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Supplementary materials

1. Supplementary materials for the methods

Preparation of protein samples

The sample preparation before MS analysis was performed as follows. After sample thawing, 100 µl of joint fluid was reduced with DTT, alkylated with IAA, and protein sample was digested by FASP (filter-aided sample preparation) protocal[1] using a 10 kDa filter, trypsin was added with 1: 50 of total protein. Protein digestion was stopped by the addition of 1% formic acid after overnight at 37 °C, then peptide mixture was eluted by centrifugation and dried under vacuum.

Liquid chromatography (LC)-MS/MS

Samples were analyzed on Orbitrap Fusion Lumos (Thermo) coupled with an Easy-nLC 1000 liquid chromatography system (Thermo). Peptide mixtures were re-dissolved in loading buffer (0.1% formic acid in water), and eluted from a 360-µm I.D. x 2 cm C18 trap column and separated on a homemade 150 µm I.D. x 15 cm column (C18, 1.9 µm, 120 Å, Dr. Maisch GmbH) with a gradient of 5-35% mobile phase (acetonitrile and 0.1% formic acid) at a flow rate of 600 nl/min. Data-dependent acquisition (DDA) performed 120K resolution MS scan and top speed mode was selected for fragmentation in the higher-energy collisional dissociation (HCD) cell at a normalized collision energy of 32%, and then fragment ions were measured in the linear ion trap, the MS or MS/MS AGC target value was set at 5e5 with 50ms or 5e3 with 35ms of max injection time, respectively. Dynamic exclusion time was set as 18s.

Protein identification and quantification

MS raw files were searched against the National Center for Biotechnology Information (NCBI) Ref-seq human proteome database (updated on 04/07/2013, 32,015 entries) using the Proteome Discoverer (version 2.0, Thermo Fisher Scientific, Rockford, IL, USA) with the Mascot search engine (Matrix Science, version 2.3.01). The mass tolerances were 20 ppm for precursor ions and 0.5 Da for product ions. Oxidation (M), Acetyl (Protein-N term) were chosen as variable modifications, carbamidomethyl (C) was set as a fixed modification, and two missed cleavage sites for trypsin was allowed. The data were searched against a decoy database and peptide false discovery rate (FDR) was set at 1% using percolator validation. Proteins with at least one unique and two strict peptides were selected for further analysis. A label-free, intensity-based absolute quantification (iBAQ) approach[2] was used to quantify proteins based on the area under the curve (AUC) of the precursor ions. The fraction of total (FOT) was used to represent the normalized abundance of a protein across experiments, which was defined as a protein's iBAQ divided by the total iBAQ of all identified proteins in one experiment. The FOT was further multiplied by 10^5 to obtain the intensity-based FOT (iFOT) and all missing values were replaced with zeros[3].

Quantitative verification of selected biomarkers with PRM methods

The sequences of the labeled peptides used for PRM are shown in **Table S1.**

For data-dependent acquisition, peptides that contained labeled peptides were loaded on a trap column (150 μm I.D. × 2 cm C18) and separated on a homemade 150 μm I.D. × 10 cm column (C18, 1.9 μm, 120 Å, Dr. Maisch GmbH) with a nonlinear 5–35% acetonitrile (ACN) gradient at 800 nl/min for 30 min. MS scan performed 120K resolution at AGC target of $3e^6$ with a maximum injection time of 80 ms and followed by up to 20 MS/MS scans with higher-energy collisional dissociation(AGC target 2e4, maximum injection time 20 ms, isolation window 1.6 m/z, and normalized collision energy 27%), detected in Orbitrap (R = 15,000 at 200 m/z). The dynamic exclusion time was enabled at 12 s.

The peptides of the target protein were identified and focused in the exact retain time windows with a DDA scan. The parent ions in the table were monitored on an Easy-nLC system (Thermo) coupled with a Q-Exactive HF mass spectrometer (Thermo). Peptides were separated on a homemade 150 μm I.D. × 10 cm column (C18, 1.9 μm, 120 Å, Dr. Maisch GmbH) with a nonlinear 5–35% ACN gradient at 800 nl/min for 30 min.

The peptides were analyzed using full scan plus PRM modes. MS scan performed 60K resolution at AGC target of $3e^6$ with a maximum injection time of 80 ms and followed by 20 PRM scans with higher-energy collisional dissociation (AGC target 5e4, maximum injection time 100 ms, isolation window 1.6 m/z, and normalized collision energy 27%), detected in Orbitrap (R = 15,000 at 200 m/z). MS2 methods were followed by a timed inclusion list containing the target precursor m/z value and charge and a 2-min retention time window that was determined from the DDA results containing the unlabeled and labeled peptides of the target protein. All of the raw files were processed using Skyline 3.1 (Skyline is a freely-available, open-source Windows client application at https://skyline.ms.). The intensities of the top-three fragment ions were summed for peptide quantification. The absolute quantification results of each peptide were calculated using the linear calibration curve of the labeled peptide.

