Longitudinal Assessment of Immunoglobulin Response and Disease Progression in Critically ill Patients with Community Acquired Pneumonia

Supplementary material

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Supplemental methods

1. Diagnostic and microbiological criteria for Community Acquired Pneumonia (CAP)

1.1 Diagnostic criteria for Community Acquired Pneumonia

In the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) cohort we prospectively collected data on infections of patients admitted to the ICU. We performed a post-hoc adjudication of the likelihood that an infection had truly occurred. The diagnostic criteria used in the MARS cohort for the adjudication of a CAP diagnosis are outlined below. In our current study, we selected only patients with CAP and an infection likelihood of probable or definite. We only selected patients with an infection likelihood of probable or definite, as a well-curated cohort is preferred for etiological research questions. Additionally, other criteria applied to be included in this study (disease severity criteria and >72 hours of follow-up).

Definition: Pneumonia that develops in the home environment or within the first 48 hours of hospital/ICU admission.

Possible

Ambiguous abnormalities on radiological examination

AND

Low clinical suspicion with one or more of the following clinical criteria:

− Cough
− (New or renewed) production of purulent sputum or a change in the nature of sputum
− Fever or hypothermia
− Leukocytosis
− Elevated CRP (>30 mg/L)
− Hypoxemia (pO2 < 60 mm Hg)

Probable

Clear new or progressive infiltrative abnormalities, consolidation, haziness, cavitation, or pleural effusion on radiological examination

AND

High clinical suspicion with two or more of the above clinical criteria, with one of the following findings:

− (Coarse) crackles or dullness on physical examination
− Positive rapid antigen tests (*S. pneumoniae, Legionella pneumophila*)

Definite

Clear new or progressive infiltrative abnormalities, consolidation, haziness, cavitation, or pleural effusion on radiological examination

AND

High clinical suspicion (see criteria under "probable")

AND

Proven pathogen, with one or more of the following criteria:

− Positive blood culture with a pathogen consistent with pneumonia (note: this excludes pathogens like CNS and enterococcus spp.)
− Detection based on quantitative culture of a pathogen in a bronchoscopic aspirate (thresholds of 10^4 in BAL* and 10^3 in PSB)
− Isolation of a virus or detection of viral antigen in respiratory secretions
− Positive serology, i.e., a diagnostic antibody titer in a single serum sample (IgM) or a fourfold rise in titer in a paired serum (IgG) for a pathogenic microorganism
− Histopathological evidence of pneumonia

1.2 Diagnostic criteria for identification of causative pathogen type

In our study we used the data available from routine microbiological testing. In the contributing tertiary intensive care units, the following microbiological tests are obtained for all admitted patients:

1. 2 sets of blood cultures
2. 1 sputum culture
3. qPCR during influenza (and later SARS-CoV-2) season
4. Urine antigen tests

When these tests are unable to identify a causative pathogen additional testing is performed:

1. With Multiplex PCR* (in all patients where the causative pathogen could not be identified)
2. With bronchoalveolar lavage for PCR and additional cultures (only in patients where the causative pathogen could not be identified AND who do not recover sufficiently)

Causative pathogens were allocated based on the following:
- Any pathogen, known to cause CAP, that was identified from a blood culture (above relevant quantification thresholds) was allocated as the causative pathogen.
- Any pathogen, known to cause CAP, that was identified from a sputum culture (above quantification thresholds), in absence of a positive blood culture, regardless of known prior colonization, was allocated as a causative pathogen.
- Any pathogen identified using a urine antigen test or PCR was considered a causative pathogen. However, if *S. pneumoniae* was identified using a urine antigen test, this could be considered a non-relevant finding if the patient had a known recent pneumococcal infection, and another causative pathogen had been identified.

2. Statistical analysis

2.1 Rationale for the primary and reciprocal model

We focused on estimating the relationship between Ig-level temporal trends and disease severity directly, rather than their association with mortality. Since Ig-level trends are closely tied to disease severity, analyzing their association with mortality could introduce bias. That is, as patients' conditions worsen their Ig-levels tend to decline, due to factors like increased vascular leakage and higher cumulative fluid balance. Therefore, assessing mortality without accounting for disease progression would not reflect whether low Ig-levels contribute to a poorer disease course, but rather reflect the impact of worsening disease on Ig-levels. However, since disease progression lies in the causal pathway between Ig levels and mortality, adjusting for it would remove any existing association between Ig levels and mortality.

To avoid these issues that arise from assessing longitudinal measurements, we assessed the direct relation between low Ig-levels and disease progression. We estimated the effect of longitudinal Ig-levels (measured on days 0, 3, and 7) on the temporal trend of SOFA scores (measured on days 2, 4, and 8), while adjusting for SOFA score at day 1 (see 1.3). To estimate the residual bias caused by the reciprocal

effect of disease progression on Ig-levels (e.g., SOFA score on day 2 influencing SOFA score on day 4 and Ig levels on day 3), we conducted a sensitivity analysis using a reciprocal model (see 1.2).

