Supplementary Material 1

Mass spectrometry (MS)

Materials. Water (Optima LCMS grade), acetonitrile (Optima LCMS grade), formic acid (Optima HPLC MS, grade ≥99.5%), Pierce MS grade trypsin protease and dithiothreitol (DTT) were purchased from Thermo Fisher Scientific (Waltham, USA). Iodoacetamide was purchased from Sigma-Aldrich (Steinheim, Germany). Heavy isotope-labeled sequences of the target peptides were purchased from Pepscan (Lelystad, Netherlands) with 13C- and 15N- labeled C-terminal Arginine or Lysine. These peptides serving as internal standards (called stable isotope standard (SIS) or heavy peptides) were spiked into the sample digests, and the ratio between endogenous (native, light) and internal standard peptide was recorded for subsequent analysis.

Samples preparation. The 259 individual samples and pooled QC samples were processed concomitantly and digested in 96-well plates according to the protocol described previously (1). All the samples were distributed in a random order into three different 96 well plates. 5μL of each clinical plasma sample was added to 20μL of 8M Urea/2.5% n-propanol/300mM Tris/10mM DTT pH 8.5 and incubated at 37°C for one hour. 500mM iodoacetamide prepared in 1M ammonium bicarbonate was added to each sample well and incubated in the dark at room temperature for one hour, and 50mM Tris/5mM CaCl2 pH 8.0 were then added to each well. Trypsin was rehydrated with 25mM acetic acid, mixed at a ratio of 1/10 (total protein content/protease) and incubated at 37°C for 20 hours. The digestion process was finally quenched by adding 2μL formic acid. Glucagon (1ng/μL) and standard heavy peptides were then added before the preparation was injected.

Multi reaction monitoring (MRM) assays optimization. 62 proteins known to be involved in inflammatory or infection processes were selected based on data in the literature. In order to optimize the MRM assays, up to 4 proteotypic peptides corresponding to each of the proteins on the list were selected *in silico* using the SRM Atlas database (http://www.srmatlas.org). Tryptic digest predictions were performed with Skyline software (MacCoss Lab Software) using the UniProt accession number for each of the proteins targeted. Only the peptides ranging between 5 and 20 amino acids in size and having no predictable missed-cleavage sites were selected. Peptides described with potential modifications (PTMs, oxidation sites) were avoided as much as possible. Only 10 peptides were kept

in the final panel of 62 peptides because of their analytical performances and were carefully analyzed. Peptides fulfilling the criteria mentioned above with the highest final suitability score (Adj SS) given by SRM Atlas software were selected. A total number of 230 peptides were selected and then synthesized in heavy isotope labeled crude quality form for developing the MRM assays.

MRM assays were optimized on a TSQ Quantiva (Thermo Fisher Scientific) triple quadrupole mass spectrometer coupled with HPLC Ultimate 3000 (Thermo Fisher Scientific). Reverse phase separation was carried out on a 150 mm×2.1 mm Accucore aQ column (Thermo Fisher Scientific) at a temperature of 50°C in a 20-minute linear gradient from 5 to 40% B, with a total run time of 45 minutes (solvent A: water/0.2% formic acid, solvent B: acetonitrile/0.2% formic acid). The flow rate was set at 240 µL/min during the linear gradient. 160 µL of digested sample were injected each time, and two columns were run in parallel. The first one was regenerated while the elution gradient was running on the second one.

MRM transitions were acquired in Q1 and Q3 operating at unit resolution (m/z window 0.7 full width at half maximum), the collision gas pressure in Q2 was set to 1.5 mTorr, and the collision energies were predicted by Skyline software using the equations related to the mass to charge ratios for TSQ Quantiva. Spray voltage was set at 3500 V, sheath gas at 50 (Arb), auxiliary gas at 30 (Arb), sweep gas at 4 (Arb), the ion transfer tube at 400 °C, and the vaporizer at 400°C. A scheduled MRM mode with two-minute detection windows was used. The cycle time was 1.3s, and calibrated radiofrequency and S-lens values were used.

Optimization of MRM assays was performed on the heavy labeled synthetic peptides both in water/0.1% formic acid and serum matrix. The four highest transitions that did not show any interferences at the expected retention time in serum were chosen for clinical samples measurement, and the best performing proteotypic peptide (intensity, signal to noise ratio, no interference) was chosen to quantify the protein.