2. Supplementary materials for the tables

Table S1 Sequence of the labeled peptides for parallel reaction monitoring

Peptides	Gene	Sequence
MNDA -P1	MNDA	EASSVSDFNQNFEVPNR*
MNDA -P2	MNDA	VFDINLK*
PRTN3-P1	PRTN3	LFPDFFTR*
PRTN3-P2	PRTN3	LVNVVLGAHNVR*

*labeled amino acid. MNDA, myeloid nuclear differentiation antigen; and PRTN3, polymorphonuclear leukocyte serine protease 3

Table S2 Details of the cases with inconsistent results between cluster analysis and the MSIS diagnosis

Patient number	infected 44	infected 33	infected 49	infected 19	infected 51	noninfected 38	noninfected 63
Diagnosis*	PJI	PJI	PJI	PJI	PJI	non-PJI	non-PJI
Cluster analysis#	non-PJI	non-PJI	non-PJI	non-PJI	non-PJI	PJI	PJI
Joint	Knee	Hip	Hip	Knee	Hip	Knee	Knee
Sex	male	female	male	female	female	female	male
Age (years)	70	67	72	67	63	72	56
Height (m)	1.7	1.56	1.67	1.65	1.62	1.55	1.8
Weight (kg)	85	59	70	80	55	54	105
BMI (kg/m^2)	29.41	24.24	25.10	29.38	20.96	22.48	32.41
Brief history	Sixteen-month history of left knee arthroplasty and a 3-month history of left knee revision arthroplasty; Now, the patient	Five-year history of right hip arthroplasty; The patient presents with a 2-year history of right hip pain without obvious reasons	Twenty-two-month history of left hip arthroplasty; A 6-month history of left hip pain; The patient suffered from	One-month history of incision disunion after left knee arthroplasty	Four-year history of right hip arthroplasty; The patient presents with a 2-month history of right hip pain and swelling	Patient had left knee arthroplasty 28 days prior in a local hospital and suffered from fever (the maximum temperature was 39 degrees Celsius) on the 3rd day after surgery; Then, she underwent DAIR surgery	One month after right knee arthroplasty with persistent pain in the joint, the patient

	presents with a 3-month history of left knee pain and swelling		left hip swelling and fever for more than 1 month			11 days prior and suffered from fever for an additional 6 days	presents with right knee swelling for 5 days
Comorbidity	Mildly damaged liver function because of antifungal drug usage; Mild hypertension	Denied any comorbidity	Forty-year history of right tibial fracture	Eight years after right knee arthroplasty	One month after radiofrequency ablation of the heart; a 3-year history of hysterectomy because of cervical cancer; 4 years of diabetes with satisfactory control; a history of anemia, thrombocytosis and reflux esophagitis	Denied any comorbidity	Mild hypertension
History of inflammatory arthritis	Deny	Deny	Deny	Deny	Deny	Deny	Deny
Recent antibiotic usage (within 2 weeks)	voriconazole	without	without	without	without	vancomycin (No obvious effect)	cephalosporin
Sinus track	without	without	without	with	without	without	without
ESR (mm/h)	65	68	95	11	98	17	7
CRP (mg/L)	4.28	33.17	22.71	1.00	58.37	9.60	3.13
IL-6 (pg/ml)	4.78	19.28	39.4	1.73	62.32	12.59	2
Fibrinogen (g/L)	4.08	5.03	7.82	5.44	NA	3.85	2.5
D-dimer (μg/ml)	1.70	1.52	3.41	0.78	NA	1.71	NA
LE strip test (Before centrifugation)†	500	500	500	NA	500	500	25
LE strip test (After centrifugation)†	250	500	25	25	25	75	25
Synovial WBC count (cells/μL)	2880	16000	24800	1250	12000	Not reported by the clinical medical center because the synovial fluid was too thick (approximately 25-30/HP)	3-5/HP, Not reported by the clinical medical center because the synovial fluid was too thick (approximately 3-5/HP)
PMN%	0.72	0.82	Not reported by the clinical medical center	Not reported by the clinical medical center	0.30	Not reported by the clinical medical center because the synovial fluid was too thick	Not reported by the clinical medical center because the synovial fluid was too thick
α-Defensin (μg/mL)	40.17	1045.48	0.863	NA	7.44	139.10	1.421
Histology§	NA	<5	>15	<5	NA	NA	NA
Culture	Candida parapsilosis	Staphylococcus epidermidis	negative	Staphylococcus sciuri	Streptococcus agalactiae	Negative	Negative
Treatment	Two-stage revision	Two-stage revision	Two-stage revision	DAIR	No show anymore	Suspicion of drug fever and achieved remission after drug withdrawal	symptomatic treatment (without antibiotic usage)
Follow-up	cure	cure	cure	cure	lost to follow-up	cure	cure

