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Comparison of Immunoglobulin G Subclass Concentrations in Severe Community-Acquired Pneumonia and Severe Pandemic 2009 Influenza A (H1N1) Infection

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We compared immunoglobulin G (IgG) subclasses in patients with severe noninfluenza community-acquired pneumonia (CAP) to those in patients with severe pandemic 2009 influenza (H1N1) virus infection. Low IgG1 and IgG2 levels occurred often in the CAP group; however, H1N1 patients had lower IgG1 and IgG2 levels (5.4 versus 3.3 g/liter [$P = 0.008$] and 2.5 versus 1.2 g/liter [$P < 0.001$], respectively). Low IgG2 levels may be specifically linked to severe H1N1; however, it is not clear whether this association is related to H1N1 or to other features of severity.

We recently reported an association between pandemic 2009 influenza A (H1N1) virus infection severity and immunoglobulin G2 (IgG2) subclass deficiency (5). However, it is not clear whether IgG2 deficiency is unique to severe H1N1 virus infection or whether it is also associated with other forms of severe noninfluenza community-acquired pneumonia (CAP). We retrospectively assessed IgG subclass concentrations in patients with severe noninfluenza CAP who participated in the Australian CAP Study (ACAPS) (2) and compared this cohort to our original cohort of patients with severe H1N1 virus infection (5).

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ACAPS was a large, prospective, multicenter study of CAP etiology in hospitalized patients (2), while our H1N1 study focused on a multicenter observational cohort of hospitalized nonpregnant and pregnant patients with H1N1 virus infection (5). Only patients with severe infection, defined as patients who required intensive respiratory and/or vasopressor support (2), were included in this further analysis. ACAPS patients diagnosed with influenza were excluded (6 patients: 4 with influenza A, 2 with influenza B). Demographic and clinical details were collected from both cohorts. CAP was defined according to Infectious Diseases Society of America/American Thoracic Society guidelines (11). At the time of study enrolment, acute-phase sera were collected and either frozen at −20°C (ACAPS cohort) or used fresh for assessment of immunoglobulin concentrations (H1N1 cohort). Stored frozen sera did not undergo repeated cycles of thawing. Total IgG and IgG subclass concentrations were assessed using the same assay (Beckman IMMAGE 800 analyzer; Beckman Coulter Inc., Brea, CA) for both cohorts. As previously (5), the reference ranges for normal adults were as follows: total IgG, 7.0 to 16.5 g/liter; IgG1, 3.8 to 9.3 g/liter; IgG2, 2.4 to 7.0 g/liter; IgG3, 0.22 to 1.76 g/liter; IgG4, 0.04 to 0.86 g/liter.

Clinical and laboratory features of the severe noninfluenza CAP cohort and the severe H1N1 cohort are shown in Table 1. Acute-phase sera from 65 (74%) of 88 ACAPS patients with severe noninfluenza CAP were available. Of the remaining 23 patients without stored sera, no factor could be identified to explain the lack of stored serum. IgG subclass concentrations in the severe noninfluenza cohort did not differ if the etiology of the CAP was known or unknown. In this cohort, *Streptococcus pneumoniae* was the most common pathogen, followed by picornaviruses and *Haemophilus influenzae*, and compared to patients who were not infected with that pathogen, none of these infections were associated with low IgG1 concentrations (16/54 versus 5/11 [$P = 0.24$], 18/56 versus 3/9 [$P = 0.61$], and 19/59 versus 2/6 [$P = 0.64$], respectively) or low IgG2 concentrations (24/54 versus 6/11 [$P = 0.39$], 25/56 versus 5/9 [$P = 0.40$], and 28/59 versus 2/6 [$P = 0.41$], respectively).

Compared to patients with severe noninfluenza CAP, the H1N1 cohort was younger and more likely to be female, to be pregnant, to require extracorporeal membrane oxygenation (ECMO), and to have a lower serum albumin level (Table 1). Serum immunoglobulin concentrations were assessed a mean of 1.5 days later in the severe H1N1 group than in the severe CAP group. IgG subclass concentrations of severe noninfluenza CAP and H1N1 patients are shown in Table 1 and Fig. 1. Compared to patients with severe noninfluenza CAP, a significantly higher proportion of patients with severe H1N1 virus infection had total IgG, IgG1, and IgG2 concentrations in the deficient range. Furthermore, the median concentrations of total IgG, IgG1, and especially IgG2 were significantly lower among H1N1 patients. To remove any potential confounding effect of pregnancy, the analysis was repeated after pregnant women were excluded. Although low IgG2 levels were again observed in the H1N1 cohort, they did not reach statistical significance ($P = 0.07$).

