Prediction of acute GVHD and relapse by metabolic biomarkers after allogeneic hematopoietic stem cell transplantation

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Clinical outcomes

The median follow-up time for the whole cohort was 42 months (range, 0.2 to 60 months). The median time for neutrophil recovery was 12 days (range, 10 to 37 days), and the median time for platelet recovery was 14 days (range, 8 to 90 days). The cumulative incidence of CMV reactivation was 53.67%. The median time for CMV reactivation was 2.9 months. The cumulative incidence of EBV infection was 17.32%. The median time to EBV reactivation was 8 months. The cumulative incidence of bacterial infection within 30 days post transplantation was 51.35%. Of the 114 patients evaluated for aGVHD, the cumulative incidence of grade II-IV aGVHD at day 100 was 53.63%. The median day for developing aGVHD was day 30. Fifty-one patients passed away and the 5-year probability of overall survival (OS) was 54.74% and disease free survival (DFS) was 51.60% for the whole cohort. In the 114 patients who survived and were in remission beyond 100 days, the cumulative incidence of cGVHD at 5 years was 52.63%. Thirty-two patients had relapsed by the time of the last follow-up. The 5-year cumulative incidence of relapse was 31.18%.
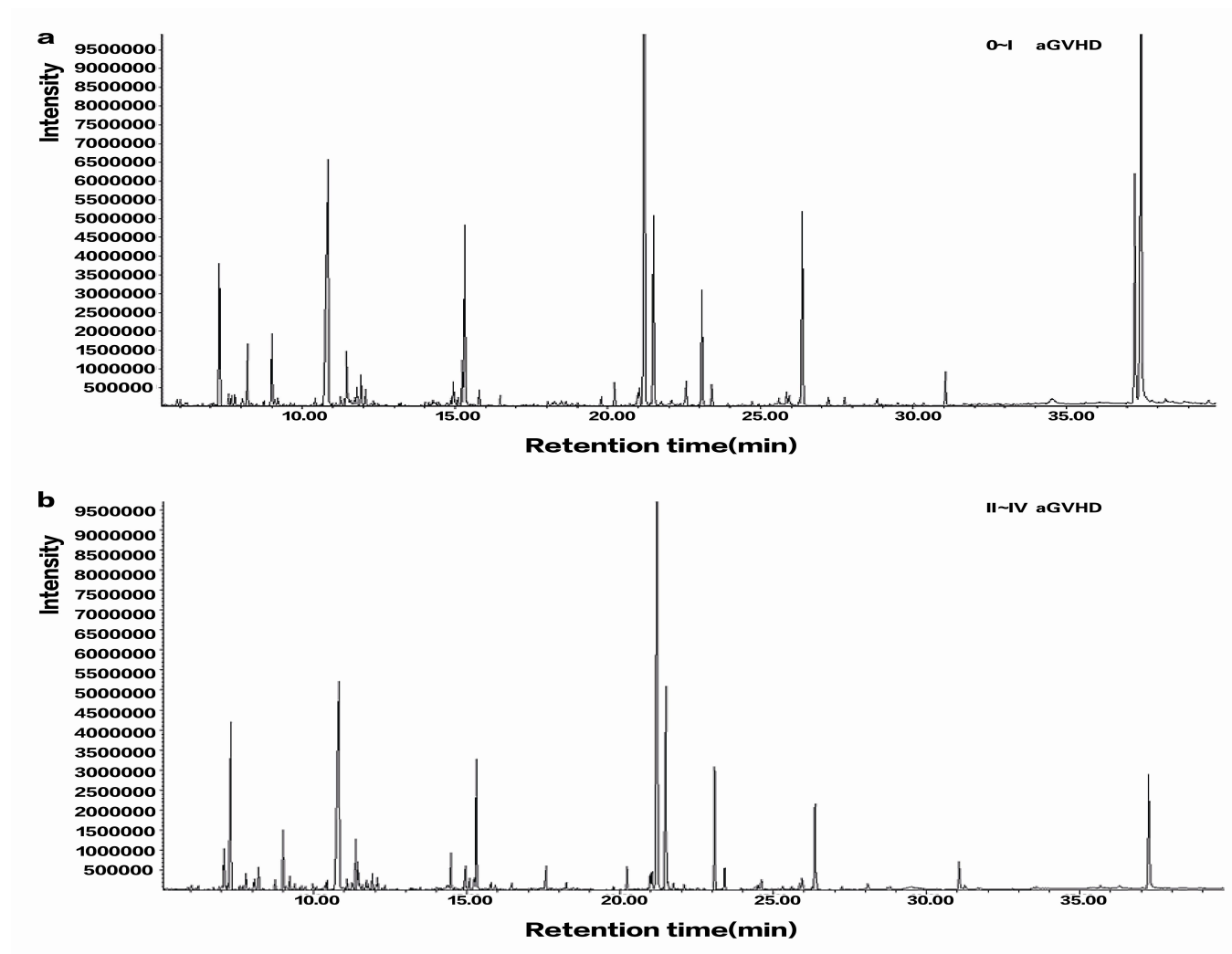
Figure S2. Reproducibility of metabolic profiling platform used in this study. Metabolite abundance quantified in serum samples over two QC replicates are shown. The Spearman correlation coefficient between technical replicates varied between 0.91 and 0.97, depending on the pair of replicates used. To obtain reliable data, samples from each group were injected in a random order. Thirteen repeated injections of the same QC samples were inserted into the analytical sequence during the GC-MS analysis to trace the batch-to-batch variability and reliability of the metabolic data.
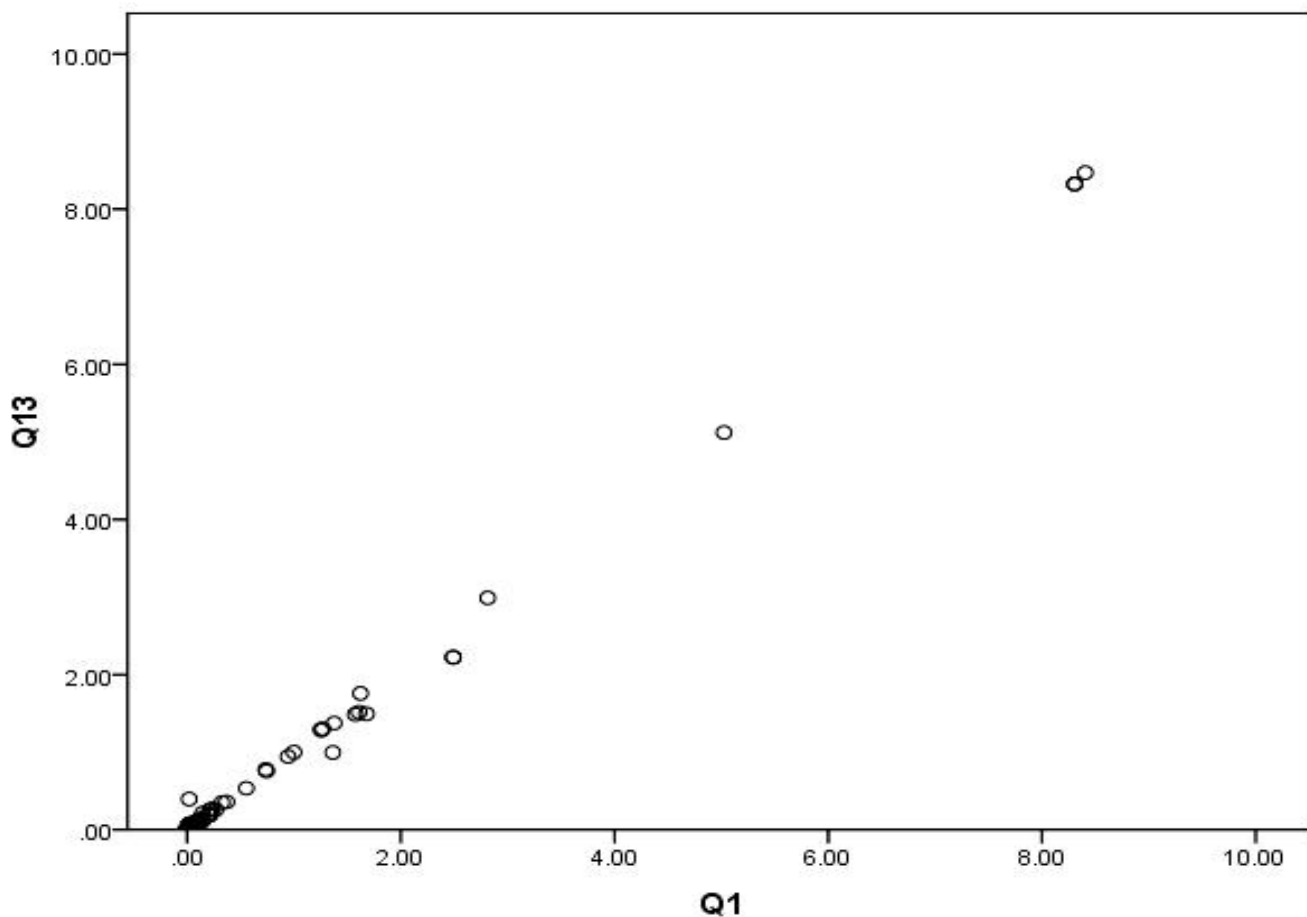
Table S1. Comparison of sixteen detectable metabolites between 0-I and II-IV aGVHD groups of patients.