MS data analysis. All the raw data generated on the triple quadrupole mass spectrometer were imported to Skyline software for data analysis. All the peptide transitions detected were summed and the ratio between endogenous transitions and internal standard transitions sum was calculated and used as an arbitrary unit (Arb unit) for the subsequent analysis.

References

1.   A. Incamps, C. Saez-Boiteau, S. L. Tiede, P. Rebillard, J. Schulte, J. Vialaret, A. Beinrucker, S. Lehmann and C. Hirtz: Impact of biological matrix on inflammatory protein biomarker quantification based on targeted mass spectrometry. *Bioanalysis* 10(17):1383-1399, 2018.

Supplementary Material 2

MS measurement quality control.

To check the quality of the data and the performances of the instrument used, QC samples (sample pools) spiked with the same SIS set of peptides as the clinical samples were monitored throughout the LC-MRM runs. One blank injection of water/0.2% formic acid and one QC sample were analyzed every sixth sample. A total number of 50 QCs were quantified on 62 peptides, but 15 peptides could not be quantified because the endogenous peptide concentrations present in the QC samples were too low. The LC-MRM variability of the 47 peptides measured was found to be less than 17%. The intra-plate coefficients of variation (CVs) ranged between 1.2% and 16.9% and the total CVs including all the QCs ranged between 1.4% and 16.6%, with a median value of 8%. The light/heavy CRP peptide ratio in the 50 QCs showed a CV of 4.0%, which confirms the quality of our technical approach. A PCA did not reveal any clusters attributable to the number of plates. LC-MRM quality assessment is a key to biomarkers research (1), and the present results confirm that the method used was reproducible.

References

1.   M. Vogeser and C. Seger: Quality management in clinical application of mass spectrometry measurement systems. *Clin Biochem* 49(13-14):947-54, 2016.
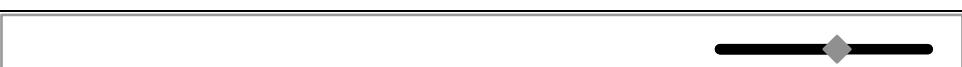
Supplementary Material Table 1. Example of 8 out of the 62 proteins screened, with the peptide monitored, m/z of the precursor, collision energy, charge and retention time.

Uniprot ID	Protein Name	Gene Name	Peptide Sequence	Precursor Mz	Collision Energy	Precursor Charge	Retention Time
P02765	Alpha-2-HS-glycoprotein	AHSG	FSVVYAK	407.2289	17.2	2	13.7
P04196	Histidine-rich glycoprotein	HRG	DGYLFQLLR	562.8086	22.4	2	20.6
P05362	**Intercellular adhesion molecule 1**	**ICAM1**	**LLGIETPLPK**	**540.8368**	**21.7**	**2**	**17.9**
P07148	Fatty acid-binding protein, liver	FABP1	AIGLPEELIQK	605.8557	23.9	2	17.6
P07996	**Thrombospondin-1**	**THBS1**	**FVFGTTPEDILR**	**697.8694**	**27**	**2**	**19.5**
P15169	Carboxypeptidase N catalytic chain	CPN1	VQNECPGITR	587.2877	23.3	2	11.0
P62834	Ras-related protein Rap-1A	RAP1A	EQGQNLAR	458.2358	18.9	2	9.6
P18206	**Vinculin**	**VCL**	**GNDIIAAAK**	**436.7454**	**18.2**	**2**	**12.2**

Biomarker	Timepoint	AUC (with 95%-CI)	Sensitivity	Specificity
		0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1		
PCT & sICAM-1	T0		0.700	0.833
	T1		0.800	0.792
	T2		0.700	1.000
	T3		0.900	0.917
	T4		0.900	0.750
PCT & thrombospondin-1	T0		0.700	0.333
	T1		0.400	0.792
	T2		0.900	0.375
	T3		1.000	0.750
	T4		0.900	0.708
PCT & vinculin	T0		0.900	0.417
	T1		0.800	0.625
	T2		0.900	0.667
	T3		1.000	0.583
	T4		0.900	0.750

