Supplementary Online Content

Edgren G, Ullum H, Rostgaard K, et al. Association of donor age and sex with survival of patients receiving transfusions. *JAMA Intern Med.* Published online April 24, 2017. doi:10.1001/jamainternmed.2017.0890

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Detailed Methodological Considerations

Why is meticulous adjustment for total number of transfusions so important in this situation?

Blood allocation routines (which typically follows a first in-first out principle) whereby only donor blood group is considered when selecting a blood unit for a patient in need of transfusion ensures that non-blood group factors are randomly distributed between patients. However, as has been noted, the probability of receiving a blood unit with a particular characteristic will increase with the cumulative number of transfusions.[1] The causal diagram in eFigure 1,[2] depicts the causal structure of the studied association. There are causal pathways from "patient disease severity" to both the exposure ("exposure to uncommon blood unit") and the outcome ("death"). Thus, "patient disease severity" fulfils the criteria of a genuine confounding factor. However, on account of the blood allocation principles, the causal pathway to the exposure goes only via "total number of transfusions", where the latter variable determines the probability that a patient receives one or more units with an uncommon trait. Total number of transfusions thus acts as a mediator of the causal effect of "patient disease severity" on the exposure. It is thus clear that careful adjustment for the comparatively simple variable "number of transfusions" can serve to remove all confounding from the more complex "patient disease severity".

Therefore, if meticulous adjustment for "total number of transfusions" can be attained, the causal link between "patient disease severity" and the exposure will be blocked (see eFigure 2). Then, "patient disease severity" is no longer a confounding factor for the studied association. In fact, given that the nebulous concept "patient disease severity" is notoriously difficult to measure exactly, let alone adjust for, adjustment for "total number of transfusions" is effectively the only efficient way of controlling for "patient disease severity" in this situation. Indeed, this is supported by the lack of effect of further adjustment for

Charlson comorbidity index once the models sufficiently carefully accounted for "total number of transfusions" (data not shown).

Why adjustments accommodating non-linear relationships?

The relationship between "total number of transfusions" and "exposure to uncommon blood unit" is based on simple mathematics and clearly linear. On the other hand, the causal pathway from "patient disease severity" to "total number of transfusions" cannot be easily assessed due to the measurement problems alluded to in the foregoing. As part of the preparatory work for the present analyses, we instead investigated the relationship between "total number of transfusions" and "death". This relationship was markedly non-linear (eFigure 3). As can be predicted from eFigure 1, it is likely that most or all of the observed relationship between "total number of transfusions" and "death" is in fact driven by "patient disease severity".

If the latter variable (i.e., "patient disease severity") is operationalized as "the condition-specific, inherent risk of dying" (which captures the essence of the variable better), a linear relationship with the outcome "death" can be assumed. Then, the observed non-linearity of the association between "total number of transfusions" and "death" must be attributed to the non-linear relationship between "patient disease severity" and "total number of transfusions". Clearly, the strongest support for the assumption that the analyses must account for the non-linear nature of the relationship derives from our observation that adjustments accommodating non-linear relationships were needed in order to break the link and fully eliminate the confounding in the simulated analyses.

As verified both in our real data and in our simulation with age and sex being allocated randomly, the effect sizes (i.e. the magnitude of the confounding) increased with increasing

rarity of the blood product. Intuitively, this is easy to comprehend – the more uncommon the blood product, the more important is the total number of transfusions. Since being exposed to two, three or more uncommon blood products will be even more improbable than being exposed to one, fictitious dose-response relationships might also arise.[1]

Choice of spline models

The main analyses (presented in Table 2) were adjusted for number of transfusions using restricted cubic splines with 5 knots (placed at 5, 20, 50, 150, and 250 transfusions). To verify the robustness of the spline function setup, we repeated the analyses for the main model, but with both increased and decreased number knots (3 and 7 knots). We also ran models where the knots were placed strictly equally (resulting in knots placed at 55, 110, 165, 220, and 275 transfusions). These analyses produced very similar results.

eReferences

1. Edgren G, Rostgaard K, Hjalgrim H. Methodological challenges in observational transfusion research: lessons learned from the Scandinavian donations and transfusions (SCANDAT) database. *ISBT Sci Ser.* 2017;12(1):191-195 doi:doi:10.1111/voxs.12342.

2. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* Jan 1999;10(1):37-48.

eTable 1. Diagnosis Codes for Derivation of Charlson Comorbidity Index	
Diagnosis	**Diagnosis codes (according to *International Classification of Disease, revision 10*)**
Acute myocardial infarction	I21, I22, I252
Congestive heart failure	I50
Peripheral vascular disease	I71, I790, I739, R02, Z958, Z959
Cerebrovascular disease	I60, I61, I62, I63, I65, I66,G450, G451, G452, G458, G459, G46, I64, G454, I670, I671, I672, I674, I675, I676, I677 I678, I679, I681, I682, I688, I69
Dementia	F00, F01, F02, F051
Respiratory disease	J40, J41, J42, J44, J43, J45, J46, J47, J67, J44, J60, J61, J62, J63, J66, J64, J65
Connective tissue disorder	M32, M34, M332, M053, M058, M059, M060, M063, M069, M050, M052, M051, M353
Peptic ulcer	K25, K26, K27, K28
Liver disease	K702, K703, K73, K717, K740, K742, K746, K743, K744, K745
Diabetes	E109, E119, E139, E149, E101, E111, E131, E141, E105, E115, E135, E145
Diabetes with complications	E102, E112, E132, E142 E103, E113, E133, E143 E104, E114, E134, E144
Paraplegia	G81 G041, G820, G821, G822
Renal disease	N03, N052, N053, N054, N055, N056, N072, N073, N074, N01, N18, N19, N25
Cancer	C01-C76, C81-C96
Metastatic cancer	C77, C78, C79, C80
Severe liver disease	K729, K766, K767, K721
Human immunodeficiency virus	B20, B21, B22, B23, B24

eTable 2. Hazard Ratios of Death in Relation to Number of Red Cell Transfusions From Donors of Different Age and Sex

30-day follow-up					
Donor age	**Unadjusted: with no adjustment for number of transfusions***		**Model 1: adjusting for number of transfusions using log-linear term ***		**Model 2: adjusting for number of transfusions using spline ***
	Hazard ratio (95% confidence interval)				
<20 years	1.45 (1.42-1.48)		1.09 (1.07-1.12)		0.99 (0.97-1.01)
20-29 years	1.12 (1.12-1.12)		0.91 (0.91-0.92)		1.00 (0.99-1.00)
30-39 years	1.11 (1.11-1.11)		0.94 (0.94-0.95)		1.00 (1.00-1.01)
40-49 years	1.13 (1.13-1.13)		1.06 (1.06-1.07)		1.00 (1.00-1.01)
50-59 years	1.15 (1.15-1.15)		1.05 (1.04-1.05)		1.00 (1.00-1.01)
60-69 years	1.31 (1.30-1.32)		1.07 (1.06-1.08)		0.99 (0.98-1.00)
≥70 years	1.59 (1.37-1.83)		1.32 (1.14-1.52)		1.03 (0.89-1.19)
Donor sex					
Female	1.07 (1.07-1.07)		0.96 (0.96-0.97)		0.99 (0.99-1.00)
Male	0.93 (0.93-0.93)		1.04 (1.03-1.04)		1.01 (1.00-1.01)
Unrestricted follow-up					
Donor age	**Unadjusted: with no adjustment for number of transfusions***		**Model 1: adjusting for number of transfusions using log-linear term***		**Model 2: adjusting for number of transfusions using spline ***
	Hazard ratio (95% confidence interval)				
<20 years	1.48 (1.47-1.49)		1.08 (1.07-1.09)		1.00 (1.00-1.01)
20-29 years	1.09 (1.08-1.09)		1.02 (1.02-1.03)		0.99 (0.99-1.00)
30-39 years	1.07 (1.07-1.07)		1.01 (1.01-1.02)		0.99 (0.99-1.00)
40-49 years	1.05 (1.05-1.05)		0.98 (0.98-0.98)		1.00 (1.00-1.00)
50-59 years	1.05 (1.05-1.05)		0.98 (0.98-0.98)		1.01 (1.00-1.01)
60-69 years	1.12 (1.11-1.12)		0.98 (0.97-0.98)		1.01 (1.00-1.02)
≥70 years	1.31 (1.28-1.34)		0.82 (0.80-0.84)		0.96 (0.92-1.00)
Donor sex					
Female	1.04 (1.04-1.04)		1.01 (1.01-1.01)		1.00 (1.00-1.00)
Male	0.96 (0.96-0.96)		0.99 (0.99-0.99)		1.00 (1.00-1.00)

