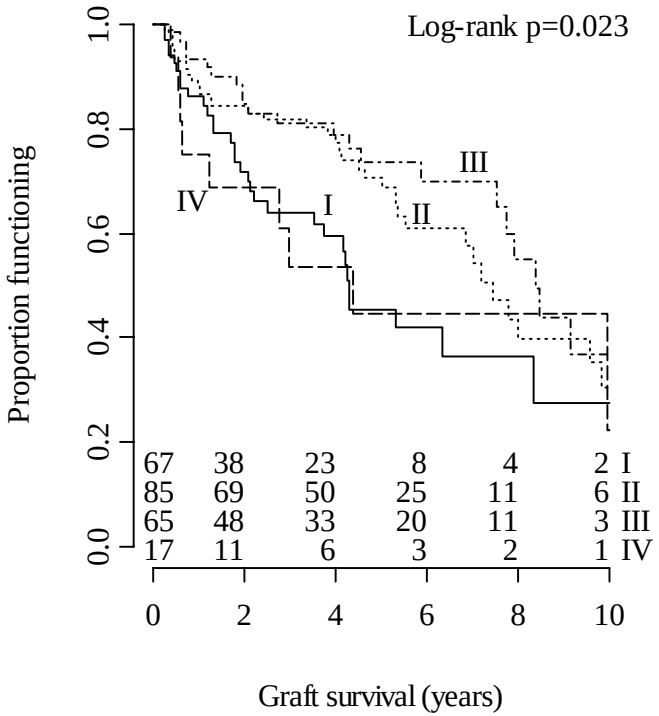
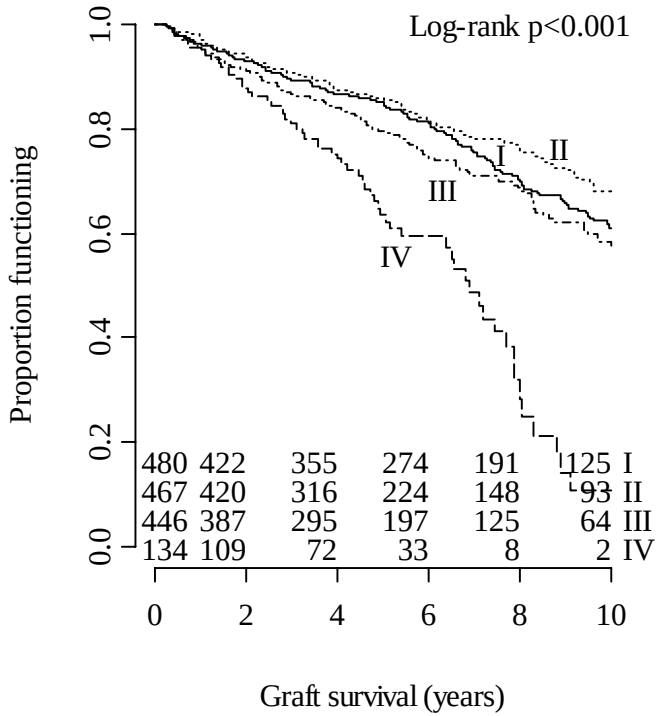
Actual graft survival (event=death or graft failure; N=1829, 587 events)

Kaplan-Meier-curves:

CNI-:

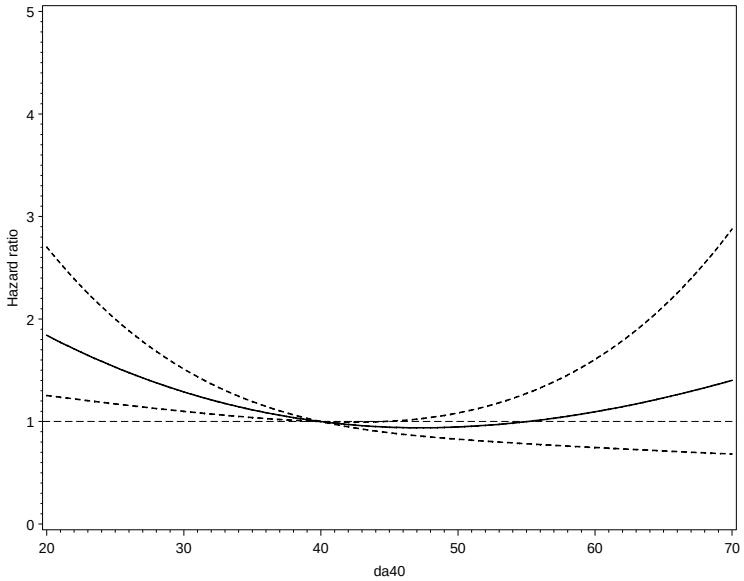


CNI+:

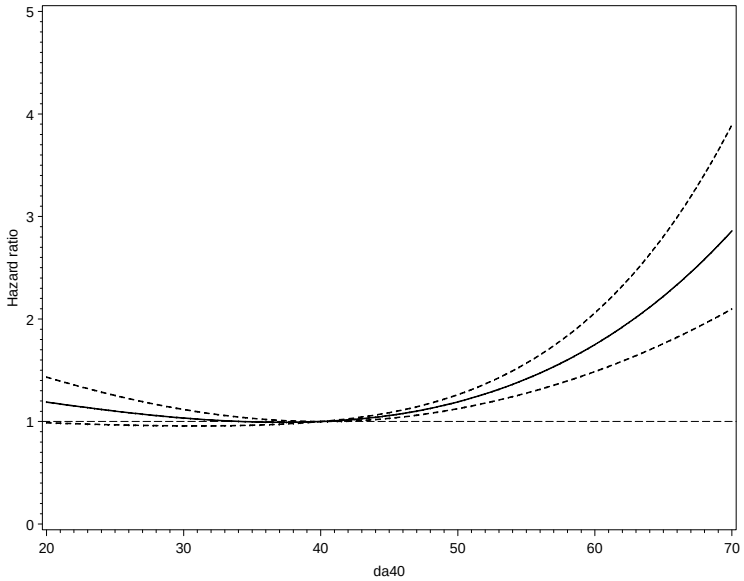


Crude hazard ratio estimates (reference: donor age=40 yrs):

CNI-:



CNI+:



Variable	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > Chi-Square
immg90	0.721	0.358	1.453	0.3601
noCA_DA1	1.051	0.501	2.203	0.8952
noCA_DA2	0.577	0.278	1.199	0.1405
noCA_DA3	0.476	0.219	1.034	0.0606
CA_DA1	0.373	0.276	0.504	<.0001
CA_DA2	0.310	0.226	0.425	<.0001
CA_DA3	0.431	0.318	0.585	<.0001

Adjusted hazard ratios

(all confounders, multiple imputation, 20 imputed data sets):

Actual graft survival (N=1829, >90 days of follow-up, 587 events):

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.70209	0.33190	1.48520	0.3546
noCA_DA1	1.21513	0.54405	2.71396	0.6343
noCA_DA2	0.65645	0.30158	1.42888	0.2887
noCA_DA3	0.58771	0.25736	1.34211	0.2070
CA_DA1	0.43564	0.31704	0.59860	<.0001
CA_DA2	0.39837	0.28672	0.55350	<.0001
CA_DA3	0.53502	0.39108	0.73195	<.0001
age_tpl10	1.18691	1.11028	1.26883	<.0001
khk	1.11101	0.88355	1.39703	0.3667
cmp	1.19764	0.90867	1.57852	0.1992
vascid1	1.13361	0.83110	1.54623	0.4249
vascid2	1.28206	0.98747	1.66454	0.0620
year_tpl	0.94486	0.91902	0.97142	<.0001
dialysezeit	1.02637	0.98381	1.07078	0.2284
cholest10	0.99789	0.98521	1.01073	0.7456
map10	1.01199	0.99394	1.03037	0.1940
diabetes	1.43944	1.16663	1.77606	0.0007
cad	0.76449	0.53602	1.09034	0.1382

Using different parametrization to assess the effect of IS in various donor age groups (IS_DAx refers to the HR of CNI+ vs. CNI- in donor age group x):

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
donorage1	1.21513	0.54405	2.71396	0.6343
donorage2	0.65645	0.30158	1.42888	0.2887
donorage3	0.58771	0.25736	1.34211	0.2070
IS_DA1	0.25171	0.16891	0.37510	<.0001
IS_DA2	0.42607	0.29124	0.62333	<.0001
IS_DA3	0.63915	0.40395	1.01130	0.0559
IS_DA4	0.70209	0.33190	1.48520	0.3546
age_tpl10	1.18691	1.11028	1.26883	<.0001
khk	1.11101	0.88355	1.39703	0.3667
cmp	1.19764	0.90867	1.57852	0.1992
vascid1	1.13361	0.83110	1.54623	0.4249
vascid2	1.28206	0.98747	1.66454	0.0620
year_tpl	0.94486	0.91902	0.97142	<.0001
dialysezeit	1.02637	0.98381	1.07078	0.2284
cholest10	0.99789	0.98521	1.01073	0.7456
map10	1.01199	0.99394	1.03037	0.1940
diabetes	1.43944	1.16663	1.77606	0.0007
cad	0.76449	0.53602	1.09034	0.1382