MR-proADM & thrombospondin	T0		0.500	0.913
	T1		0.800	0.565
	T2		1.000	0.565
	T3		0.800	0.783
	T4		0.900	0.913
MR-proADM & vinculin	T0		0.700	0.783
	T1		0.700	0.826
	T2		0.900	0.609
	T3		0.600	0.913
	T4		1.000	0.739
PCT, sICAM-1 & MR-proADM	T0		0.900	0.739
	T1		0.800	0.870
	T2		0.800	0.957
	T3		0.900	0.913
	T4		0.700	0.913
IL-17A, sICAM-1 & MR-proADM	T0		0.889	0.682
	T1		0.778	0.864
	T2		0.778	0.955
	T3		0.889	0.864

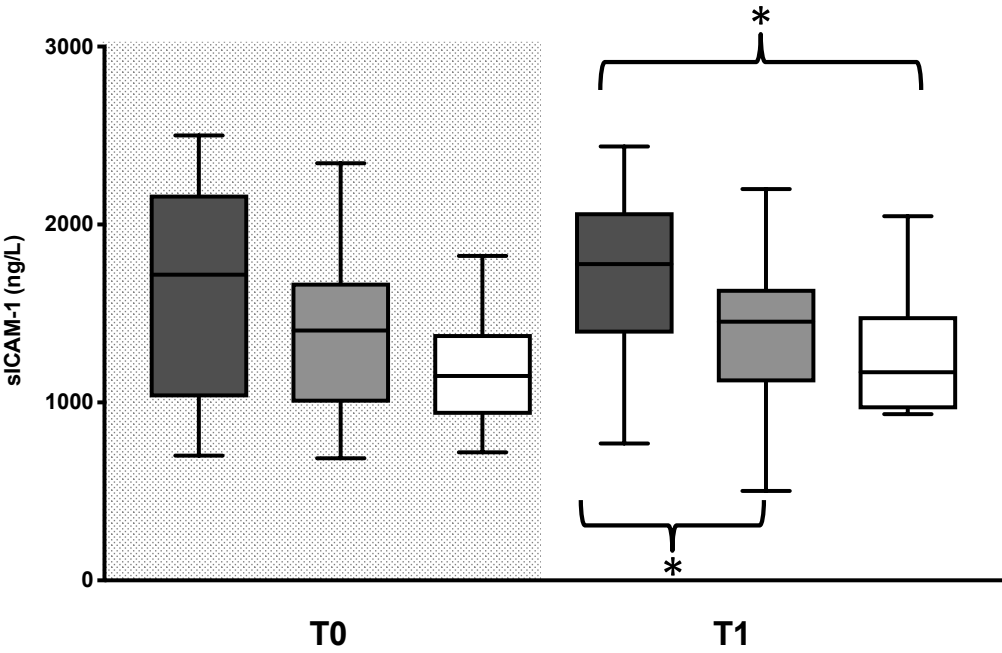|			Sensitivity	Specificity
	T4		0.778	0.909
IL-17A, sICAM-1, MR-proADM & PCT	T0		0.889	0.682
	T1		0.667	0.955
	T2		0.778	0.955
	T3		0.778	1.000
	T4		0.778	0.909

Legends: ROC-analyses for fungally infected vs. fungally colonized or patients without any fungal findings. Data are given as AUCs with 95%-confidence intervals (CI) or absolute values for sensitivity and specificity. Abbreviations: AUC, area under the curve; CI, confidence interval