*Analyses were run with separate models for each category, with recipients of all other blood unit types as reference. Hazard ratios are presented per transfused unit. The unadjusted model only included terms for donor age/or sex. In Model 1 we adjusted for number of red-cell transfusions as a log-linear term. In Model 2 we adjusted for number of red-cell transfusions as a restricted cubic spline with 5 knots. All three models were stratified by hospital to account for regional differences. Both Model 1 and Model 2 also included parameters for Charlson comorbidity score, sex and age.

eTable 3. Hazard Ratios of Death Over 30 Days in Relation to Categorized Number of Red Cell Transfusions From Donors of Different Age Group and Sex, With Recipients Who Received No Blood Unit of Each Type as Reference

Donor age	**None**			**1-2 units**			**≥3 units**	
	Number of deaths; hazard ratio (95% confidence interval)							
<20 years	89 358	1.00 (ref)		7 646	0.98 (0.96-1.01)		217	1.00 (0.79-1.27)
20-29 years	55 569	1.00 (ref)		34 315	0.99 (0.97-1.00)		7 337	1.00 (0.96-1.04)
30-39 years	45 189	1.00 (ref)		41 864	1.01 (0.99-1.02)		10 168	1.03 (1.00-1.07)
40-49 years	37 114	1.00 (ref)		46 759	0.99 (0.97-1.00)		13 348	0.99 (0.96-1.02)
50-59 years	42 822	1.00 (ref)		43 549	0.99 (0.97-1.00)		10 850	0.99 (0.96-1.03)
60-69 years	70 662	1.00 (ref)		24 081	1.00 (0.98-1.01)		2 478	0.98 (0.92-1.04)
≥70 years	97 048	1.00 (ref)		169	0.95 (0.81-1.11)		4	n.e.
Donor sex								
Female	25 287	1.00 (ref)		50 733	0.99 (0.98-1.01)		21 201	0.98 (0.96-1.01)
Male	12 029	1.00 (ref)		54 540	1.01 (0.99-1.02)		30 652	1.02 (0.99-1.05)

*Estimated using fully adjusted statistical models, with one model per donor age group. Hazard ratios were computed from the Model 2 in Supplementary Table 2. Analyses were thus adjusted for number of red-cell transfusions as a restricted cubic spline with 5 knots, hospital as a stratum, Charlson comorbidity score, sex as well as age.
n.e. denotes not estimated.

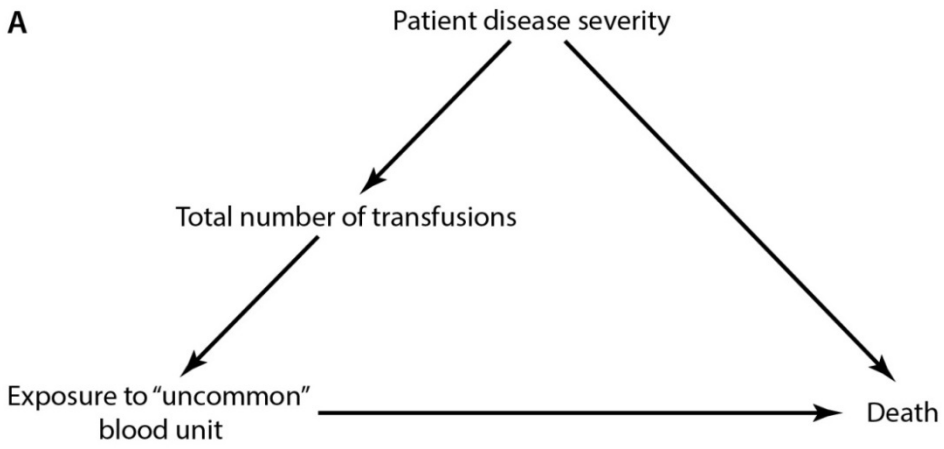
eTable 4. Cumulative Mortality Over 30 Days With Corresponding Cumulative Mortality Difference in Relation to Categorized Number Of red Cell Transfusions From Donors of Different Age Group and Sex, With Recipients Who Received No Blood Unit of Each Type as Reference

