0)	4 186 (7.1)	4 288 (7.4)	
Other malignant disease	1 233 (6.2)	3 664 (6.2)	3 717 (6.4)	
All other indications	4 441 (22.4)	13 652 (23.1)	13 220 (22.9)	

n/a, not applicable.
**P* values obtained using conditional linear model.

shown). No interaction between donor age and number of transfusions was observed, indicating that donor age did not affect patient survival even among those who received higher numbers of transfusions. Thus, our overall interpretation is that both 30-day and 1-year mortality is independent of blood donor age for both plasma and red cell transfusion recipients.

The key strength of our investigation, in addition to the large sample size and reliable transfusion and follow-up data, is an effect of the administrative principles for allocation of blood units. Because blood donor age is not considered when a unit is selected for a particular patient, the allocation of blood units from donors of different age groups should thus effectively be randomly distributed among patients transfused in the same circumstances, leaving little scope for confounding.

Although systemic factors in the blood of young mice have been shown to have a rejuvenating effect in older mice on a range of organs including neurons, vascular endothelium, cardiomyocytes, and muscle,[3,5,12] we find no plausible evidence of such effects on life span, either overall or in patients with known dementia, cardiovascular disease, or prior cerebrovascular events who might benefit more from rejuvenating effects. That said, our results may not be directly comparable to data from mouse models. First, in experimental parabiotic models, a continuous circulatory circuit is typically maintained for several weeks because it takes at least 1 to 2 weeks for vascular connections to develop and any biologic effect to be observed.[13] Because of the observational design of our study, we were forced to restrict the analyses to 1 transfusion episode and it is thus possible that we missed clinically relevant effects which could occur in chronically transfused patients. Similarly, as we selected patients who had only received blood units from donors of the same age and matched the 3 exposure groups to have received the exact same number of transfusions, the included patients at most only received modest numbers of transfusions. It is possible that there may be beneficial effects of transfusions from young donors among patients who receive larger numbers of transfusions, which we acknowledge as a limitation. Second, there may have been some degree of degradation of the possibly rejuvenating factors during manufacture and storage of blood products. Third, although we classified young donors as being younger than 25 years, which should correspond to mice aged 3 to 4 months as in previous experiments,[14] differences in the life span and metabolic capacity in the 2 species may render the results biologically incomparable.[15]

To conclude, this first epidemiological study of rejuvenating effects in humans does not support the notion that donor age has positive effects on prolonging life span among transfused patients.

Table 2. Short- and long-term mortality in the plasma and red cell cohorts, in relation to age of contributing blood donors

	Recipients of blood from donors aged <25 y			Recipients of blood from donors aged 25-50 y			Recipients of blood from donors aged >50 y		
	No. of patients	No. of deaths (%)	HR (95% CI)	No. of patients	No. of deaths (%)	HR (95% CI)	No. of patients	No. of deaths (%)	HR (95% CI)
Plasma recipients									
30-d mortality									
All transfused patients	5 334	707 (13.3)	0.99 (0.91-1.07)	20 273	2 722 (13.4)	1.00 (ref)	19 607	2 575 (13.1)	1.01 (0.96-1.07)
Patients with known dementia	109	27 (24.8)	1.22 (0.71-2.09)	178	41 (23.0)	1.00 (ref)	87	24 (27.6)	1.05 (0.61-1.80)
Patients with known cardiovascular disease	540	118 (21.9)	1.14 (0.93-1.41)	1 378	240 (17.4)	1.00 (ref)	1 075	172 (16.0)	0.93 (0.77-1.12)
Patients with known cerebrovascular disease	393	80 (20.4)	0.99 (0.78-1.27)	947	184 (19.4)	1.00 (ref)	590	95 (16.1)	0.76 (0.59-0.97)
1-y mortality									
All transfused patients	5 334	1458 (27.3)	1.01 (0.96-1.07)	20 273	5 500 (27.1)	1.00 (ref)	19 607	5 370 (27.4)	1.05 (1.01-1.09)
Patients with known dementia	109	52 (47.7)	0.97 (0.67-1.40)	178	88 (49.4)	1.00 (ref)	87	48 (55.2)	0.95 (0.65-1.38)
Patients with known cardiovascular disease	540	199 (36.9)	1.02 (0.94-1.29)	1 378	426 (30.9)	1.00 (ref)	1 075	324 (30.1)	0.92 (0.79-1.06)
Patients with known cerebrovascular disease	393	149 (37.9)	1.05 (0.87-1.26)	947	344 (36.3)	1.00 (ref)	590	209 (35.4)	0.90 (0.76-1.07)
Red cell recipients									
30-d mortality									
All transfused patients	19 828	1927 (9.7)	1.02 (0.97-1.08)	58 978	5 471 (9.3)	1.00 (ref)	59 336	4 994 (8.4)	1.00 (0.97-1.04)
Patients with known dementia	858	150 (17.5)	0.95 (0.79-1.13)	2 229	397 (17.8)	1.00 (ref)	2 482	404 (16.3)	1.06 (0.83-1.34)
Patients with known cardiovascular disease	1 793	297 (16.6)	1.02 (0.90-1.15)	6 748	1 085 (16.1)	1.00 (ref)	7 078	1 011 (14.3)	1.03 (0.88-1.21)
Patients with known cerebrovascular disease	1 296	211 (16.3)	1.05 (0.90-1.21)	4 673	711 (15.2)	1.00 (ref)	3 473	537 (15.5)	1.01 (0.91-1.13)
1-y mortality									
All transfused patients	19 828	4885 (24.6)	1.00 (0.97-1.03)	58 978	14 283 (24.2)	1.00 (ref)	57 830	13 709 (23.7)	1.00 (0.97-1.02)
Patients with known dementia	858	385 (44.9)	0.90 (0.81-1.01)	2 229	1 043 (46.8)	1.00 (ref)	1 622	732 (45.1)	0.93 (0.85-1.03)
Patients with known cardiovascular disease	1 793	602 (33.6)	1.01 (0.93-1.10)	6 748	2 176 (32.2)	1.00 (ref)	5 630	1 753 (31.1)	0.99 (0.94-1.06)
Patients with known cerebrovascular disease	1 296	480 (37.0)	0.99 (0.90-1.09)	4 673	1 713 (36.7)	1.00 (ref)	3 473	1 297 (37.3)	1.00 (0.93-1.07)