2.2 Visual representation of the primary model and reciprocal model

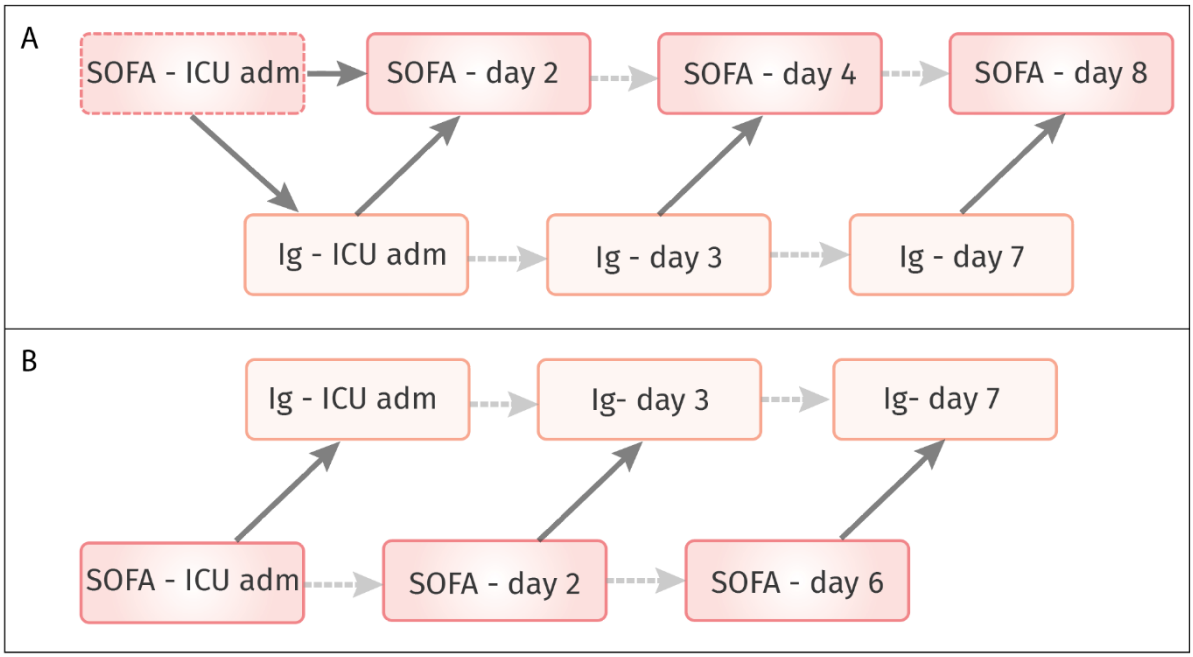

Figure represents the primary model and the reciprocal model. Ig: immunoglobulin G or M plasma level. SOFA: Sequential organ failure score (minus central nervous system score). The dashed line represents the inclusion of ICU admission SOFA score as a confounder (note that this model also included age as a confounder).

2.3 Within- and between-patient coefficient

We used a time-varying covariate (Ig level) to estimate SOFA score trends over time. In a time-varying covariate model, the coefficient reflects both the effect of Ig level differences between patients and the effect of changes in Ig levels within a patient. To distinguish these effects, we separated the time-varying covariate into two components: between-patient and within-patient.

The mean Ig level per patient, representing the between-patient component, is not time-varying. The within-patient component is the difference between the Ig level on days 0, 3, or 7 and the patient's mean Ig level, making it time-varying (with the between-patient difference being 0).

2.4 Numerical example of the between-patient and within-patient effects of IgG on SOFA score

There was a statistically significant, but not clinically relevant between-patient effect of IgG on SOFA scores, and no observable effect of a further within-patient decline. For example: if patient A has a mean IgG-level of 3 g/L, and patient B has a mean IgG-level of 16 g/L, there was an SOFA-score difference of 0.07*13=0.9 at ICU admission. Thus, a very large difference in overall IgG-level per patient had a very small effect on SOFA scores. A within-patient significant effect would have suggested an instantaneous effect on SOFA-score: if the IgG-level of patient A or B decreased with 1 g/L from day 0 to day 1, their SOFA-score increased with 0.04, instantaneously, on day 1, but this effect was not significant.

2.5 Handling of missing data

The percentage of missing data was low. There was no missingness in demographics, disease severity scores, or outcome data. Ig-levels could be determined on nearly all target sample days: 241/255 measurements on day 1, 255/255 on day 3 and 177/177 on day 7. As mixed models are known to sufficiently handle missing data, we did not impute the missing measurement data at day 0.

