Low levels of immunoglobulins and mannose-binding lectin are not associated with etiology, severity, or outcome in community-acquired pneumonia

Siljan, William W.; Holter, Jan C.; Nymo, Ståle H.; Husebye, Einar; Ueland, Thor; Skattum, Lillemor; Bosnes, Vidar; Garred, Peter; Frøland, Stig S.; Mollnes, Tom E.; Aukrust, Pål; Heggelund, Lars

Published in:
Open Forum Infectious Diseases

DOI:
10.1093/ofid/ofy002

Publication date:
2018

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC-ND

Citation for published version (APA):
Siljan, W. W., Holter, J. C., Nymo, S. H., Husebye, E., Ueland, T., Skattum, L., ... Heggelund, L. (2018). Low levels of immunoglobulins and mannose-binding lectin are not associated with etiology, severity, or outcome in community-acquired pneumonia. Open Forum Infectious Diseases, 5(2), [ofy002].
https://doi.org/10.1093/ofid/ofy002
Low Levels of Immunoglobulins and Mannose-Binding Lectin Are Not Associated With Etiology, Severity, or Outcome in Community-Acquired Pneumonia

William W. Siljan, Jan C. Holter, Ståle H. Nymo, Einar Husebye, Thor Ueland, Lillemor Skattum, Vidar Bosnes, Peter Garred, Stig S. Froland, Tom E. Mollnes, Pål Aukrust, and Lars Heggelund

Background. Disease severity and outcome in community-acquired pneumonia (CAP) depend on the host and on the challenge of the causative microorganism(s). We measured levels of immunoglobulins (Igs) and complement in 257 hospitalized adults with CAP and examined the association of low levels of Igs or complement to microbial etiology, disease severity, and short-term and long-term outcome.

Methods. Serum Igs were analyzed in blood samples obtained at admission and at 6 weeks postdischarge if admission levels were low. Serum complement deficiencies were screened with a total complement activity enzyme-linked immunosorbent assay (ELISA), with further analyses performed if justified. Disease severity was assessed by the CURB-65 severity score. Short-term outcome was defined as a composite endpoint of intensive care unit (ICU) admission and 30-day mortality, and long-term outcome as 5-year all-cause mortality.

Results. At admission, 87 (34%) patients had low levels of at least 1 Ig, with low IgG2 as the most prevalent finding (55/21%). IgG levels were lower in bacterial than viral CAP (8.48 vs 9.97 g/L, P = 0.023), but low Igs were not associated with microbial etiology. Fifty-five (21%) patients had low lectin pathway activity, of which 33 (13%) were mannose-binding lectin (MBL) deficient. Low admission levels of any Ig or MBL were not associated with disease severity, short-term outcome, or long-term outcome. Excluding patients defined as immunocompromised from analysis did not substantially affect these results.

Conclusion. In hospitalized adults with CAP, low admission levels of Igs or complement were in general not associated with microbial etiology, disease severity, short-term outcome, or long-term outcome.

Keywords. complement; etiology; immunoglobulin; mannose-binding lectin; mannose-binding protein-associated serine proteases; mortality; pneumonia.

Community-acquired pneumonia (CAP) still has high morbidity and mortality worldwide despite advances in its management [1], resulting in an increasing number of hospital and intensive care unit (ICU) admissions [2, 3]. Patients with primary or secondary immunodeficiencies are more susceptible to pulmonary infections, and the occurrence of secondary immunodeficiencies is rising, caused by wider use of immunosuppressive medications [4].

Immunoglobulins (Igs) are fundamental mediators of humoral immunity by neutralization, opsonization, and phagocytosis of pathogens and by contributing to complement activation [5]. Deficiencies in this antibody-mediated immune system implicate a significant risk of recurrent infections, in particular those caused by encapsulated bacteria in the respiratory tract [6, 7]. In the acute phase of infectious diseases such as sepsis and severe influenza, low levels of Igs have been associated with an unfavorable outcome [8–10]. Correspondingly, an association between subnormal levels of Igs, predominantly total IgG and IgG1/2 subclasses, and disease severity has been reported in CAP and influenza A H1N1 cohorts [11–13]. However, the relevance of Ig levels in relation to microbial etiology in CAP is less clear, although it is well known that IgG and in particular IgG2 are of major importance for the pulmonary defense against encapsulated bacteria [14, 15]. Furthermore, whereas an association has been established in primary hypogammaglobulinemia, the significance of...
a transient decrease in IgG/IgG2 levels during infections like CAP is more uncertain.