Supplementary Material Figure 1

(A)
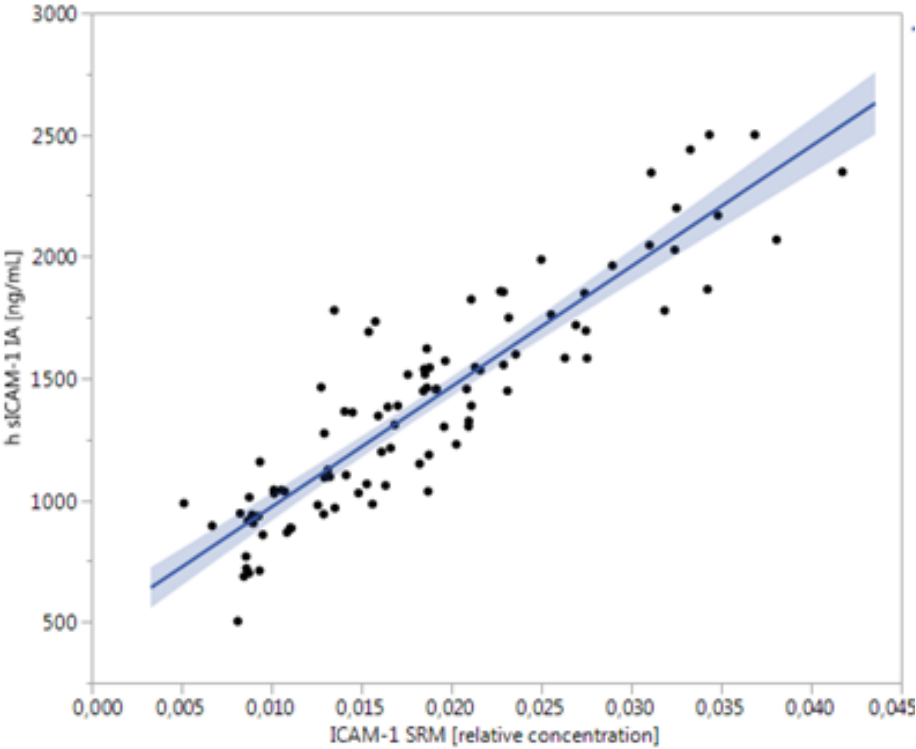
(B)

(C)
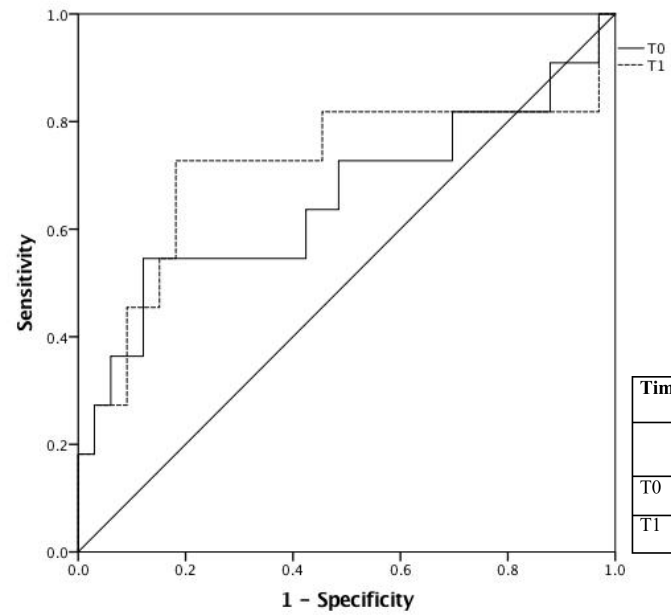
Timepoint	AUC (with 95%-CI)											Sensitivity	Specificity	Best cut-off value (ng/l)
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1			
T0												0.545	0.879	1705
T1												0.727	0.818	1591

Supplementary Material Figure 1: Immunoassay-based measurements of plasmatic sICAM-1 concentrations for the detection of an IFI in patients with septic shock.

(A) Correlation of a subset of 95 samples (time points T0 and T1) measured by Platinum ELISA (eBioscience, Thermo Fisher Scientific) and mass spectrometry (MRM analysis). Spearman correlation provide a correlation factor of 0.89 (Fisher 95% confidence interval 0.837 to 0.927)

(B) Plasma concentrations of sICAM-1 were measured in patients suffering from septic shock with an invasive fungal infection (IFI, dark grey box), a fungal colonization (light grey box) or without any fungal findings (white box). Plasma samples were collected at the onset of septic shock (T0) and 1 day (T1) afterwards. Data in box plots are given as median, 25th percentile, 75th percentile with the 10th as well as 90th percentile at the end of the whiskers. Concerning symbolism and higher orders of significance: $p < 0.05$: *.