Analysis including mediators:

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.70031	0.32771	1.49654	0.3575
noCA_DA1	1.29585	0.58331	2.87878	0.5243
noCA_DA2	0.71526	0.32019	1.59781	0.4134
noCA_DA3	0.57948	0.24981	1.34422	0.2035
CA_DA1	0.48087	0.34088	0.67833	<.0001
CA_DA2	0.42732	0.30394	0.60080	<.0001
CA_DA3	0.55800	0.40481	0.76916	0.0004
age_tpl10	1.19376	1.11531	1.27772	<.0001
khk	1.07208	0.84953	1.35292	0.5566
cmp	1.23459	0.93460	1.63086	0.1369
vascid1	1.15666	0.86924	1.53912	0.3162
vascid2	1.23724	0.96369	1.58845	0.0945
year_tpl	0.94354	0.91764	0.97017	<.0001
dialysezeit	1.02422	0.98166	1.06863	0.2690
cholest10	0.99779	0.98517	1.01057	0.7331
map10	1.01205	0.99347	1.03097	0.2047
diabetes	1.46093	1.18500	1.80111	0.0004
cad	0.75780	0.53318	1.07705	0.1221
bcar1	0.96746	0.80717	1.15959	0.7204
dgf	1.11297	0.89278	1.38746	0.3411
logcreat90	1.09452	0.98856	1.21185	0.0821

Analysis without multiple imputation (N=801, Events=181):

Crude effect, only data for which complete covariate information is available:

Variable	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > Chi-Square
immg90	0.315	0.092	1.072	0.0646
noCA_DA1	0.227	0.054	0.955	0.0430
noCA_DA2	0.307	0.086	1.094	0.0685
noCA_DA3	0.237	0.062	0.901	0.0347
CA_DA1	0.447	0.258	0.775	0.0041
CA_DA2	0.374	0.210	0.667	0.0009
CA_DA3	0.548	0.315	0.954	0.0335

Adjusted effect, only data with complete covariate information:

Variable	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > Chi-Square
immg90	0.312	0.088	1.106	0.0713
noCA_DA1	0.193	0.042	0.881	0.0337
noCA_DA2	0.320	0.089	1.159	0.0827
noCA_DA3	0.307	0.079	1.197	0.0890
CA_DA1	0.445	0.246	0.805	0.0074
CA_DA2	0.387	0.207	0.723	0.0029
CA_DA3	0.588	0.326	1.061	0.0780
age_tpl10	0.972	0.853	1.107	0.6674
khk	1.118	0.730	1.711	0.6077
cmp	1.047	0.565	1.939	0.8847
vascid1	0.900	0.518	1.564	0.7094
vascid2	1.475	0.937	2.322	0.0933
year_tpl	0.996	0.950	1.045	0.8687
dialysezeit	1.133	1.040	1.235	0.0042
cholest10	1.015	0.996	1.035	0.1303
map10	1.035	0.996	1.076	0.0776
Diabetes	1.747	1.203	2.537	0.0034
cad	0.678	0.398	1.156	0.1539

Conclusion: Restricting the analysis to patients with complete covariate information changes the crude effect substantially. Therefore, the observed change in the adjusted effect, which is of the same magnitude as that of the crude effect, is considered a consequence of the greatly reduced sample size (43.8%) and number of events (30.8%) and of a possible selection bias due to the restriction on complete cases.