*Diagnosis was based on the modified MSIS criteria; #Classified by label-free cluster analysis; †The brand of leukocyte esterase strip used for testing was AUTION Sticks, 10 PA, ARKRAY, Japan; §Neutrophils per high-power field in 5 high-power fields observed from the histologic analysis of periprosthetic tissue at 400× magnification. PJI, periprosthetic joint infection; DAIR, debridement and implant retention; NA, not available.

3. Supplementary materials for the figures

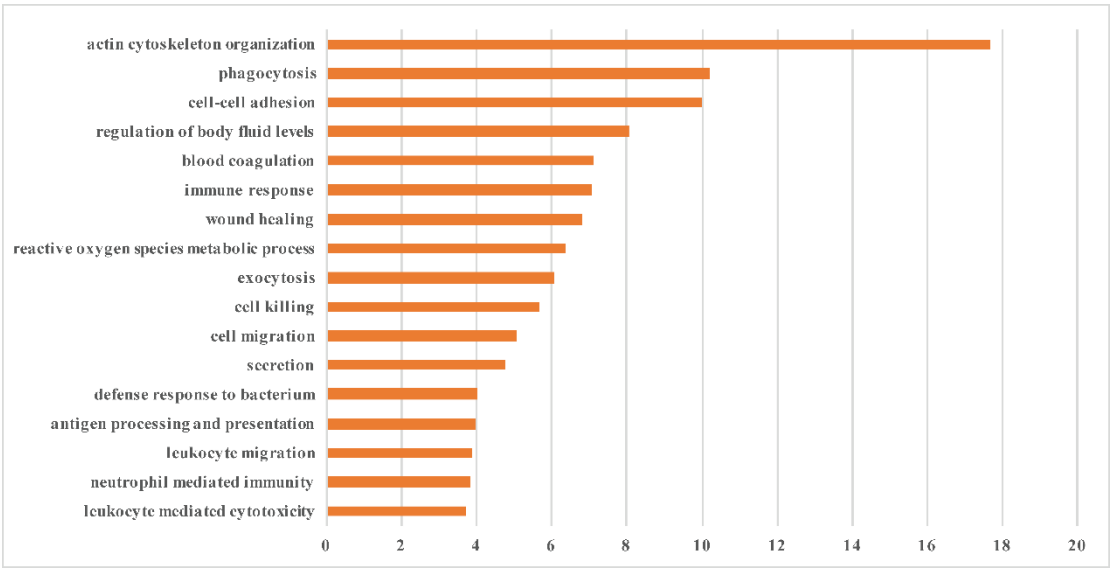

Figure S1 GO analysis of the upregulated proteins.

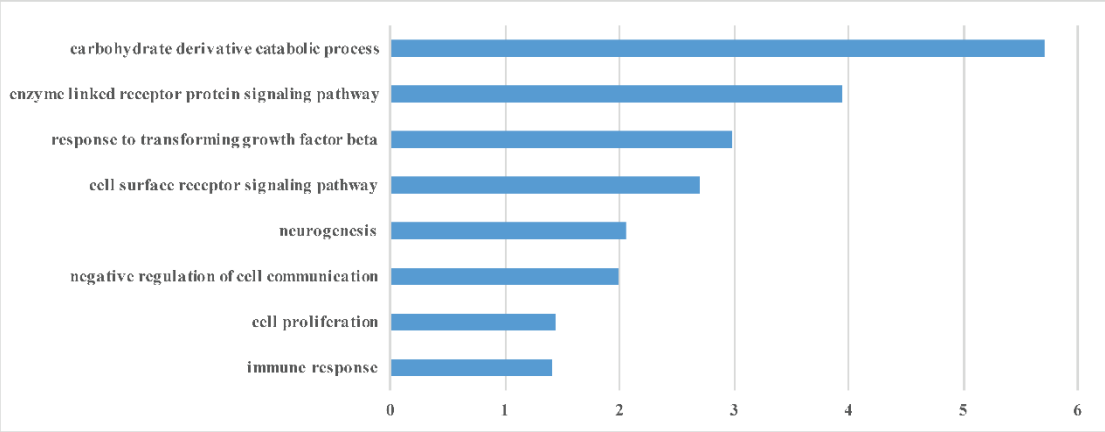

Figure S2 GO analysis of the downregulated proteins.

References

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