Although IgG2 subclass deficiency has recently been associated with severe H1N1 virus infection (5), IgG subclass concentrations

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TABLE 1 Comparison of clinical and laboratory features of patients with severe noninfluenza CAP and those with severe pandemic 2009 H1N1 virus infection

Patient feature	Severe CAP[a]	Severe H1N1[b]	*P* value
Total no. (%)	65	19	
Median age, yr (range)	69 (19–76)	36 (16–79)	<0.001
No. of males	40	7	0.05
No. pregnant	1	7	<0.001
Comorbidities			
No. with malignancy	3	1	0.65
No. with immunosuppression	8[e]	1[e]	0.35
No. with asthma	21	3	0.13
No. with obesity	—[f]	1	
No. with diabetes mellitus	11	3	0.61
No. with pneumonia	65	16	
ICU management			
No. with endotracheal intubation/ventilation alone	27	12	0.08
No. with endotracheal intubation/ventilation plus ECMO	0	2	0.05
No. with noninvasive ventilation/high-flow O_2	38	5	0.013
No. who died within 30 days	18	2	0.10
Laboratory features			
No. with a known infectious pathogen[c]	31		
No. with *Streptococcus pneumoniae*	11		
No. with picornavirus	9		
No. with *Haemophilus influenzae*	6		
No. with *Mycoplasma pneumoniae*	4		
No. with *Legionella pneumophila*	4		
No. with ≥1 pathogens	9	1[d]	0.28
Mean serum albumin level (g/liter) ± SD	31 ± 6	23 ± 5	<0.001
Immunoglobulin data (all patients)			
Mean no. of days after admission when serum immunoglobulins were assessed ± SD	1.2 ± 1.8	2.6 ± 2.1	0.005
No. (%) with low total IgG level	23 (35)	12 (63)	0.03
Median (range) total IgG level (g/liter)	8.7 (0.6–28.7)	6.1 (1.9–25.2)	0.002
No. (%) with low IgG1 level	21 (32)	11 (58)	0.025
Median (range) IgG1 level (g/liter)	5.4 (0.3–24.6)	3.3 (1.1–17.8)	0.008
No. (%) with low IgG2 level	30 (46)	15 (79)	0.011
Median (range) IgG2 level (g/liter)	2.5 (0.2–10.5)	1.2 (0.4–6.6)	<0.001
No. (%) with low IgG3 level	5 (8)	2 (11)	0.50
Median (range) IgG3 level (g/liter)	0.68 (0.04–3.2)	0.42 (0.21–1.4)	0.06
No. (%) with low IgG4	4 (6)	3 (16)	0.18
Median (range) IgG4 level (g/liter)	0.21 (0.01–2.7)	0.15 (0.1–2.0)	0.16
Immunoglobulin data (nonpregnant patients)			
Total no. not pregnant	64	12	
No. (%) with low total IgG level	23 (36)	6 (50)	0.27
Median (range) total IgG level (g/liter)	8.7 (0.6–28.7)	6.6 (2.2–25.2)	0.12
No. (%) with low IgG1 level	21 (33)	6 (50)	0.21
Median (range) IgG1 level (g/liter)	5.4 (0.3–24.6)	3.7 (1.3–17.8)	0.15
No. (%) with low IgG2 level	30 (47)	9 (75)	0.07
Median (range) IgG2 level (g/liter)	2.5 (0.2–10.5)	1.5 (0.4–6.6)	0.06
No. (%) with low IgG3 level	5 (8)	1 (8)	0.66
Median (range) IgG3 level (g/liter)	0.69 (0.04–3.2)	0.46 (0.21–1.4)	0.22
No. (%) with low IgG4 level	4 (6)	1 (8)	0.59
Median (range) IgG4 level (g/liter)	0.22 (0.01–2.7)	0.23 (0.1–2.0)	0.80

[a] Influenza patients excluded.
[b] H1N1 cohort data adapted from Gordon et al. (5).
[c] Additional culture results: 2 patients had *Pseudomonas aeruginosa*, 2 patients had *Staphylococcus aureus*, 1 patient had *Acinetobacter baumannii*, and 1 patient had *Escherichia coli*.
[d] *Pseudomonas aeruginosa*.
[e] In the H1N1 group, one patient was receiving prednisolone. In the ACAPS group, eight patients were receiving prednisolone and one patient was receiving dexamethasone.
[f] —, Obesity was not recorded in the ACAPS study.