Donor age	**None**		**1-2 units**		**≥3 units**		
	Adjusted cumulative mortality (95% confidence interval) and Adjusted cumulative mortality difference (95% confidence interval)						
<20 years	10.8% (10.7-10.9%)	0.0% (ref)	10.6% (10.4-10.9%)	-0.1% (-0.4-0.1%)	10.4% (8.94-11.9%)	-0.4% (-1.8-1.1%)	
20-29 years	10.8% (10.7-10.9%)	0.0% (ref)	10.7% (10.6-10.8%)	-0.1% (-0.2-0.0%)	10.9% (10.6-11.2%)	0.0% (-0.3-0.4%)	
30-39 years	10.7% (10.6-10.8%)	0.0% (ref)	10.8% (10.7-10.9%)	0.1% (-0.16-0.2%)	11.1% (10.8-11.3%)	0.4% (0.0-0.6%)	
40-49 years	10.8% (10.7-10.9%)	0.0% (ref)	10.7% (10.6-10.8%)	-0.1% (-0.3-0.0%)	11.0% (10.8-11.3%)	0.2% (-0.1-0.5%)	
50-59 years	10.8% (10.7-10.9%)	0.0% (ref)	10.7% (10.6-10.8%)	-0.1% (-0.3-0.0%)	11.1% (10.8-11.3%)	0.3% (-0.1-0.6%)	
60-69 years	10.8% (10.7-10.9%)	0.0% (ref)	10.8% (10.6-10.9%)	0.0% (-0.2-0.1%)	10.9% (10.4-11.3%)	0.1% (-0.4-0.6%)	
≥70 years	10.8% (10.7-10.8%)	0.0% (ref)	10.3% (8.85-11.7%)	-0.5% (-1.9-1.0%)	n.e.		
Donor sex							
Female	10.9% (10.7-11.0%)	0.0% (ref)	10.8% (10.7-10.9%)	-0.1% (-0.2-0.1%)	10.7% (10.5-10.9%)	-0.2% (-0.4-0.1%)	
Male	10.6% (10.4-10.8%)	0.0% (ref)	10.8% (10.7-10.9%)	0.2% (0.0-0.4%)	10.8% (10.7-11.0%)	0.2% (-0.1-0.5%)	

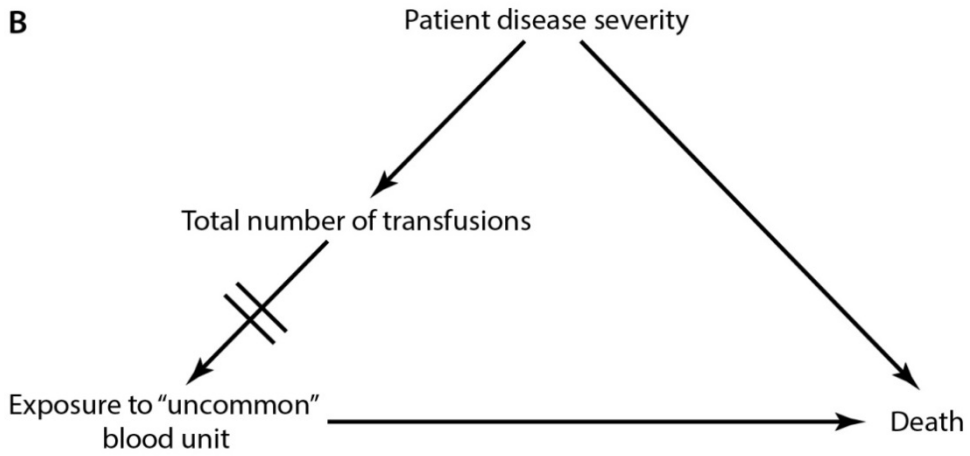
*Estimated using fully adjusted statistical models, with one model per donor age group. Hazard ratios were computed from Model 2 in Supplementary Table 2. Analyses were thus adjusted for number of red-cell transfusions as a restricted cubic spline with 5 knots, Charlson comorbidity score, sex as well as age.

n.e. denotes not estimated.

eFigure 1. Causal Structure of the Studied Association



eFigure 2. Blockage of Causal Link



eFigure 3. Estimated Association Between Cumulative Number of Red Blood Cell Transfusions and Hazard Ratio of Death