Subgroup analysis (IS=CNI- or CNI+)

IS=CNI-:

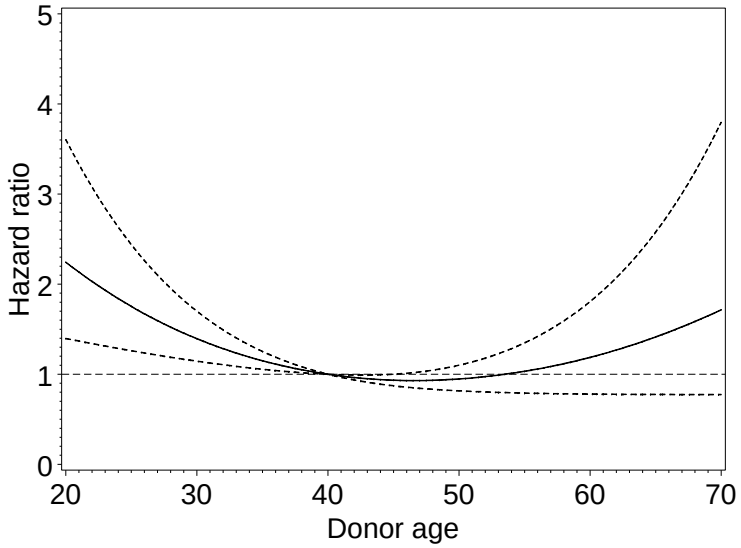
Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
donorage1	1.02871	0.42909	2.46627	0.9493
donorage2	0.59607	0.26022	1.36538	0.2210
donorage3	0.53258	0.22431	1.26450	0.1532
age_tpl10	1.15838	0.98667	1.35997	0.0725
khk	1.05341	0.44479	2.49483	0.9050
cmp	1.06154	0.42900	2.62672	0.8968
vascid1	1.79577	0.40763	7.91109	0.4357
vascid2	1.12597	0.45218	2.80376	0.7971
year_firstrrt	0.88818	0.83163	0.94857	0.0004
dialysezeit	0.87483	0.77052	0.99327	0.0390
cholest10	1.00146	0.97479	1.02885	0.9156
map10	1.02134	0.97913	1.06537	0.3262
diabetes	1.16724	0.65748	2.07222	0.5973
cad	0.75222	0.28395	1.99271	0.5667

IS=CNI+:

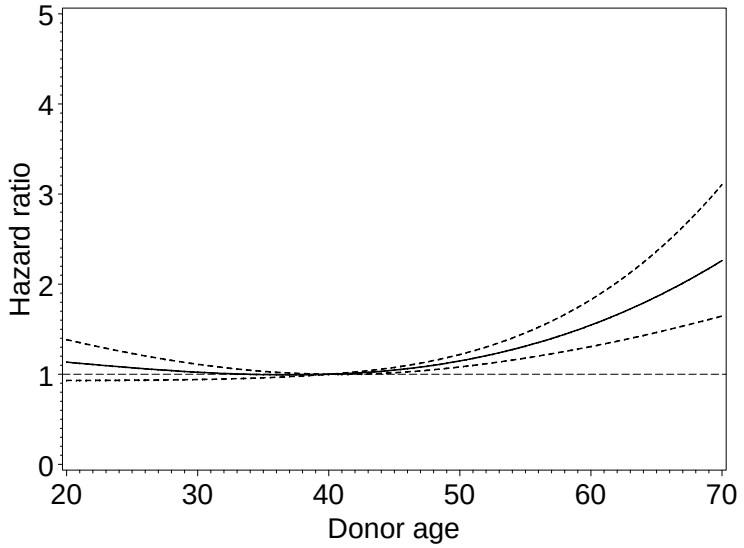
Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
donorage1	0.45825	0.33054	0.63529	<.0001
donorage2	0.41685	0.29986	0.57949	<.0001
donorage3	0.55540	0.40356	0.76436	0.0003
age_tpl10	1.20018	1.11299	1.29421	<.0001
khk	1.06092	0.81093	1.38796	0.6643
cmp	1.20605	0.85835	1.69459	0.2761
vascid1	1.13582	0.80790	1.59684	0.4592
vascid2	1.32344	0.99602	1.75849	0.0532
year_firstrrt	0.96504	0.93486	0.99619	0.0282
dialysezeit	0.99828	0.94263	1.05721	0.9530
cholest10	1.00012	0.98529	1.01517	0.9877
map10	1.01033	0.99054	1.03052	0.3085
diabetes	1.48447	1.18606	1.85795	0.0006
cad	0.77838	0.52897	1.14540	0.2037

Adjusted hazard ratio estimates:

CNI-:



CNI+:



Assessment of proportional hazards assumption: interaction of variables with log(time):