The complement system is a key part of immune defense, constituting a functional bridge between innate and humoral immunity [16]. Complement may be activated through 3 major pathways; the classical, lectin, and alternative, with considerable crosstalk between the pathways and with other branches of the immune system. Complement deficiencies have been associated with increased susceptibility to bacterial infections [17]. The most prevalent deficiencies of the complement system are mannose-binding lectin (MBL) and complement component 2 (C2), and it has been shown that individuals with C2 deficiency are more prone to infections and severe outcome in pneumonia [17]. MBL is a soluble pattern recognition molecule, mainly exerting its effects through opsonization of pathogens and subsequent activation of the lectin complement pathway [18]. Genetically determined MBL deficiency is very prevalent, as complete deficiency affects 5% to 10% and low levels are seen in up to 30% of the Caucasian population [19]. With the exception of patients with cystic fibrosis [20] and, potentially, common variable immunodeficiency (CVID) [21], the significance of MBL deficiency in relation to CAP remains unclear.

We have previously reported that by using extended diagnostics, a high microbial yield was achieved in a well-defined cohort of patients with CAP [22]. The objective of this study was to assess the presence of low Ig levels and decreased activation of the 3 major complement activation pathways at hospital admission and, if present, examine their association to microbial etiology, disease severity, and short-term and long-term outcome. We hypothesized that low Ig levels, especially low IgG2, and decreased complement activation at admission would be associated with bacterial etiology and unfavorable outcome in CAP.

**MATERIALS AND METHODS**

**Study Population and Design**

The study was performed in an acute care 270-bed general hospital in Drammen, Vestre Viken Hospital Trust, in South-Eastern Norway between January 1, 2008, and January 31, 2011; 267 patients aged ≥18 years admitted with suspected pneumonia to the Department of Internal Medicine were consecutively included. Patients were screened for eligibility within the first 48 hours of hospital admission by determining presence of CAP criteria, defined by (i) a new pulmonary infiltrate on chest radiograph, (ii) rectal temperature >38.0°C, and (iii) at least 1 of the following symptoms or signs: cough (productive or nonproductive), dyspnea, respiratory chest pain, crackles, or reduced respiratory sounds. If the chest radiographic examination uncovered noninfectious causes such as pulmonary infarction, tumor, or bronchiectasis, or if the patient had been hospitalized within the past 2 weeks, the patient was excluded from the study.

In the current study, patients with missing Ig and complement analyses at hospital admission (n = 10) were excluded, leaving a sample of 257 (ie, analysis cohort). (The inclusion process is summarized in Supplementary Appendix.) Patients were invited to an outpatient follow-up approximately 6 weeks after hospital discharge (convalescent phase, n = 220). When analyzing associations with long-term outcome, patients who died were considered responders at their death dates, and those who survived after the closing date were considered censored; patients who died within 30 days after hospital admission (n = 10) were excluded. Patients lost to follow-up were censored at the time of last contact.

Of the 257 patients in this study, 45 were considered immunocompromised. An immunocompromised host included the occurrence of (i) primary or secondary immunodeficiency, defined as antibody deficiency, human immunodeficiency virus (HIV), organ transplant, and/or receiving chemotherapy and/or radiation therapy within the past 3 months; (ii) active malignancy, defined as any cancer except basal—or squamous—cell cancer of the skin that was active at the time of presentation or diagnosed within 1 year of presentation; or (iii) immunosuppressive drug use, defined as any use of systemic steroids, Azathioprine, TNF inhibitor, Cyclosporine, Cyclophosphamide, or Methotrexate within the past 3 months.

All patients provided written informed consent. The study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (ref. number S-06266a), and a waiver of consent was obtained from the committee to link patient data to death certificates (2012/467 A).

**Data Collection and Definitions**

Baseline data collection and definitions have been described elsewhere [22, 23]. In brief, demographic, clinical, and laboratory data were collected within 48 hours of admission. The mean time from hospital admission to study inclusion was 0.6 ± 0.5 days, and 250 of 257 (97%) patients were included within 24 hours. The microbial etiology of CAP was established by use of comprehensive microbiological testing (ie, bacterial cultures, serology, urinary antigen tests, and polymerase chain reaction [PCR]).

**Blood Sampling**

Blood samples were obtained at hospital admission and at the scheduled 6-week follow-up, with serum and plasma samples drawn into pyrogen-free vacutainer tubes. Tubes for plasma samples contained ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Serum or plasma was separated from whole blood within 60 minutes by refrigerated centrifugation at 2000 g for 12 minutes and stored in several aliquots at –80°C. Samples were thawed only once.