(C) Receiver operating characteristic (ROC) analysis with sICAM-1 in all participating patients at septic shock onset (T0) and 1 day (T1) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both, patients with a fungal colonization as well as patients without any fungal isolates served as controls for this ROC-analysis. Abbreviations: AUC, area under the curve; CI, confidence interval

Supplementary Material Figure 2

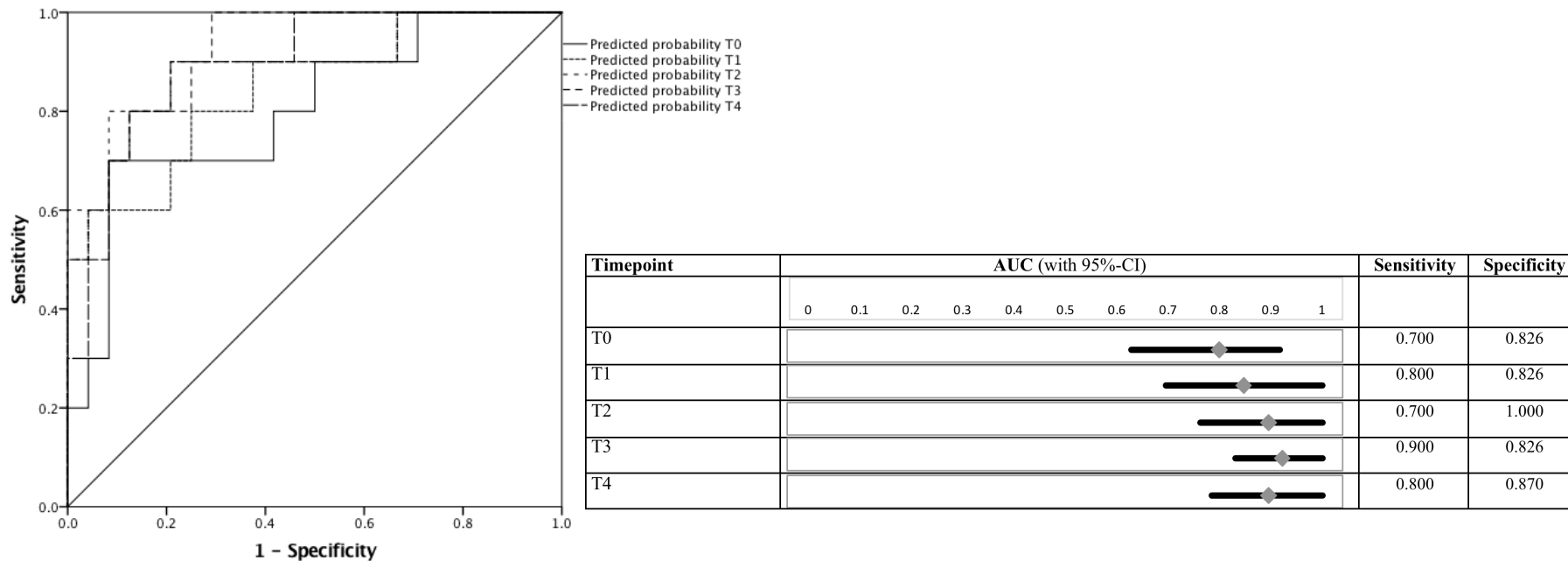| Timepoint	AUC (with 95%-CI)											Sensitivity	Specificity
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1		
T0												0.700	0.826
T1												0.800	0.826
T2												0.700	1.000
T3												0.900	0.826
T4												0.800	0.870

Supplementary Material Figure 2: ROC-analyses for combined measurements of sICAM-1, thrombospondin-1 and vinculin for the detection of an IFI in patients with septic shock.

Receiver operating characteristic (ROC) analyses with sICAM-1, thrombospondin-1 and vinculin in all participating patients at septic shock onset (T0), day 1 (T1), day 2 (T2), day 7 (T3) and 14 day (T4) afterwards with regard to the prediction of an invasive fungal infection (IFI) up to day 28. Patients suffering from an invasive fungal infection (IFI) represented the target group, whereas both, patients with a fungal colonization as well as patients without any fungal isolates served as controls for these ROC-analyses. Abbreviations: AUC, area under the curve; CI, confidence interval

Supplementary Material 3: STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5-6*
Bias	9	Describe any efforts to address potential sources of bias	-
Study size	10	Explain how the study size was arrived at	-

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	-
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-22
		(*b*) Report category boundaries when continuous variables were categorized	-
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-22
Discussion			
Key results	18	Summarise key results with reference to study objectives	23
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	26
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	23-26
Generalisability	21	Discuss the generalisability (external validity) of the study results	26
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	27

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.