Supplemental Figure

Figure S1. Study flow-chart

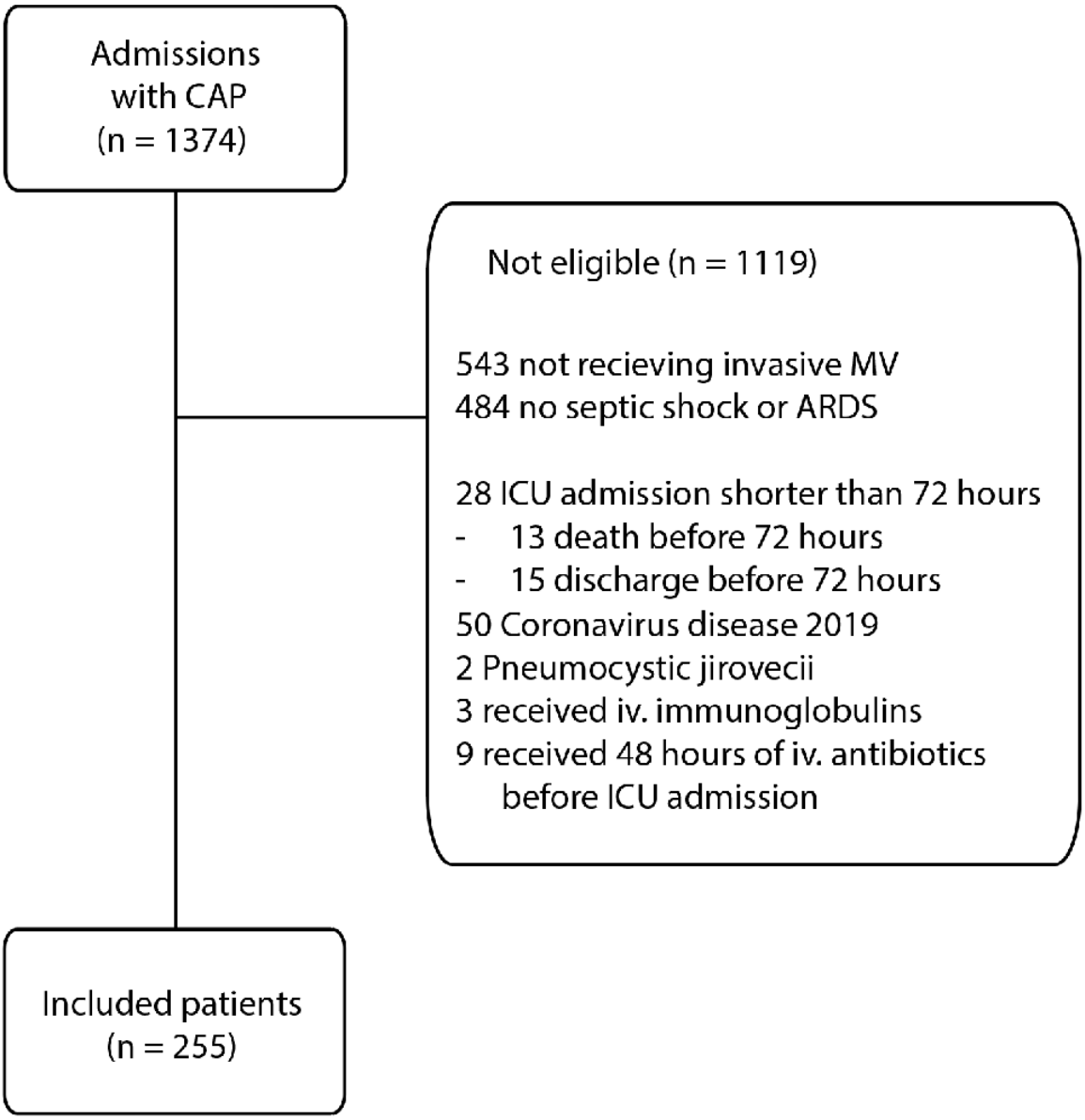

Figure S2. Patients status during ICU admission

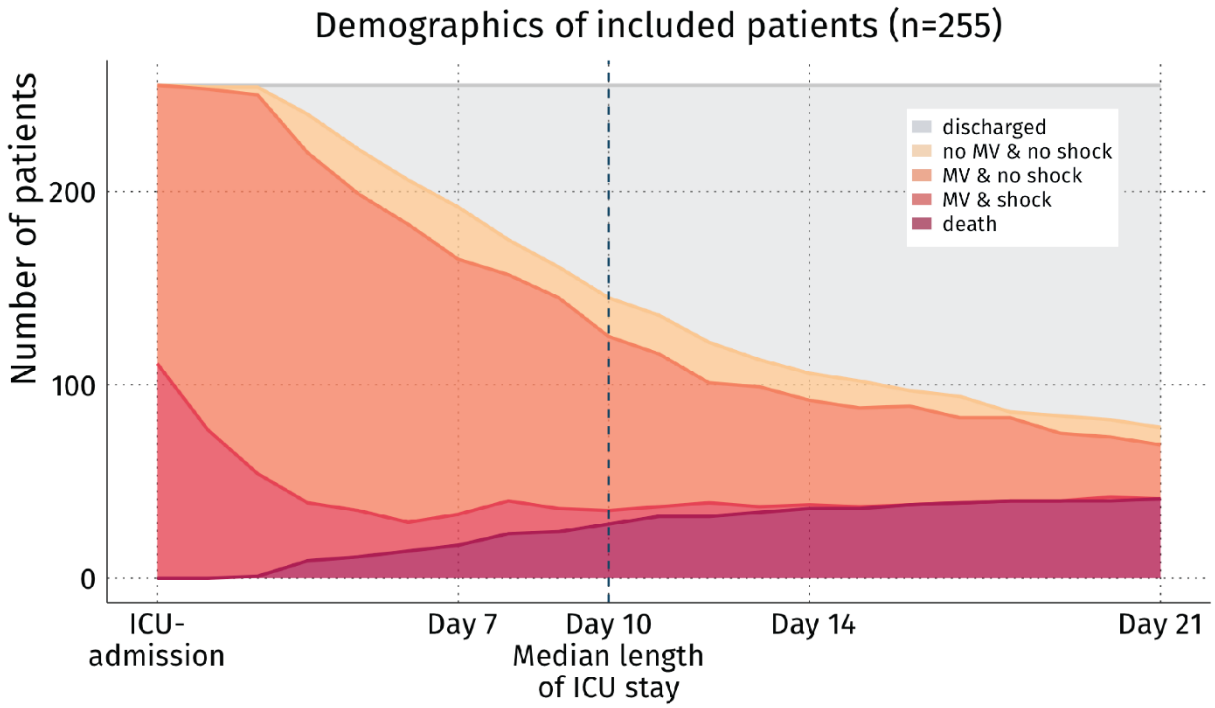

Figure S2 represents the status of the included patients during their ICU admission until ICU day 21. Shock refers to septic shock, defined as sepsis (an infection with a sequential organ failure assessment score increase >2), and requirement for vasopressors, and lactate > 2 mmol/L. ICU: Intensive Care Unit; MV: invasive mechanical ventilation.