**Ig and Complement Analyses**

Serum levels of IgM, IgA, IgG, and IgG subclasses (IgG1, IgG2, IgG3, IgG4) at hospital admission were quantified by
immunonephelometry (BN ProSpec nephelometer, Siemens AG, Germany, and Cobas 8000 analyzer, Roche AG, Switzerland). The reference values are given in Supplementary Appendix. In patients with levels of IgG or IgG2 below reference range at hospital admission, samples from the 6-week follow-up were analyzed.

Complement deficiencies were screened in serum with a total complement activity enzyme-linked immunosorbent assay (ELISA; Complement system Screen, Wieslab, Euro Diagnostica, Malmö, Sweden). The method includes classical pathway (CP), lectin pathway (LP), and alternative pathway (AP) activity with a common readout for terminal pathway activity and has been described in detail previously [24]. The values are given in percentage related to a standard serum defined to contain 100% activity. The reference ranges are given in the Figure 2 legend. Patients with indications of complement deficiency from the screening were further analyzed for the complement proteins described below.

MBL was quantified using an ELISA described in detail previously [19]; low levels were defined as <50 ng/mL. C1q and C2 were quantified at Region Skåne, Lund, Sweden, and C3 and C4 at Oslo University Hospital, Oslo, Norway, using standard immunochemical techniques.

In brief, mannose-binding lectin–associated serine protease 1 and 2 (MASP-1 and MASP-2, respectively) deficiencies were identified by genotyping for MASP1 G426E (rs28945068) and MASP2 D120G (rs72550870), while analysis of MASP-2 binding to MBL or ficolins was performed in serum with an assay from Hycult Biotech (HM2190-IA, Uden, the Netherlands) [25, 26]. Plasma levels of MASP-2 were quantified by an ELISA kit (Hycult Biotech), with a reference range of 170–1196 ng/mL.

Outcome Measures
Based on the etiology, patients were categorized into 4 groups: (i) bacterial, (ii) viral, (iii) viral-bacterial, and (iv) unknown. In addition, patients with Streptococcus pneumoniae or Haemophilus influenzae were studied separately. Disease severity was evaluated by the validated CURB-65 scoring system [27]; patients with a CURB-65 score of <3 were classified into a low-risk group and ≥3 into a high-risk group. Short-term outcome was defined as a composite end point of ICU admission and 30-day mortality [28]. Long-term outcome was defined as 5-year all-cause mortality.

Statistical Analysis
Categorical variables were expressed as counts (percentages), and continuous variables were presented as mean (standard deviation) for normally distributed data or median (25th–75th percentiles) for visually skewed data. Continuous variables were analyzed using a t test, and comparison of categorical variables were compared using the χ² test or 1-way ANOVA, where appropriate, whereas Tukey's test was performed for post hoc test of differences between pair of groups. Univariate and adjusted logistic regressions were used to assess the association between Ig or complement parameters and short-term outcome. Adjusted Cox regression analysis was used to assess the association between Ig or complement parameters and long-term outcome. A 2-sided P value <.05 was considered significant. Statistical analyses were performed using STATA, version 14.0, for Windows (Stata Corp LP, College Station, TX) and SPSS, version 23.0, for Windows (IBM Corp, Armonk, NY).

RESULTS
Ig Levels Below Reference Range
At hospital admission, 87 (34%) patients had levels below the reference range of at least 1 Ig (IgM, IgA, IgG, IgG1, IgG2, IgG3, or IgG4), with low IgG2 levels as the most prevalent finding (55 patients/21%) (Figure 1). At the 6-week follow-up, IgG levels remained low in more than one-third of patients with sub-normal levels at admission (12 patients, 40%). In particular, 39 out of the 50 (78%) patients with low IgG2 at admission (2 deceased, 3 missing samples) had levels below the reference range at the 6-week follow-up (Figure 1). We found no increase in comorbidities (cardiovascular disease [CVD], chronic obstructive pulmonary disease [COPD], autoimmune disease, diabetes mellitus, renal disease, neurological disease) among patients with low IgM, IgA, IgG, or IgG2 levels compared with the rest of the study population.