Metabolites	Retention time (min)	m/z	P^A	FDR	AUC
Palmitic acid （PA）	23.08	313	0.02*	0.16	0.75
Stearic acid(SA)	26.36	341	0.03*	0.17	0.72
Galactose	20.98	319	0.08	0.26	0.70
D-glucose	21.20	319	0.08	0.26	0.70
L-phenylalanine	16.48	218	0.11	0.30	0.67
Lactic acid	7.30	117	0.15	0.34	0.67
Pyruvic acid	7.00	174	0.18	0.35	0.61
L-Leucine	9.22	86	0.23	0.35	0.72
Glycerol	11.46	205	0.25	0.35	0.65
Oleic acid	25.94	339	0.25	0.35	0.63
L-Valine	7.80	72	0.25	0.35	0.69
Cholesterol	37.24	329	0.55	0.71	0.62
Linoleic acid	25.85	337	0.64	0.72	0.60
Mannose	23.42	204	0.68	0.72	0.50
Urea	10.84	189	0.68	0.72	0.57
L-Proline	14.95	156	0.80	0.80	0.46
SA: PA			0.0009*	0.02	0.84

[A]PA, SA, and SA:PA ratio were significantly different between the two groups. *$P < 0.05$.

Table S2. Effect of potential risk factors, including the SA:PA ratio, on day 7 post-transplantation on aGVHD risk.