Figure S3. Estimated disease progression across different clinical scenarios for IgM

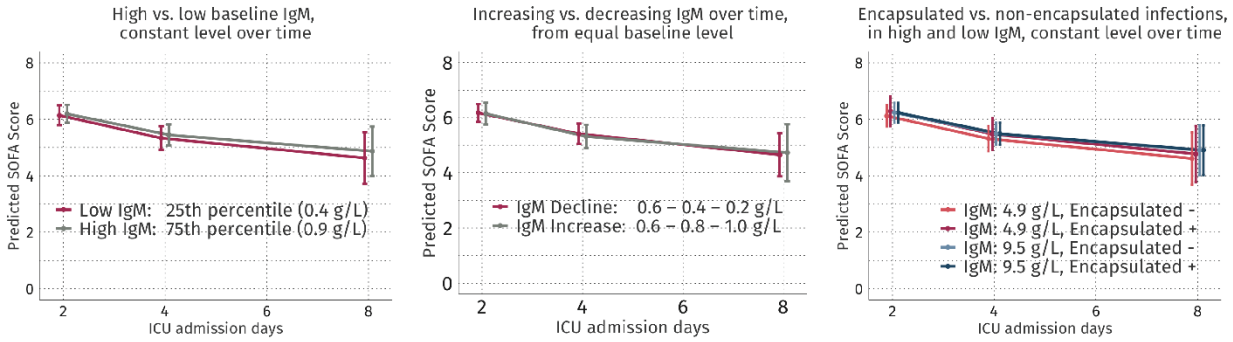

Figure S3 represents mean changes in predicted SOFA-score estimated with a generalized linear mixed-effects model for repeated measures, reported for all patients with (A) high vs. low baseline IgM which remains constant over time, (B) increasing vs decreasing IgM over time, from an equal baseline level, and (C) patients with encapsulated vs. non-encapsulated infections in the context of a high or low constant IgG-level. The I bars represent 95% confidence intervals

Supplemental tables

Table S1. Patient characteristics stratified for encapsulated vs. non-encapsulated infections

	Infection caused by *S. pneumoniae or H. influenzae* (n = 82)	**Other or no causative pathogen identified (n = 173)**	**p-value[(a)]**
Demographics			
Age (median, IQR)	63 [52.2 – 70]	61 [48 – 70]	0.28
Male (n,%)	52 (63)	107 (62)	0.89
Comorbidities (n,%)			
Diabetes mellitus	*13 (20)*	*32 (22)*	*0.72*
Chronic obstructive pulmonary disease	*23 (35)*	*29 (20)*	*0.026*
Congestive heart failure	*3 (5)*	*16 (11)*	*0.19*
Chronic renal insufficiency	*7 (11)*	*18 (13)*	*0.82*
Cancer	*12 (18)*	*23 (16)*	*0.69*
Asplenia	*1 (2)*	*2 (1)*	*1*
Immune deficiency	*17 (26)*	*39 (27)*	*0.87*
Disease severity at ICU admission[(b)] (median, IQR)			
Sequential Organ Failure Assessment score[(c)]	8 [7 – 9]	7 [5 – 9]	<0.001
PaO_2/FiO_2	174 [125 – 226] (n=76)	198 [142 – 257] (n=163)	<0.001
C-Reactive Protein (mg/L)	275 [152 – 382] (n=67)	182 [113 – 280] (n=155)	<0.001
Lactate (mmol/L)	2.9 [2.1 – 4.3] (n=59)	2.3 [1.6 – 4.6] (n=138)	0.06
Laboratory measurements (g/L) (median, IQR)			
IgG – day 1	6.47 [4.63 – 9.16] (n=76)	7.27 [5.03 – 9.70] (n=165)	0.23
IgG – day 3	6.14 [4.65 – 8.98] (n=82)	6.48 [5.03 – 8.98] (n=173)	0.31
IgG – day 7	7.26 [5.91 – 10.2] (n=59)	7.51 [5.57 – 10.3] (n=118)	0.97
IgM – day 1	0.61 [0.44 – 0.88] (n=76)	0.65 [0.38 – 0.97] (n=165)	0.90
IgM – day 3	0.57 [0.42 – 0.83] (n=82)	0.63 [0.38 – 0.97] (n=173)	0.86
IgM – day 7	0.79 [0.54 – 1.10] (n=59)	0.85 [0.55 – 1.20] (n=118)	0.76
Albumin day 1	22.8 [18.5 – 28.6] (n=77)	24.5 [20.7 – 28.0] (n=165)	0.13
Albumin – day 3	20.4 [17.3 – 23.4] (n=82)	22.6 [18.4 – 25.7] (n=173)	0.044
Albumin – day 7	20.4 [17.3 – 23.4] (n=59)	22.8 [19.0 – 26.9] (n=118)	0.016
Outcomes			
ICU mortality (n, %)	13 (16)	34 (20)	0.55
ICU LOS in survivors (median, IQR)	11 [6 – 14]	10 [6 –17]	0.53

Table S1. Data are presented as medians [IQR: interquartile range] or absolute numbers (%); Between-group differences were tested using a Fisher's exact test for categorical data or Mann-Whitney U test for continuous data (b) These values represent the worst observed values of the first 48 hours of ICU admission; (c) Sequential Organ Failure Score (SOFA) is represented as the total SOFA without the value for central nervous system; ICU: Intensive Care Unit; LOS: Length Of Stay.