Ig Levels in Relation to Microbial Etiology
Low levels of any Ig at hospital admission were not related to a specific group of microbial etiology. However, serum levels of IgG differed significantly between the groups of etiology (P = .027), with lower levels seen in bacterial than viral CAP (P = .023). For IgA, IgM, or any of the IgG subclasses, no relation to microbial etiology was seen (Table 1). Additionally, in patients with low levels of any Ig, CAP caused by encapsulated bacteria (S. pneumoniae and H. Influenzae) did not occur more frequently (P = .734).

Ig Levels in Relation to Disease Severity, Short-term Outcome, and Long-term Outcome
Patients with low IgG or IgG2 at both time points did not present with a higher CURB-65 severity score (P = .594) than other CAP patients. Moreover, there were no differences in Ig levels between patients with high (≥3) vs low (<3) CURB-65 severity score (Table 2).

In all, 39 (15%) patients were survivors requiring ICU admission, while 10 (4%) patients died within 30 days of hospital admission. In univariate logistic regression analysis, Igs were not associated with an adverse short-term outcome when assessed as continuous variables (Table 3). Similarly, Igs below the reference range were not associated with an adverse short-term outcome (Table 3).

Excluding patients who died within 30 days of hospital admission, 5-year mortality was 26% (65/246 patients, 1 lost
to follow-up). In age- and gender-adjusted Cox regression analysis, low levels of IgGs were not associated with increased 5-year mortality (Supplementary Table 2). Adjustment for clinically significant comorbidities (CVD, COPD, active malignancy, and renal disease) did not affect results for disease severity, short-term outcome, or long-term outcome.

Ig Results Excluding Immunocompromised Patients

Forty-five patients were defined as immunocompromised at study inclusion, leaving 212 patients in the study population when excluding these. In this assumed nonimmunocompromised group, Ig levels at hospital admission differed significantly between groups of microbial etiology for IgA ($P = .048$) and IgG2 ($P = .021$), with lower levels seen in bacterial than in viral-bacterial CAP for IgA (mean 2.55 ± 1.13 g/L vs 3.34 ± 2.13 g/L, $P = .039$) and in bacterial vs viral CAP for IgG2 (mean 2.51 ± 0.99 g/L vs 3.22 ± 1.45 g/L, $P = .018$). By contrast, low levels of any Ig were not associated with a higher CURB-65 severity score. The number of adverse outcomes in this subgroup was too low to perform outcome analyses.

### Complement Deficiencies

Screening of serum samples documented 55 (21%) patients with low or undetectable lectin pathway activity, of which 3 also had a defective classical pathway activity (Figure 2). One of the 3 patients had an MBL deficiency, was quantified for C1q, and had only a slightly reduced level. The other 2 had normal MBL and were quantified for C2 and C4, both with normal levels. Abnormalities in functional activity of the alternative pathway were not observed in our patient cohort (Figure 2).

### MASP-2 Deficiency

One patient with a lectin pathway deficiency, normal MBL levels (1134 ng/mL), and normal classical and alternative pathway activity was found to be homozygote MASP-2 deficient. MASP-2 in serum did not form complexes with MBL or ficolins. Protein quantification of MASP-2 was in accordance with a defect, being 63 and 29 ng/mL at hospital admission and 6-week follow-up, respectively, well below the reference range of 170–1196 ng/mL. The patient was a previously healthy female aged 66 years without tendency to infection. The CURB-65 severity score at

### Table 1. Serum Immunoglobulin Levels (g/L) and Number of Patients With Serum Immunoglobulin Levels Below Reference Range at Hospital Admission in 257 Hospitalized Patients With Community-Acquired Pneumonia, Stratified by Etiology

| Total (n = 257) | Bacterial (n = 73) | Viral-Bacterial (n = 49) | Viral (n = 39) | Unknown (n = 96) | P |
|-----------------|-------------------|-------------------------|---------------|-----------------|---|
| IgM 0.86 ± 0.53 | 0.91 ± 0.59       | 0.84 ± 0.51             | 0.81 ± 0.41   | 0.95 ± 0.66     | .657 |
| IgA 2.83 ± 1.64 | 2.57 ± 1.09       | 3.16 ± 2.09             | 2.91 ± 1.82   | 2.43 ± 1.25     | .139 |
| IgG 9.08 ± 2.87 | 8.48 ± 2.71       | 9.26 ± 2.82             | 9.97 ± 3.04   | 8.72 ± 2.81     | .027 |
| IgG1 6.44 ± 2.53 | 6.01 ± 2.14     | 6.51 ± 2.51             | 7.16 ± 3.06   | 6.25 ± 2.29     | .071 |
| IgG2 2.70 ± 1.23 | 2.49 ± 1.10     | 2.81 ± 1.18             | 2.96 ± 1.46   | 2.49 ± 1.22     | .123 |
| IgG3 0.45 ± 0.31 | 0.44 ± 0.25     | 0.48 ± 0.37             | 0.46 ± 0.33   | 0.38 ± 0.23     | .764 |
| IgG4 0.55 ± 0.55 | 0.56 ± 0.56     | 0.51 ± 0.40             | 0.59 ± 0.68   | 0.57 ± 0.60     | .762 |
| IgG < ref. range* | 30 (100)        | 13 (36.7)               | 3 (10.0)      | 11 (43.3)       | .099 |
| IgG2 < ref. range* | 55 (100)        | 17 (30.9)               | 6 (10.9)      | 25 (45.5)       | .313 |