Interactions with log(_time_)

var1	raw_p	False Discovery Rate p-value
	.	.
immg90	0.13459	0.66755
noCA_DA1	0.57667	0.78997
noCA_DA2	0.39672	0.78997
noCA_DA3	0.89452	0.89452
CA_DA1	0.59434	0.78997
CA_DA2	0.60409	0.78997
CA_DA3	0.32232	0.78997
age_tpl	0.72791	0.88389
khk	0.19634	0.66755
cmp	0.89271	0.89452
vascid1	0.16488	0.66755
vascid2	0.40658	0.78997
year_tpl	0.48741	0.78997
dialysezeit	0.79850	0.89452
cholest	0.00008	0.00140
diabetes	0.07220	0.61369
map	0.49375	0.78997

Model with cholest*log(time):

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.66811	0.32423	1.37668	0.2742
noCA_DA1	1.17063	0.54366	2.52064	0.6872
noCA_DA2	0.64567	0.30469	1.36826	0.2536
noCA_DA3	0.56110	0.25260	1.24638	0.1559
CA_DA1	0.42978	0.31292	0.59029	<.0001
CA_DA2	0.38580	0.27771	0.53595	<.0001
CA_DA3	0.53314	0.38916	0.73038	<.0001
age_tpl	1.01732	1.01034	1.02434	<.0001
khk	1.10855	0.87428	1.40558	0.3936
cmp	1.21822	0.90489	1.64005	0.1913
vascid1	1.16625	0.84510	1.60943	0.3455
vascid2	1.20470	0.90940	1.59589	0.1920
year_tpl	0.93909	0.91344	0.96545	<.0001
dialysezeit	1.02381	0.98139	1.06806	0.2757
cholest	0.99678	0.99484	0.99872	0.0012
cholest_time	1.00236	1.00117	1.00355	0.0001
diabetes	1.46395	1.18565	1.80758	0.0004
map	1.00106	0.99918	1.00294	0.2696

Conclusion: Adequate modeling of the effect of cholesterol has no impact on the findings on the interaction of Donorage and IS.

Model with HR for cholest divided into two parts: follow-up 0-3 years, and more than 3 years:

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.67258	0.32644	1.38574	0.2822
noCA_DA1	1.18801	0.55193	2.55715	0.6596
noCA_DA2	0.64308	0.30339	1.36309	0.2494
noCA_DA3	0.56439	0.25396	1.25427	0.1603
CA_DA1	0.42875	0.31214	0.58894	<.0001
CA_DA2	0.38592	0.27783	0.53606	<.0001
CA_DA3	0.53071	0.38736	0.72710	<.0001
age_tpl	1.01719	1.01023	1.02419	<.0001
khk	1.10525	0.87189	1.40105	0.4070
cmp	1.21686	0.90314	1.63955	0.1950
vascid1	1.16870	0.84663	1.61328	0.3394
vascid2	1.20486	0.90913	1.59680	0.1924
year_tpl	0.94013	0.91443	0.96655	<.0001
dialysezeit	1.02379	0.98145	1.06796	0.2753
cholest_0_3	0.99750	0.99547	0.99952	0.0155
cholest_3_on	1.00101	0.99956	1.00247	0.1731
diabetes	1.46402	1.18575	1.80759	0.0004
map	1.00109	0.99922	1.00296	0.2519

Conclusion: modeling of the time-dependent effect of cholesterol by partitioning the time axis into two parts (</> 3 years after TPL) has no impact on the findings on the interaction of Donorage and IS.

Analysis of interactions:

Do any covariates modify the combined effects of IS and donor age? The corresponding interaction tests are carried out using one of 20 imputed data sets because of feasibility:

Obs	variable	with_variable	Pr > Chi-Square	DF	False Discovery Rate p-value
1	age_tpl	immg90 noC...	0.0246	7	0.0739
2	khk	immg90 noC...	0.1105	7	0.1657
4	vascid1	immg90 noC...	0.5839	7	0.5839
5	vascid2	immg90 noC...	0.4798	7	0.5397
6	year_tpl	immg90 noC...	0.0955	7	0.1657
7	dialysezeit	immg90 noC...	0.2089	7	0.2686
8	cholest	immg90 noC...	0.0216	7	0.0739
9	map	immg90 noC...	0.0353	7	0.0795
10	diabetes	immg90 noC...	0.0093	7	0.0739

To avoid spurious findings, the false discovery rate p-values (which correct for multiple testing) should be used. No significances are found. Nevertheless, subgroup analysis were carried out for non-diabetic and diabetic patients.