Estimates adjusted for age, sex, calendar period, number of transfusions, country, and hospital.
ref, reference.

However, the therapeutic implications of transfusions from young donors for the reversal of age-related effects remain scientifically intriguing, and results from the first trials in humans are eagerly awaited.

Acknowledgments: The authors thank Prof Jonas Frisén, Karolinska Institutet, for insightful comments on an earlier version of this manuscript. The authors are greatly indebted to all of the blood banks in Sweden and Denmark for both collecting and contributing data to this study.

The SCANDAT2 database assembly was funded by the Swedish Research Council (2011-30405, 2007-7469), the Swedish Heart-Lung Foundation (20090710), the Swedish Society for Medical Research (G.E.), and the Danish Council for Independent Research (09-066021) (H.H.).

Contribution: All authors were involved in data acquisition; S.K.V., F.C., H.H., and G.E. designed the study, analyzed the data, and drafted the manuscript; K.R. provided statistical expertise; and all authors critically revised the manuscript and approved the final version of the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Gustaf Edgren, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; e-mail: gustaf.edgren@ki.se.

References

1. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature.* 2005;433(7027):760-764.
2. Villeda SA, Plambeck KE, Middeldorp J, et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat Med.* 2014; 20(6):659-663.
3. Loffredo FS, Steinhauser ML, Jay SM, et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell.* 2013; 153(4):828-839.
4. Baht GS, Silkstone D, Vi L, et al. Exposure to a youthful circulaton rejuvenates bone repair through modulation of β-catenin [published correction appears in *Nat Commun.* 2015;6:7761]. *Nat Commun.* 2015; 6:7131.
5. Katsimpardi L, Litterman NK, Schein PA, et al. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science.* 2014;344(6184):630-634.
6. Sinha M, Jang YC, Oh J, et al. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science.* 2014;344(6184): 649-652.
7. Najean Y, Rain JD, Billotey C. Epidemiological data in polycythaemia vera: a study of 842 cases. *Hematol Cell Ther.* 1998;40(4):159-165.
8. Edgren G, Rostgaard K, Vasan SK, et al. The new Scandinavian Donations and Transfusions database (SCANDAT2): a blood safety resource with added versatility. *Transfusion.* 2015;55(7):1600-1606.
9. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol.* 2009;24(11):659-667.
10. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol.* 2014;29(8):541-549.
11. Zetterqvist J, Vansteelandt S, Pawitan Y, Sjölander A. Doubly robust methods for handling confounding by cluster [published online ahead of print October 26, 2015]. *Biostatistics.* doi:10.1093/biostatistics/kxv041.
12. Villeda SA, Luo J, Mosher KI, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature.* 2011;477(7362): 90-94.

13. Conboy MJ, Conboy IM, Rando TA. Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. *Aging Cell.* 2013;12(3):525-530.

14. Geifman N, Rubin E. The mouse age phenome knowledgebase and disease-specific inter-species age mapping. *PLoS One.* 2013;8(12): e81114.

15. Demetrius L. Aging in mouse and human systems: a comparative study. *Ann N Y Acad Sci.* 2006;1067:66-82.

DOI 10.1182/blood-2015-11-683862