Variable	Univariate[A] Odds Ratio (95%CI)	P	Multivariate[B] Odds Ratio (95%CI)	P	Multivariate[B] PA Odds Ratio (95%CI)	P	Multivariate[B] SA Odds Ratio (95%CI)	P	Multivariate[C] SA:PA Odds Ratio (95%CI)	P
Sex (Female vs. Male)	1.01 (0.46,2.19)	0.98	NA	NA	NA	NA	NA	NA	NA	NA
Age (Continuous)	1.02 (0.98,1.05)	0.36	NA	NA	NA	NA	NA	NA	NA	NA
Disease (ALL vs. AML)	1.43 (0.67,3.07)	0.46	NA	NA	NA	NA	NA	NA	NA	NA
Disease (NHL vs. ALL)	0.62 (0.15,2.46)	0.66	NA	NA	NA	NA	NA	NA	NA	NA
Status (Other vs. CR1)	0.54 (0.19, 1.5)	0.23	NA	NA	NA	NA	NA	NA	NA	NA
Risk (High vs. Low& Intermediate)	0.97 (0.44,2.11)	0.93	NA	NA	NA	NA	NA	NA	NA	NA
Source (BM and PSC vs. BM or PSC)	3.75 (1.6, 8.81)	0.002*	3.44 (1.45, 8.18)	0.005*	3.46 (1.42, 8.4)	0.006*	3.56 (1.37,9.27)	0.009*	5.13 (1.78,14.76)	0.002*
HLA (Mismatch vs. Match)	1.82 (0.82,4.03)	0.13	NA	NA	NA	NA	NA	NA	NA	NA

ABO blood type (Mismatch vs. Match)	1.48 (0.69,3.19)	0.31	NA	NA	NA	NA	NA	NA	NA	NA
Donor / recipient sex (Mismatch vs. Match)	2.11 (0.96,4.66)	0.06	1.82 (0.80,4.14)	0.15	1.75 (0.76,4.06)	0.19	2.08 (0.85,5.14)	0.11	2.14 (0.79, 5.77)	0.133
Donor sex (Female vs. Male)	0.95 (0.44,2.06)	0.9	NA	NA	NA	NA	NA	NA	NA	NA
Female to male (Yes vs. No)	1.34 (0.58, 3.13)	0.49	NA	NA	NA	NA	NA	NA	NA	NA
MNC (Continuous)	1.03 (0.94, 1.13)	0.48	NA	NA	NA	NA	NA	NA	NA	NA
CD34(+) cells (Continuous)	1.11 (0.89, 1.38)	0.36	NA	NA	NA	NA	NA	NA	NA	NA
Conditioning (TBI / Cy^j vs. BUCy)	0.71 (0.31, 1.61)	0.41	NA	NA	NA	NA	NA	NA	NA	NA
PA (Continuous)	4.42 (1.1, 17.71)	0.03*	NA	NA	4.24 (0.98, 18.35)	0.05	NA	NA	NA	NA
SA (Continuous)	0.08 (0.02, 0.29)	< 0.001*	NA	NA	NA	NA	0.07 (0.02, 0.28)	< 0.001*	NA	NA
SA:PA (High vs. Low, Cutoff = 0.731)	0.09 (0.03, 0.22)	< 0.001*	NA	NA	NA	NA	NS	NS	0.06 (0.02, 0.18)	< 0.001*

Table S3. Effect of potential risk factors, including the SA:PA ratio, on day 7 post-transplantation on overall survival.

Variable	Univariate[A]		Multivariate		Multivariate SA		Multivariate[B] SA:PA	
	Odds Ratio (95%CI)	P	Odds Ratio (95%CI)	P	Odds Ratio (95%CI)	P	Odds Ratio (95%CI)	P
Sex (Female vs. Male)	0.80 (0.45, 1.41)	0.44	NA	NA	NA	NA	NA	NA
Age (Continuous)	0.99 (0.97, 1.01)	0.43	NA	NA	NA	NA	NA	NA
Disease (ALL vs. AML)	0.98 (0.54, 1.79)	0.96	1.36 (0.69, 2.67)	0.37	1.43 (0.74, 2.78)	0.29	1.45 (0.75, 2.82)	0.27