Data are presented as means and ± standard deviation or No. (%). Group comparison performed with 1-way analysis of variance or $\chi^2$ test as appropriate. Patients with unknown etiology excluded from statistical analysis.

Abbreviations: Ig, immunoglobulin.

*Assessed as dichotomous variables.
admission was 1. She was treated with intravenous antibiotics at a general ward for 5 days before discharge. The microbial etiology of CAP was not identified.

**MBL Deficiency in Relation to Microbial Etiology, Disease Severity, and Outcome**

MBL-deficient serum samples were not related to microbial etiology, disease severity, or short-term or long-term outcome (Supplementary Table 3, A–D). A similar pattern was seen when excluding immunocompromised patients, rendering results for disease severity, short-term outcome, or long-term outcome.

**Table 2. Serum Immunoglobulin Levels (g/L) or Number of Patients With Low Immunoglobulin Levels at Hospital Admission Stratified by CURB-65 <3 vs CURB-65 ≥3 in 257 Hospitalized Patients With Community-Acquired Pneumonia**

| Immunoglobulin | CURB-65 <3 (n = 157) | CURB-65 ≥3 (n = 95) | P  |
|---------------|---------------------|---------------------|----|
| IgM           | 0.91 ± 0.53         | 0.88 ± 0.66         | .737|
| IgA           | 2.64 ± 1.67         | 2.74 ± 1.19         | .610|
| IgG           | 8.74 ± 2.88         | 9.17 ± 2.77         | .249|
| IgG1          | 6.19 ± 2.54         | 6.56 ± 2.22         | .241|
| IgG2          | 2.59 ± 1.23         | 2.67 ± 1.23         | .601|
| IgG3          | 0.42 ± 0.24         | 0.44 ± 0.35         | .454|
| IgG4          | 0.53 ± 0.54         | 0.59 ± 0.59         | .409|
| IgG < ref. range* | 20 (66.7)   | 10 (33.3)           | .690|
| IgG2 < ref. range* | 35 (64.8)   | 19 (35.2)           | .752|

Data presented as means and ± standard deviation or No. (%). Group comparison performed with t test or χ² test, as appropriate. Data on CURB-65 severity score are missing in 5 patients.

Abbreviations: CURB-65, Confusion, Urea, Respiratory rate, Blood pressure, Age ≥65; Ig, immunoglobulin.

*Assessed as dichotomous variables.

**Table 3. Univariate Logistic Regression Analysis of Serum Immunoglobulin Levels at Hospital Admission and Association to ICU Admission/30-Day Mortality in 257 Hospitalized Patients With Community-Acquired Pneumonia**

| Immunoglobulins | OR (95% CI) | P  |
|-----------------|-------------|----|
| IgM             | 1.16 (0.69–1.93) | .579|
| IgA             | 0.95 (0.77–1.18) | .632|
| IgG             | 0.94 (0.84–1.06) | .315|
| IgG1            | 0.99 (0.87–1.12) | .843|
| IgG2            | 0.85 (0.64–1.11) | .232|
| IgG3            | 0.99 (0.33–3.03) | .993|
| IgG4            | 1.07 (0.63–1.82) | .811|
| IgG < ref. range* | 1.64 (0.68–3.94) | .268|
| IgG2 < ref. range* | 1.42 (0.69–2.91) | .340|

Abbreviations: CI; confidence interval; ICU, intensive care unit; Ig, immunoglobulin; OR, odds ratio.

*Assessed as dichotomous variables.