Table S2. Causative pathogens identified

No. (%)	All patients (n = 255)
Streptococcus pneumoniae	58 (23)
Staphylococcus aureus	36 (14)
Haemophilus influenzae	29 (11)
Pseudomonas aeruginosa	21 (8)
Escherichia coli	11 (4)
Legionella pneumophila	8 (3)
Moraxella catarrhalis	5 (2)
Klebsiella pneumoniae	4 (2)
Klebsiella oxytoca	4 (2)
Acinetobacter spp.	3 (1)
Streptococcus pyogenes	3 (1)
Streptococcus viridans	2 (1)
Streptococcus agalactiae	2 (1)
Mycoplasma pneumoniae	2 (1)
Proteus mirabilis	2 (1)
Chlamydia psittaci	1 (0)
Influenza virus	28 (11)
Other respiratory virus	31 (12)
Other pathogens	28 (11)
Unknown	43 (17)

Prevalence of the causative pathogens identified in the study population.
Data are presented as absolute numbers (%). Multiple causative pathogens could be identified per patient

Table S3A. Association between IgG-levels and outcomes

	Baseline SOFA-score	p-value	Baseline PaO_2/FiO_2	p-value
Patient-mean IgG-level (g/L)[a]	-0.07 (-0.13 – -0.01)	0.03	-0.8 (-3.2 – 1.6)	0.52
Deviation from patient-mean IgG-level over time (g/L)[b]	-0.04 (-0.20 – 0.11)	0.56	5.8 (-1.7 – 13.2)	0.13
	SOFA-score changes over time	**p-value**	**PaO_2/FiO_2 changes over time**	**p-value**
Patient-mean IgG-level (g/L)[c]	0.01 (-0.01 – 0.03)	0.32	0.2 (-0.6 – 0.9)	0.69
Deviation from patient-mean IgG-level over time (g/L)[d]	-0.03 (-0.08 – 0.02)	0.30	-0.7 (-2.9 – 1.6)	0.56

Table S3A. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score or PaO_2/FiO_2-ratio) and IgG-levels, with random effects for intercept and time-trend. Age and baseline disease severity (measured by SOFA-score or PaO_2/FiO_2 ratio) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score or PaO_2/FiO_2; (b) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2; (c) Represents an effect on SOFA-score or PaO2/FiO2-ratio slope at baseline; (d) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2-ratio slope. This table only presents the coefficients of interest, the full model (including a quadratic time effect) is presented in table S6B.

Table S3B. Association between IgM-levels and outcomes

	Baseline SOFA-score	p-value	Baseline PaO_2/FiO_2	p-value
Patient-mean IgM-level (g/L)[a]	-0.01 (-0.20 – 0.18)	0.93	-7.0 (-14.7 – 0.7)	0.07
Deviation from patient-mean IgM-level over time (g/L)[b]	0.30 (-0.60 – 1.21)	0.51	1.3 (-43.5 – 46.1)	0.95
	SOFA-score changes over time	**p-value**	**PaO_2/FiO_2 changes over time**	**p-value**
Patient-mean IgM-level (g/L)[c]	0.04 (-0.03 – 0.11)	0.24	-0.8 (-11.2 – 9.6)	0.89
Deviation from patient-mean IgM-level over time (g/L)[d]	-0.13 (-0.37 – -0.13)	0.29	1.1 (-1.5 – 3.6)	0.40

Table S3B. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score or PaO_2/FiO_2) and IgM-levels, with random effects for SOFA-score intercept and time-trend. Age and baseline disease severity (in SOFA-score or PaO_2/FiO_2) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score or PaO_2/FiO_2; (b) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2; (c) Represents an effect on SOFA-score or PaO2/FiO2-ratio slope at baseline; (d) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2-ratio slope. This table only presents the coefficients of interest, the full model (including a quadratic time effect) is presented in table S6B.

Table S4A. Subgroup analysis – Association between IgG and outcome in patients with septic shock vs. no septic shock

In patients with septic shock (n = 111)		
	Baseline SOFA-score	
Patient-mean IgG-level (g/L)[(a)]	-0.07 (-0.16 – 0.02)	0.15
Deviation from patient-mean IgG-level over time (g/L)[(b)]	**-0.21 (-0.39 – -0.02)**	**0.03**
	SOFA-score changes over time	
Patient-mean IgG-level (g/L)[(c)]	-0.02 (-0.05 – 0.01)	0.30
Deviation from patient-mean IgG-level over time (g/L)[(d)]	-0.00 (-0.07 – 0.08)	0.90

In patients without septic shock (n = 144)		
	Baseline SOFA-score	
Patient-mean IgG-level (g/L)[(a)]	-0.05 (-0.13 – 0.02)	0.16
Deviation from patient-mean IgG-level over time (g/L)[(b)]	**0.21 (-0.02 – 0.46)**	**0.07**
	SOFA-score changes over time	
Patient-mean IgG-level (g/L)[(c)]	0.02 (-0.00 – 0.05)	0.06
Deviation from patient-mean IgG-level over time (g/L)[(d)]	-0.07 (-0.15 – 0.00)	0.06

Table S4A. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score) and Ig-levels, with random effects for SOFA-score intercept and time-trend. Age and baseline disease severity (in SOFA-score) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score or PaO_2/FiO_2; (b) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2; (c) Represents an effect on SOFA-score or PaO2/FiO2-ratio slope at baseline; (d) Represents an instantaneous effect per day on SOFA-score or PaO_2/FiO_2-ratio slope.