Non-diabetic (N=1399, 419 events):

Variable	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > Chi-Square
immg90	0.774	0.344	1.743	0.5365
noCA_DA1	1.289	0.543	3.063	0.5652
noCA_DA2	0.617	0.268	1.417	0.2548
noCA_DA3	0.640	0.268	1.531	0.3159
CA_DA1	0.358	0.247	0.519	<.0001
CA_DA2	0.274	0.186	0.402	<.0001
CA_DA3	0.437	0.304	0.628	<.0001
age_tpl	1.021	1.014	1.028	<.0001
khk	0.998	0.779	1.280	0.9903
cmp	1.362	1.050	1.768	0.0201
year_tpl	0.946	0.916	0.977	0.0007
vascid1	1.047	0.773	1.417	0.7673
vascid2	1.163	0.886	1.526	0.2757
cholest	1.000	0.998	1.001	0.6114
MAP	1.002	1.000	1.004	0.0233

Diabetic (N=362, 152 events):

Variable	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > Chi-Square
immg90	0.438	0.097	1.981	0.2838
noCA_DA1	0.894	0.189	4.236	0.8878
noCA_DA2	0.443	0.087	2.263	0.3281
noCA_DA3	0.134	0.018	0.994	0.0493
CA_DA1	0.545	0.294	1.010	0.0538
CA_DA2	0.757	0.400	1.432	0.3919
CA_DA3	0.752	0.404	1.403	0.3707
age_tpl	1.002	0.987	1.018	0.7560
khk	1.106	0.758	1.615	0.6017
cmp	1.600	1.009	2.539	0.0457
year_tpl	0.941	0.890	0.995	0.0330
vascid1	1.173	0.768	1.790	0.4609
vascid2	1.387	0.983	1.957	0.0628
cholest	1.001	0.998	1.003	0.5175
MAP	0.999	0.994	1.004	0.5953

The effects of IS and donor age on actual graft survival differ in their magnitude but not in their direction between diabetic and non-diabetic patients.

KHK and CMP show a higher effect in diabetic than in non-diabetic persons.

Detailed description of our approach to handle missing data

Missing values are a problem commonly encountered in studies like ours. Particularly in the analysis of multivariable models one may end up with 30-40% complete observations even if each variable has no more than 5-10% missing values. Therefore, we applied multiple imputation (Ref.) to our data set, which is currently the state-of-the-art technique to deal with missing values.

Multiple imputation is carried out in three steps. In step one, regression models are estimated from the non-missing observations which use each covariate in turn as the dependent variable and all other covariates as independent variables. In step two, multiple versions of the original data set are produced by replacing the missing values of covariates by values that follow the regression models of step 1. These so-called imputations vary between the different versions of the reconstructed data set because the regression models of step 1 cannot perfectly predict missing covariate values, and the imputation procedure reflects the model uncertainty properly by randomly drawing values from the predicted distribution instead of imputing the expected covariate value. In step 3, each imputed data set is analyzed by Cox regression and then the regression results are combined, properly taking into account the within-data set variation and the between-data set variation when computing confidence intervals for hazard ratios. The between-data set variation will be low if a covariate can be well predicted by other covariates, but high if the prediction model of a covariate lacks precision. For the former case, multiple imputation provides a powerful analysis. In the latter case, multiple imputation analysis will not be much better than an analysis of the complete observations, but still improves the estimation of parameters related to covariates with small amounts of missing data in terms of precision.

If for some covariates the amount of missing data exceeds a certain level (10% say), results obtained by multiple imputation can be biased if the assumption that covariate values are missing just randomly does not hold. Therefore, we assessed the sensitivity of our initial results on the assumption of randomly missing data by artificially doubling the amount of missing data in each covariate in two ways: first purely at random and second, only in subjects with covariate values higher than the median. The results from both analyses differed only marginally from our initial results (the detailed results are given in our supplemental data file), suggesting that any violation of the assumption of randomly missing data that could be present in our data would not affect our conclusions.