**DISCUSSION**

In the present study, admission Ig levels below reference range were not associated with a specific group of microbial etiology in CAP, but IgG and IgG2 levels were significantly lower in bacterial than in viral CAP. Second, Ig levels below the reference range at hospital admission were not associated with disease severity, short-term outcome, or long-term outcome. Third, MBL deficiency was not associated with microbial etiology, disease severity, or outcome measures.

Even though admission Ig levels below the reference range were not associated with a specific microbial etiology in our study, IgG levels were significantly lower in bacterial than in viral CAP, and when excluding immunocompromised patients, we found lower levels of IgG2 in bacterial vs viral CAP. Still, the IgG2 associations with bacterial CAP in the present cohort may be regarded as modest, and therefore in line with results from an Australian CAP study [11], where no single pathogen was associated with low IgG subclass levels, illustrating an uncertain impact of IgG2 deficiency. Antibodies to polysaccharide antigens of encapsulated bacteria are largely, but not entirely, of the IgG2 subclass, while antibodies to protein antigens of viruses are IgG1 and IgG3 [29]. Previously, increased susceptibility to infections caused by encapsulated bacteria in patients with low IgG2 has been demonstrated [14, 15, 30], suggesting that inherently IgG2-deficient patients have a reduced ability to mount an antibody response to certain polysaccharide antigens, leading to an inadequate defense against invading pathogens like *S. pneumoniae* and *H. influenzae*. Yet, an appropriate immune response may remain intact in patients with low IgG2 levels, as the cause of reduced levels remains unclear. Another possible reason for low IgG2 levels may be consumption of antibodies in the acute phase of CAP, with low levels subsequently seen at hospital admission.

The causes and clinical importance of our IgG and IgG2 findings are therefore uncertain.

Several recent studies have reported an association between low Ig levels, disease severity, and unfavorable outcome in patients with sepsis [8]. In CAP, however, this association has not been explored to the same extent. Feldman et al. could not find any differences in IgG subclass levels between 66 non-ICU and ICU patients with CAP [31]. By contrast, a recent Spanish study of 418 CAP patients showed a significant association between low Ig levels (IgG/IgG1) in patients with a CURB-65 score of 4 and 5, in ICU patients vs non-ICU patients (IgG/IgG1/IgG2), and with 30-day mortality (IgG/IgG1/IgG2) [13]. Low levels of IgG2 have been observed in patients with severe H1N1 influenza in China [32] and Australia [10, 11]. Of note, in the Chinese study, the lowest IgG2 levels were seen in seriously ill influenza patients with a bacterial co-infection [32]. In our study, low serum levels of Igs at admission were associated neither with disease severity
nor with ICU admission and 30-mortality. The low number of severely ill patients may have influenced our observations regarding outcome. Nonetheless, the relationship between low admission Ig levels and adverse short-term outcome does not seem to be as close in CAP as in sepsis, at least not in patients with a nonsevere clinical course.

Despite the considerable morbidity in primary hypogammaglobulinemia, especially recurrent respiratory infections [6], the impact on the long-term outcome of a transient decrease in Igs during infections like pneumonia is not clear. In the present cohort, we did not find significant associations between low levels of Igs at hospital admission and 5-year all-cause mortality, suggesting a less important role of low admission Ig levels per se on long-term immune responses.

Although deficiency of the pattern recognition molecule MBL has been associated with respiratory infections in cystic fibrosis [20] and CVID [21], a consistent predisposition to pneumonia in MBL-deficient or -insufficient patients has not been documented. A meta-analysis indicated that MBL-deficient patients were predisposed to invasive pneumococcal infections [33], but in 3 CAP studies of low MBL, including a more recent meta-analysis, no associations to S. pneumoniae [33], but in 3 CAP studies of low MBL, including a more recent meta-analysis, no associations to S. pneumoniae or other pathogens in CAP have been found [34–36]. In our study population, MBL deficiency was not associated with microbial etiology. Consequently, our observations support the hypothesis that MBL deficiency is not an important factor for microbial vulnerability in CAP [37, 38].

A large Spanish study of 848 CAP patients found MBL insufficiency to be associated with severe sepsis, multiorgan dysfunction syndrome, ICU admission, and 90-day mortality, but not 28-day mortality [37]. In later studies, however, these findings have not been reproduced [36, 38]. Similarly, an association between MBL deficiency and disease severity or outcome measures was not seen in our CAP cohort, but in our cohort fewer patients had a very high CURB-65 score and the short-term mortality rate was fairly low. Thus, the study populations are not completely comparable. In conclusion, a link between MBL deficiency and CAP severity and outcome may exist, even though our results do not support an association.