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Variable	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Disease (NHL[c] vs. ALL)	2.61 (1.15, 5.96)	0.02*	1.79 (0.67, 4.75)	0.25	1.64 (0.62, 4.35)	0.32	1.57 (0.58, 4.23)	0.37
Status (Other vs. CR1)	2.69 (1.47, 4.95)	0.001*	1.70 (0.74, 3.92)	0.21	1.69 (0.75, 3.79)	0.21	1.62 (0.71, 3.69)	0.25
Risk (High vs. Low & Intermediate)	1.57 (0.91, 2.73)	0.11	0.98 (0.49, 1.97)	0.96	0.96 (0.48, 1.89)	0.89	1.01 (0.51,1.99)	0.99
Source (BM and PSC vs. BM or PSC)	1.21 (0.67, 2.20)	0.52	NA	NA	NA	NA	NA	NA
HLA (Mismatch vs. Match)	1.98 (1.14, 3.44)	0.015*	2.06 (1.15, 3.66)	0.01*	2.10 (1.18, 3.73)	0.01*	1.97 (1.10, 3.51)	0.02
ABO blood type (Mismatch vs. Match)	1.26 (0.73, 2.22)	0.40	NA	NA	NA	NA	NA	NA
Donor / recipient sex (Mismatch vs. Match)	1.07 (0.61,1.86)	0.82	NA	NA	NA	NA	NA	NA
Donor sex (Female vs. Male)	0.76 (0.43,1.36)	0.36	NA	NA	NA	NA	NA	NA
Female to male (Yes vs. No)	1.10 (0.60, 2.04)	0.75	NA	NA	NA	NA	NA	NA
MNC (Continuous)	1.02 (0.95,1.10)	0.60	NA	NA	NA	NA	NA	NA
CD34(+) cells (Continuous)	0.97 (0.82,1.15	0.74	NA	NA	NA	NA	NA	NA

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Conditioning (TBI / Cy vs. BUCy)	2.31 (1.33,4.05)	0.003*	1.71 (0.80, 3.64)	0.16	1.72 (0.81, 3.63)	0.16	1.90 (0.89, 4.05)	0.09
PA (Continuous)	0.71 (0.26,1.93)	0.50	NA	NA	NA	NA	NA	NA
SA (Continuous)	2.08 (0.94, 4.61)	0.07	NA	NA	1.97 (0.85, 4.55)	0.11	NA	NA
SA:PA (Continuous)	1.95 (0.97, 3.91)	0.06	NA	NA	NA	NA	1.95 (0.92, 4.14)	0.08

[A]In univariate analyses, disease type, disease status, risk stratification, HLA match, and conditioning were associated with overall survival. [B]Multivariate analysis showed that the SA:PA ratio had no significant impact on overall survival ($P = 0.08$). **Abbreviations used** ALL = Acute lymphoblastic leukemia; AML = Acute myelogenous leukemia; NHL = Non-hodgkin`s lymphoma; CR1 = complete remission at first course; BM = Bone marrow; PSC = Peripheral stem cells; MNC = mononuclear cells; values shown indicate number of cells in millions; BU = busulfan; Cy = cyclophosphamide; TBI = total body irradiation; NA denotes "not applicable". 95% CI = 95% confidence interval. *$P < 0.05$.

Table S4. Effect of potential risk factors, including the SA:PA ratio, on day 7 post-transplantation on the risk of relapse after transplantation.