Assessing the sensitivity of our results on the assumption of randomly missing data

Outcome: actual graft survival (event=death or graft loss)

Multiple imputation analysis with artificially generated not-randomly missing values (doubling the amount of missing data in each covariate):

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.72918	0.33908	1.56808	0.4187
noCA_DA1	1.28514	0.58274	2.83416	0.5341
noCA_DA2	0.69910	0.31837	1.53509	0.3723
noCA_DA3	0.56911	0.24642	1.31441	0.1869
CA_DA1	0.44200	0.31692	0.61646	<.0001
CA_DA2	0.40212	0.28625	0.56490	<.0001
CA_DA3	0.54993	0.39431	0.76698	0.0004
age_tpl10	1.20330	1.12693	1.28484	<.0001
khk	1.03339	0.80888	1.32022	0.7921
cmp	1.16129	0.84545	1.59511	0.3532
vascid1	1.36996	0.90534	2.07304	0.1344
vascid2	1.25250	0.93809	1.67227	0.1262
year_tpl	0.94744	0.92204	0.97354	<.0001
dialysezeit	1.02717	0.98471	1.07147	0.2133
cholest10	0.99743	0.98383	1.01121	0.7121
map10	1.01301	0.99406	1.03231	0.1791
diabetes	1.47187	1.19905	1.80678	0.0002
cad	0.76381	0.53587	1.08870	0.1362

Multiple imputation analysis with artificially generated randomly missing values (doubling the amount of missing data in each covariate):

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
immg90	0.67938	0.32742	1.40966	0.2991
noCA_DA1	1.21996	0.57042	2.60914	0.6082
noCA_DA2	0.64279	0.30193	1.36846	0.2516
noCA_DA3	0.52923	0.23505	1.19157	0.1243
CA_DA1	0.45865	0.33157	0.63444	<.0001
CA_DA2	0.41108	0.29455	0.57371	<.0001
CA_DA3	0.53213	0.38574	0.73409	0.0001
age_tpl10	1.20489	1.12795	1.28708	<.0001
khk	1.09979	0.84251	1.43565	0.4798
cmp	1.04953	0.76867	1.43301	0.7583
vascid1	1.05862	0.76904	1.45725	0.7240
vascid2	1.14789	0.85418	1.54260	0.3551
year_tpl	0.94919	0.92357	0.97552	0.0002
dialysezeit	1.02502	0.98237	1.06952	0.2544
cholest10	0.99556	0.98208	1.00921	0.5206
map10	1.01098	0.99248	1.02982	0.2464
diabetes	1.48868	1.21270	1.82747	0.0001
cad	0.77712	0.54449	1.10914	0.1647

Comparison with original multiple imputation analysis:

Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|	Not-randomly missing values Hazard Ratio	Randomly missing values Hazard Ratio
immg90	0.68519	0.32787	1.43193	0.3146	0.72918	0.67938
noCA_DA1	1.18106	0.53195	2.62227	0.6824	1.28514	1.21996
noCA_DA2	0.64164	0.29744	1.38416	0.2579	0.69910	0.64279
noCA_DA3	0.57432	0.25586	1.28919	0.1788	0.56911	0.52923
CA_DA1	0.43548	0.31670	0.59881	<.0001	0.44200	0.45865
CA_DA2	0.39972	0.28862	0.55359	<.0001	0.40212	0.41108
CA_DA3	0.53755	0.39286	0.73553	0.0001	0.54993	0.53213
age_tpl10	1.18773	1.11086	1.26992	<.0001	1.20330	1.20489
khk	1.06383	0.83769	1.35103	0.6104	1.03339	1.09979
cmp	1.19566	0.90393	1.58154	0.2090	1.16129	1.04953
vascid1	1.16924	0.87616	1.56036	0.2861	1.36996	1.05862
vascid2	1.28796	0.99769	1.66268	0.0521	1.25250	1.14789
year_tpl	0.94593	0.92054	0.97202	<.0001	0.94744	0.94919
dialysezeit	1.02614	0.98366	1.07047	0.2316	1.02717	1.02502
cholest10	0.99843	0.98658	1.01042	0.7958	0.99743	0.99556
map10	1.01260	0.99405	1.03149	0.1840	1.01301	1.01098
diabetes	1.44447	1.17431	1.77679	0.0005	1.47187	1.48868
cad	0.76119	0.53391	1.08522	0.1316	0.76381	0.77712

There is no parameter for which the estimate from the data set with artificially generated missing values lies outside the confidence interval for that parameter computed from the original data set. Moreover, the hazard ratio estimates are very close to the original estimates even in the case of not-randomly missing values. The highest deviation is observed for vascid1, for which the amount of missing values was 42%.