MASP-2, a part of the MASP family, is a protease essential for activation of the lectin complement pathway by cleavage of C4 and C2 [18]. Defects of MASP-2 have been suggested to be associated with infectious diseases [39], but with an uncertain clinical penetrance [40]. We identified a patient with a homozygous MASP-2 deficiency, to our knowledge the first case described in Norway. This patient was without tendency to recurrent infections and did not present with a severe form of CAP, in accordance with previous findings [37].

Limitations
The following limitations should be considered. First, our study was performed at a single hospital, thereby possibly limiting its generalizability. Second, blood samples were not obtained beyond the 6-week follow-up; thus we do not know the time frame of the decrease in IgG/IgG2 levels in 39 patients in this CAP cohort. Third, the outcome measure short-term outcome was defined as a composite of ICU admission and 30-day mortality. A majority of patients (39/49, 80%) were categorized with the softer ICU survivor outcome parameter.

Conclusion
In summary, neither low admission immunoglobulin levels nor MBL deficiency was associated with microbial etiology, disease severity, short-term outcome, or long-term outcome in hospitalized patients with CAP.

Supplementary Data
Supplementary materials are available at Open Forum Infectious Diseases online (http://ofid.oxfordjournals.org).

Acknowledgments
We gratefully thank Kåre Bo, Thomas Skrede, Anita Johansen, and Britt Hiaasen for collection of patient data; Ola Bjørang, Helvi H. Samdal, Carina Thilesen, and Mette Bogen for excellent laboratory assistance; and Nihal Perera for contributing to the design of the database.