Variable	Univariate[A] Odds Ratio (95% CI)	P	Multivariate Odds Ratio (95% CI)	P	Multivariate SA Odds Ratio (95% CI)	P	Multivariate[B] SA:PA Odds Ratio (95% CI)	P
Sex (Female vs. Male)	0.87 (0.43, 1.78)	0.71	NA	NA	NA	NA	NA	NA
Age (Continuous)	1 (0.97, 1.03)	0.95	NA	NA	NA	NA	NA	NA
Disease (ALL vs. AML)	0.84 (0.38, 1.85)	0.66	1.13 (0.44, 2.89)	0.80	1.16 (0.47, 2.84)	0.75	1.23 (0.51, 2.95)	0.65
Disease (NHL vs. ALL)	3.64 (1.42, 9.32)	0.007*	1.68 (0.50, 5.65)	0.40	1.82 (0.57, 5.75)	0.31	1.51 (0.45, 4.99)	0.50
Status (Other vs. CR1)	3.68 (1.75, 7.77)	0.0006*	2.39 (0.49, 11.60)	0.28	2.34 (0.53, 10.27)	0.26	2.41 (0.58, 10.04)	0.23
Risk (High vs. Low & Intermediate)	2.54 (1.26, 5.15)	0.0095*	1.66 (0.66, 4.20)	0.29	1.54 (0.64, 3.74)	0.34	1.63 (0.70, 3.82)	0.26
Source (BM and PSC vs. BM or PSC)	0.89 (0.40, 1.99)	0.77	NA	NA	NA	NA	NA	NA
HLA (Mismatch vs. Match)	0.94 (0.44, 2.00)	0.87	NA	NA	NA	NA	NA	NA

Variable	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
ABO blood type (Mismatch vs. Match)	1.5 (0.75, 3.01)	0.25	NA	NA	NA	NA	NA	NA
Donor/recipient sex (Mismatch vs. Match)	1.28 (0.62, 2.65)	0.50	NA	NA	NA	NA	NA	NA
Donor sex (Female vs. Male)	0.65 (0.31, 1.37)	0.26	NA	NA	NA	NA	NA	NA
Female to male (Yes vs. No)	1.15 (0.53, 2.48)	0.73	NA	NA	NA	NA	NA	NA
MNC (Continuous)	0.96 (0.88, 1.04)	0.28	NA	NA	NA	NA	NA	NA
CD34(+) cells (Continuous)	1.06 (0.87, 1.3)	0.57	NA	NA	NA	NA	NA	NA
Conditioning (TBI / Cy vs. BUCy)	2.42 (1.20, 4.9)	0.014*	1.03 (0.27, 3.95)	0.96	1 (0.26, 3.89)	1	1.26 (0.36, 4.39)	0.72
PA (Continuous)	0.82 (0.24, 2.75)	0.75	NA	NA	NA	NA	NA	NA
SA (Continuous)	3.2 (1.21, 8.47)	0.019*	NA	NA	2.68 (0.95, 7.58)	0.06	NA	NA
SA:PA (High vs. Low cutoff = 1.3667)	2.11 (1.03, 4.32)	0.04*	NA	NA	NA	NA	2.26 (1.04, 4.91)	0.04*

[A]In univariate analyses, disease type, disease status, risk stratification, and conditioning were associated with relapse. [B]After adjustment for these variables, patients in the group with high SA:PA ratios were significantly more likely to relapse than those in the group with low ratios ($P = 0.04$). **Abbreviations used:** ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; NHL = non-hodgkin`s lymphoma; CR1 = complete remission at first course; BM = bone marrow; PSC = peripheral stem cells; MNC = mononuclear cells; values shown indicate number of cells in millions, BU = busulfan; Cy = cyclophosphamide; TBI = total body irradiation; NA denotes "not applicable". 95% CI=95% confidence interval. *$P < 0.05$.

Table S5. Association of PA, SA, and SA:PA ratio with the occurrence of CMV or EBV infection or bacterial infection within 30 days post- transplantation[A].

Covariable	CMV reactivation			EBV reactivation			Bacterial infection		
	P	HR	95% IC	*P*	HR	95% IC	*P*	HR	95% IC
PA	0.93	0.86	0.03-22.24	0.21	0.004	0-25.41	0.48	2.64	0.18-39.86
SA	0.40	0.28	0.01-5.45	0.18	124.96	0.10-15260	0.86	0.80	0.07-9.51
SA/PA	0.56	2.53	0.11-57,54	0.25	0.01	0-21.22	0.51	2.49	0.17-36.91

[A]Abbreviations used: CMV = cytomegalovirus; EBV = Epstein-Barr virus; 95% CI = 95% confidence interval; HR = hazard ratio