Table S4B. Subgroup analysis – Relation between IgM and outcome in patients with septic shock vs. no septic shock

In patients with septic shock (n = 111)		
	Baseline SOFA-score	
Patient-mean IgM-level (g/L)[(a)]	0.14 (-0.25 – 0.54)	0.47
Deviation from patient-mean IgM-level over time (g/L)[(b)]	-0.94 (-2.55 – 0.67)	0.25
	SOFA-score changes over time	
Patient-mean IgM-level (g/L)[(c)]	0.02 (-0.10 – 0.14)	0.74
Deviation from patient-mean IgM-level over time (g/L)[(d)]	0.15 (-0.24 – 0.53)	0.46

In patients without septic shock (n = 144)		
	Baseline SOFA-score	
Patient-mean IgM-level (g/L)[(a)]	-0.02 (-0.23 – 0.19)	0.83
Deviation from patient-mean IgM-level over time (g/L)[(b)]	0.64 (-0.45 – 1.73)	0.24
	SOFA-score changes over time	
Patient-mean IgM-level (g/L)[(c)]	0.04 (-0.04 – 0.11)	0.35
Deviation from patient-mean IgM-level over time (g/L)[(d)]	-0.20 (-0.51 – 0.10)	0.19

Table S4B. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score) and Ig-levels, with random effects for SOFA-score intercept and time-trend. Age and baseline disease severity (in SOFA-score) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score; (b) Represents an instantaneous effect on daily SOFA-score; (c) Represents an effect on SOFA-score slope at baseline; (d) Represents an instantaneous effect on daily SOFA-score.

Table S4C. Subgroup analysis – Relation between IgG and outcome in immunocompromised patients vs. non-immunocompromised patients

In immunocompromised patients (n = 71)		
	Baseline SOFA-score	
Patient-mean IgG-level (g/L)[(a)]	**0.03 (-0.08 – 0.14)**	**0.61**
Deviation from patient-mean IgG-level over time (g/L)[(b)]	0.08 (-0.15 – 0.31)	0.51
	SOFA-score changes over time	
Patient-mean IgG-level (g/L)[(c)]	-0.00 (-0.05 – 0.04)	0.83
Deviation from patient-mean IgG-level over time (g/L)[(d)]	-0.08 (-0.20 – 0.04)	0.18

In non-immunocompromised patients (n = 184)		
	Baseline SOFA-score	
Patient-mean IgG-level (g/L)[(a)]	**-0.09 (-0.16 – 0.01)**	**0.02**
Deviation from patient-mean IgG-level over time (g/L)[(b)]	-0.08 (-0.27 – 0.12)	0.43
	SOFA-score changes over time	
Patient-mean IgG-level (g/L)[(c)]	0.01 (-0.00 – 0.03)	0.24
Deviation from patient-mean IgG-level over time (g/L)[(d)]	-0.01 (-0.07 – 0.05)	0.66

Table S4C. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score) and Ig-levels, with random effects for SOFA-score intercept and time-trend. Age and baseline disease severity (in SOFA-score) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score; (b) Represents an instantaneous effect on daily SOFA-score; (c) Represents an effect on SOFA-score slope at baseline; (d) Represents an instantaneous effect on daily SOFA-score.

Table S4D. Subgroup analysis – Relation between IgM and outcome in immunocompromised patients vs. non-immunocompromised patients

In immunocompromised patients (n = 71)		
	Baseline SOFA-score	
Patient-mean IgM-level (g/L)[(a)]	0.07 (-0.17 – 0.31)	0.59
Deviation from patient-mean IgM-level over time (g/L)[(b)]	-0.03 (-1.65 – 1.59)	0.97
	SOFA-score changes over time	
Patient-mean IgM-level (g/L)[(c)]	0.02 (-0.09 – 0.14)	0.68
Deviation from patient-mean IgM-level over time (g/L)[(d)]	-0.07 (-0.50 – 0.35)	0.74

In non-immunocompromised patients (n = 184)		
	Baseline SOFA-score	
Patient-mean IgM-level (g/L)[(a)]	-0.16 (-0.47 – 0.16)	0.33
Deviation from patient-mean IgM-level over time (g/L)[(b)]	0.12 (-1.00 – 1.24)	0.83
	SOFA-score changes over time	
Patient-mean IgM-level (g/L)[(c)]	0.07 (-0.01 – 0.16)	0.10
Deviation from patient-mean IgM-level over time (g/L)[(d)]	-0.15 (-0.45 – 0.14)	0.31

Table S4C. Associations were estimated from generalized linear mixed-models for repeated measures for the outcome (SOFA-score) and Ig-levels, with random effects for SOFA-score intercept and time-trend. Age and baseline disease severity (in SOFA-score) were included as confounders in all models. (a) Represents an effect on baseline SOFA-score; (b) Represents an instantaneous effect on daily SOFA-score; (c) Represents an effect on SOFA-score slope at baseline; (d) Represents an instantaneous effect on daily SOFA-score.

Table S5A. Sensitivity analysis – all encapsulated pathogens recategorized as high risk

Predictor	Inter-cept[a]	Time trend (per day)[b]	p-value	Between patient Ig-level [c]	p-value	Within patient Ig-level [d]	p-value	Between-patient Ig-level[e]	p-value	Within patient Ig-level[f]	p-value	Direct effect of SpHi+/- on outcome baseline	p-value	Difference in the effect of Ig-level between SpHi+ and SpHi-[g]	p-value
Association between Ig-levels and SOFA-score															
IgG (g/ L)	7.29	- 0.63	***	-0.03	0.462	- 0.04	0.608	0.01	0.318	-0.03	0.281	-0.17	0.447	-0.10	0.098
IgM (g/ L)	7.19	- 0. 66	***	0.08	0.477	0.24	0.597	0.04	0.236	-0.12	0.335	0.41	0.266	-0.45	0.058
Association between Ig-levels and PaO_2/FiO_2-ratio															
IgG (g/ L)	229	4.6	***	-0.13	0. 931	5.7	0.135	0.2	0.677	-0.64	0.570	-6.9	0.402	-1.80	0.426
IgM (g/ L)	238	3.7	0.087	-7.5	0.080	0.6	0.981	-0.6	0.905	1.05	0.416	-9.9	0.467	1.6	0.855