Financial support. This work was supported by Vestre Viken Hospital Trust, Norway. The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References
1. Wanderink RG, Waterer GW. Community-acquired pneumonia. N Engl J Med 2014;370:1863.
2. Trotter CL, Stuart JM, George R, Miller E. Increasing hospital admissions for pneumonia. England. Emerg Infect Dis 2008;14:727–33.
3. Woodhead M, Welch CA, Harrison DA, et al. Community-acquired pneumonia on the intensive care unit: secondary analysis of 17,869 cases in the ICNARC Case Mix Programme Database. Crit Care 2006;10(Suppl 2):S1.
4. Letourneau AR, Issa NC, Baden LR. Pneumonia in the immunocompromised host. Curr Opin Pulm Med 2014;20:272–9.
5. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol 2010;125:S41–52.
6. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol 2010;125:S182–94.
7. Olinder-Nielsen AM, Granert C, Forsberg P, et al. Immunoglobulin prophylaxis in 350 adults with IgG subclass deficiency and recurrent respiratory tract infections: a long-term follow-up. Scand J Infect Dis 2007;39:44–50.
8. Bermejo-Martín JR, Giamarellos-Bourboulis EJ. Endogenous immunoglobulins and sepsis: new perspectives for guiding replacement therapies. Int J Antimicrob Agents 2015;46(Suppl 1):S25–8.
9. Shankar-Hari M, Culshaw N, Post B, et al. Endogenous IgG hypogammaglobulinaemia in critically ill adults with sepsis: systematic review and meta-analysis. Intensive Care Med 2015;41:393–401.
10. Gordon CL, Johnson PD, Permezel M, et al. Association between severe pandemic 2009 influenza A (H1N1) virus infection and immunoglobulin G(2) subclass deficiency. Clin Infect Dis 2010;50:672–8.
11. Gordon CL, Holmes NE, Grayson ML, et al. Comparison of immunoglobulin G subclass concentrations in severe community-acquired pneumonia and severe pandemic 2009 influenza A (H1N1) infection. Clin Vaccine Immunol 2012;19:446–8.
12. de la Torre MC, Bolíbar I, Vendrell M, et al. Serum immunoglobulins in the infected and convalescent phases in community-acquired pneumonia. Respir Med 2013;107:2038–45.
13. de la Torre MC, Toran P, Serra-Prat M, et al. Serum levels of immunoglobulins and severity of community-acquired pneumonia. BMJ Open Respir Res 2016;3:e000152.
14. Ekdahl K, Bacronier JH, Svanborg C. Immunoglobulin deficiencies and impaired immune response to polysaccharide antigens in adult patients with recurrent community-acquired pneumonia. scand J Infect Dis 1997;29:401–7.
15. Ambrosino DM, Schiffman G, Gotschlich EC, et al. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. J Clin Invest 1985;75:1935–42.
16. Dunkelberger JR, Song WC. Complement and its role in innate and adaptive immune responses. Cell Res 2010;20:34–50.
17. Skaatun I, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. Mol Immunol 2011;48:1643–55.
18. Garred P, Genster N, Pilely K, et al. A journey through the lectin pathway of complement-MBL and beyond. Immunol Rev 2016;274:74–97.
19. Garred P, Madsen HO, Kurthztals JA, et al. Dialelic polymorphism may explain variations of the blood concentration of mannan-binding protein in Eskimos, but not in black Africans. Eur J Immunogenet 1992;19:403–12.
20. Garred P, Pressler T, Madsen HO, et al. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 1999;104:431–7.
21. Fevang B, Molinnes TE, Holm AM, et al. Common variable immunodeficiency and the complement system; low mannose-binding lectin levels are associated with bronchiectasis. Clin Exp Immunol 2005;142:576–84.
22. Holter JC, Müller F, Bjørgø A, et al. Etiology of community-acquired pneumonia and diagnostic yields of microbiological methods: a 3-year prospective study in Norway. BMC Infect Dis 2015;15:64.
23. Holter JC, Ueland T, Jenum PA, et al. Risk factors for long-term mortality after hospitalization for community-acquired pneumonia: a 5-year prospective follow-up study. PLoS One 2016;11:e0148741.
24. Seelen MA, Roos A, Wieland J, et al. Functional analysis of the classical, alternative, and MBL pathways of the complement system: standardization and validation of a simple ELISA. J Immunol Methods 2005;296:187–98.
25. Csuka D, Munte-Fog L, Skjødt MO, et al. A novel assay to quantitate MASP-2/ficolin-3 complexes in serum. J Immunol Methods 2013;387:237–44.
26. Weiss G, Madsen HO, Garred P. A novel mannose-binding lectin-associated serine protease 1/3 gene variant. Scand J Immunol 2007;65:430–4. 27. Lim WS, van der Eerden MM, Laing R, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax 2003;58:377–82.
28. Siljan WW, Holter JC, Nyno SH, et al. Circulating cell-free DNA is elevated in community-acquired bacterial pneumonia and predicts short-term outcome. J Infect 2016;73:383–6.
29. Vidarsson G, Dekkers G, Rispeins T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol 2014;5:520.
30. Stanley PJ, Corbo G, Cole PJ. Serum IgG subclasses in chronic and recurrent respiratory infections. Clin Exp Immunol 1984;58:703–8.
31. Feldman C, Mahomed AG, Mahida P, et al. IgG subclasses in previously healthy adult patients with acute community-acquired pneumonia. S Afr Med J 1996;86:600–2.
32. Chan JF, To KK, Tse H, et al. The lower serum immunoglobulin G2 level in severe cases than in mild cases of pandemic H1N1 2009 influenza is associated with cytokine dysregulation. Clin Vaccine Immunol 2011;18:305–10.
33. Moens L, Van Hoeveveld E, Peetersmans WE, et al. Mannose-binding lectin genotype and invasive pneumococcal infection. Hum Immunol 2006;67:605–11.
34. García-Laorden MI, Rodríguez de Castro F, Solé-Violán J, et al. The role of mannose-binding lectin in pneumococcal infection. Eur Respir J 2013;41:131–9.
35. Perez-Castellano M, Peñaranda M, Rivas A, et al. Mannose-binding lectin does not act as an acute-phase reactant in adults with community-acquired pneumococcal pneumonia. Clin Exp Immunol 2006;145:228–34.
36. van Kempen G, Meijvis S, Endeman H, et al. Mannose-binding lectin and ficolin polymorphisms in patients with community-acquired pneumonia caused by intracellular pathogens. Immunology 2017;151:81–8.
37. García-Laorden MI, Solé-Violán J, Rodríguez de Castro F, et al. Mannose-binding lectin and mannose-binding lectin-associated serine protease 2 in susceptibility, severity, and outcome of pneumonia in adults. J Allergy Clin Immunol 2008;122:368–74, 374.e1–2.
38. Endeman H, Herpers BL, de Jong BA, et al. Mannose-binding lectin polymorphisms in patients with community-acquired pneumonia caused by influenza A(H1N1)pdm09. J Infect 2015;66:214–20.
39. Garcia-Laorden MI, Garcia-Saavedra A, de Castro FR, et al. Low clinical penetration of mannose-binding lectin-associated serine protease 2 deficiency. J Allergy Clin Immunol 2006;118:1383–6.