Table S5A presents a sensitivity analysis in which all causative pathogens with certain strains known to exhibit polysaccharide capsules are reclassified into the SpHi+ group. Results of multilevel models relating the effects of Ig-level and Ig-level time trend to disease course. Age and baseline disease severity (SOFA-score or PaO_2/FiO_2-ratio) were included as confounders in all models. (a) The intercepts represent the estimated disease severity score at admission for a patient with average disease severity at baseline, average age and an average IgG- or IgM-level (without an encapsulated causative pathogen) (b) Final models for SOFA-score also included a polynomial time effect with the estimates for $days^2$ being 0.043 (p=0.008), 0.043 (p=0.008), 0.043 (p=0.008) and 0.038 (p=0.019) for the models respectively (c) Represents an effect on baseline SOFA-score or PaO_2/FiO_2-ratio (d) Represents an instantaneous effect on daily SOFA-score or PaO_2/FiO_2-ratio (e) Represents an effect on SOFA-score or PaO_2/FiO_2-ratio slope at baseline (f) Represents an instantaneous effect on daily SOFA-score or PaO_2/FiO_2-ratio slope (g) Derived from an interaction term between the patient mean Ig-level and SpHi+/-, and represents an effect on baseline SOFA-score or PaO_2/FiO_2-ratio. SOFA: total Sequential Organ Failure Assessment (minus central nervous system score). * = p<0.05; ** = p<0.01; *** = p<0.001

Table S5B. Sensitivity analysis – Reciprocal effect of SOFA score on IgG- and IgM-levels

Predictor	Intercept	Time trend (per day)	p-value	Polynomial time trend (per day)	p-value	SOFA-score effect on intercept	p-value	SOFA-score effect on slope	p-value	SOFA-score effect on polynomial slope	p-value
Association with IgG-level (g/L)											
SOFA-score	8.32	-0.388	0.029	0.078	0.002	-0.069	0.127	0.014	0.502	-0.001	0.716
Association with IgM-level (g/L)											
SOFA-score	1.050	-0.096	0.023	0.019	0.000	-0.021	0.014	0.008	0.037	-0.001	0.105

Results of sensitivity analyses that estimate the reciprocal effect of SOFA-scores measured on day 1, 2 and 6 of ICU-admission on IgG- and IgM-levels measured on day 1, 3 and 7. Age was included as a confounder in both models. Best performing models included a polynomial time trend, and random effects for the linear and polynomial time trend. SOFA-score: total Sequential Organ Failure Assessment Score (minus central nervous system score).

Table S6A. Mixed-effects model building – unconditional growth models

	Fixed effects			Random effects			
Unconditional growth models	**Intercept[(a)]**	**Time trend**	**Quadratic time trend**	**Time trend (Std deviation)**	**Quadratic time trend (Std deviation)**	**AIC**	**p-value**
SOFA-score							
Model 1	*7.14*	*-0.38*	-	-	-	*3219.1*	-
Model 2	*7.14*	*-0.38*	-	*0.27*	-	*3210.8*	*0.002*
Model 3	*7.26*	*-0.61*	*0.04*	*0.27*	-	*3206.8*	*0.014*
Model 4	7.26	-0.61	0.04	0.66	0.11	3200.0	0.005
PF-ratio							
Model 1	224.2	4.6	-	-	-	7816.5	-
Model 2	224.1	4.9	-	8.4	-	7810.2	0.006
Model 3	*223.8*	*5.4*	*-0.08*	*8.4*	-	7812.2	0.902

Table S6B. Mixed-effects model building – conditional growth models

				Effect on intercept		Effect on slope		Interaction term (person mean Ig-level*SpHi)[1]				
Conditional growth models	**Intercept**	**Time trend**	**Quadratic time trend**	**Between-patient Ig-level[†]**	**Within-patient Ig-level[†]**	**Between-patient Ig-level[†]**	**Within-patient Ig-level[†]**	**Direct effect of SpHi+ on outcome**	**Difference in the effect of Ig-level[†] between SpHi+ and SpHi-**	**Age**	**Baseline SOFA-score**	**AIC**
SOFA-score												
Model 5 - IgG	*7.26*	*-0.64****	*0.04***	*-0.14***	*-0.11*	-	-	-	-	-	-	*3116.9*
Model 6 - IgG	*7.26*	*-0.64****	*0.05***	*-0.16***	*-0.12*	*0.01*	-	-	-	-	-	*3117.3*
Model 7 - IgG	*7.26*	*-0.64****	*0.05****	*-0.16***	*-0.08*	*0.01*	*-0.01*	-	-	-	-	*3119.1*
Model 8 - IgG[2]	***7.23***	***-0.63******	***0.04*****	***-0.07****	***-0.04***	***0.01***	***-0.03***	**-**	**-**	***0.01***	***0.88******	***2844.5***
Model 9 - IgG[2]	*7.27*	*-0.63****	*0.04***	*-0.04*	*-0.05*	*0.01*	*-0.03*	*-0.17*	*-0.11*	*0.01**	*0.89****	*2845.0*
Model 5 - IgM	*7.50*	*-0.62****	*0.04***	*-0.19*	*-0.27*	-	-	-	-	-	-	*3125.4*
Model 6 - IgM	*7.59*	*-0.66****	*0.04***	*-0.26*	*-0.29*	*0.04*	-	-	-	-	-	*3126.1*

Model 7 - IgM	*7.58*	*-0.66****	*0.04**	*-0.26*	*-0.47*	*0.04*	*0.05*	*-*	*-*	*-*	*-*	*3127.9*
Model 8 - IgM	***7.26***	***-0.66******	***0.04****	***-0.01***	***0.31***	***0.04***	***-0.13***	***-***	***-***	***0.01***	***0.89******	***2850.8****
Model 9 - IgM	*7.19*	*-0.66****	*0.04**	*0.07*	*0.26*	*0.04*	*-0.12*	*0.43*	*-0.44*	*0.01*	*0.89****	*2851.2*
PF-ratio												
Model 4 - IgG	*226.0*	*4.3***	*-*	*0.1*	*4.2*	*-*	*-*	*-*	*-*	*-*	*-*	*7643.8*
Model 5 - IgG	*226.0*	*4.3**	*-*	*-0.1*	*4.1*	*0.1*	*-*	*-*	*-*	*-*	*-*	*7645.6*
Model 6 - IgG	*226.0*	*4.5**	*-*	*-0.1*	*6.3*	*-0.8*	*0.1*	*-*	*-*	*-*	*-*	*7647.1*
Model 7 - IgG	***226.0***	***4.6*****	***-***	***-0.8***	***5.8***	***0.2***	***-0.7***	***-***	***-***	***-1.0*****	***-6.6******	***7614.9***
Model 8 - IgG	*226.3*	*4.6***	*-*	*-0.8*	*5.8*	*0.2*	*-0.7*	*-1.0*	*-0.0*	*-1.0***	*-6.6****	*7618.9*
Model 4 - IgM	*228.6*	*4.9***	*-*	*-3.1*	*-2.0*	*-*	*-*	*-*	*-*	*-*	*-*	*7646.2*
Model 5 - IgM	*230.5*	*3.5*	*-*	*-4.6*	*-2.8*	*1.1*	*-*	*-*	*-*	*-*	*-*	*7647.4*
Model 6 - IgM	*230.6*	*3.5*	*-*	*-4.6*	*-0.8*	*-0.5*	*1.1*	*-*	*-*	*-*	*-*	*7649.4*
Model 7 - IgM	***233.7***	***3.6***	***-***	***-7.0***	***1.5***	***-0.9***	***1.1***	***-***	***-***	***-1.0*****	***-6.8******	***7615.7***
Model 8 - IgM	*234.1*	*3.6*	*-*	*-6.9*	*1.2*	*-0.8*	*1.1*	*-0.8*	*-0.6*	*-1.0***	*-6.7****	*7619.6*

Table S2A/B. Step by step multilevel model building. The final model was selected based on their inclusion of the covariates needed to answer the research question, and to evaluate if polynomial terms and random effects should be included Akaike's Information Criterion was used. The bold printed models are the final models. Time-varying Ig-levels were split into a person-mean Ig-level (mean of day 1, day 3 and day 7 per patient), and the deviation from the person-mean (i.e. day 1 Ig-level minus person-mean Ig-level), reported separately for IgG and IgM. All variables were centered before adding them to the model. (1) We only evaluated if the interaction term (person mean Ig-level*SpHi+) affects the model intercept. Final models; **†** **=** models are reported for both IgG and IgM; * = p <0.05; ** = p <0.01, *** = p <0.001

Syntax

```
# Unconditional MEANS model.
model0 <- lme(SOFA_total_noGCS_d248~1, random =~1|Pseudo_ID, data = CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model0)

# Unconditional GROWTH model.
model1 <- lme(SOFA_total_noGCS_d248~day0, random =~1| Pseudo_ID, data = CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model1)

# Unconditional LINEAR GROWTH model + random slope
model2 <- lme(SOFA_total_noGCS_d248~day0, random =~day0| Pseudo_ID, data = CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model2)

# Unconditional QUADRATIC GROWTH model
model3 <- lme(SOFA_total_noGCS_d248~day0 + day0_sq, random =~day0| Pseudo_ID, data = CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model3)

# Add random slope for quadratic time trend.
model4 <- lme(SOFA_total_noGCS_d248~day0 + day0_sq, random =~(day0+day0_sq)| Pseudo_ID, data = CAPIg_long_final1, method = 'ML',
na.action=na.omit)
summary(model4)
anova(model0,model1,model2,model3,model4)

# Conditional QUADRATIC GROWTH model
model5.igg <- lme(SOFA_total_noGCS_d248~(day0 + day0_sq)+ IgG.between + IgG.within, random =~day0+day0_sq| Pseudo_ID, data = CAPIg_long_final1,
method = 'ML', na.action=na.omit)
summary(model5.igg)

model6.igg <- lme(SOFA_total_noGCS_d248~(day0 + day0_sq)+ IgG.between*day0 + IgG.within, random =~day0+day0_sq| Pseudo_ID, data =
CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model6.igg)
```

```
model7.igg <- lme(SOFA_total_noGCS_d248~(day0 + day0_sq)+ IgG.between*day0 + IgG.within*day0, random =~day0+day0_sq| Pseudo_ID, data =
CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model7.igg)

model8.igg <- lme(SOFA_total_noGCS_d248~(day0 + day0_sq)+ IgG.between*day0 + IgG.within*day0 + SOFA_adm.gmc + age.gmc, random
=~day0+day0_sq| Pseudo_ID, data = CAPIg_long_final1, method = 'ML', na.action=na.omit)
summary(model8.igg)
anova(model5.igg, model6.igg, model7.igg, model8.igg)

# Update final model to REML to get better estimate of coefficients.
model8.igg.REML <- update(model8.igg, method = 'REML')
summary(model8.igg.REML)
intervals(model8.igg.REML, which = 'fixed')

# Check assumptions
qqnorm(model8.igg.REML)
plot(model8.igg.REML)
plot(model8.igg.REML, Pseudo_ID ~ resid(.))
plot(model8.igg.REML, SOFA_total_noGCS_d248 ~ fitted(.) | Pseudo_ID, abline = c(0,1))
plot(ranef(model8.igg.REML))
```