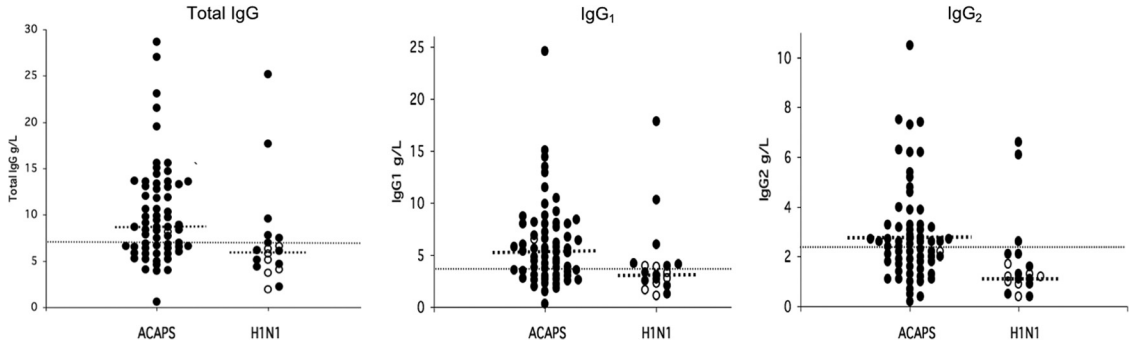

FIG 1 Total IgG, IgG1, and IgG2 concentrations in serum samples from patients with severe noninfluenza CAP and severe H1N1 virus infection. Data are shown for pregnant (○) and nonpregnant (●) patients. Dashed line, median value of each grouping; dotted line, lower limit of the normal range of the relevant immunoglobulin for adults.

in adults with severe CAP have not been extensively investigated (4). ACAPS is currently one of the largest studies of the microbial etiology of CAP (2) and provides a unique opportunity to assess IgG subclass concentrations in patients with severe CAP. Although a large proportion of patients with severe CAP had low total IgG (specifically, IgG1 and IgG2) levels, no single pathogen was associated with low IgG subclass levels, including pneumococcus, a polysaccharide-encapsulated bacterium associated with infections in IgG2-deficient individuals (3).

IgG2 deficiency has been associated with recurrent pneumonia (3); however, there are limited data on IgG subclass concentrations in previously well adults with CAP. Herer et al. (6) found low IgG2 concentrations in 38 hospitalized CAP patients; however, this difference was observed in patients with bacterial, not viral, pneumonia and pneumonia severity was not reported. In a single-center prospective study, Feldman et al. (4) found that 21% of 19 adults admitted to the intensive care unit (ICU) with severe CAP were deficient in IgG1 or IgG2—a substantially lower percentage than the 62% we identified among 65 ACAPS patients.

Compared to patients with severe CAP, patients with severe H1N1 virus infection had significantly lower total IgG (specifically, IgG1 and IgG2) concentrations. However, since low IgG subclass concentrations have been reported in the late stages of pregnancy (5, 10), we reassessed our data after excluding pregnant patients. Low IgG2 concentrations were again observed in the H1N1 group, but this difference did not reach statistical significance ($P = 0.07$). In addition, other factors may have confounded any association between low IgG subclasses and severe H1N1 virus infection—for instance, severe H1N1 patients were younger, had more-severe illness, had IgG concentrations assessed later, and had lower serum albumin levels than the ACAPS cohort. Although a detailed multivariate analysis adjusting for all significant univariate variables could be undertaken, we considered this statistically inappropriate given the small numbers associated with some variables. Thus, we are unable to say with absolute certainty whether the lower IgG2 levels associated with severe H1N1 disease are solely specific to H1N1 virus infection or are potentially confounded by the other clinical features known to be specifically associated with this strain of influenza A virus (7, 9, 12). Other authors have recently described low IgG2 levels in severe cases of H1N1 virus infection and noted an association with cytokine dysregulation (1), while the use of convalescent-phase plasma from patients recovering from H1N1 virus infection appears to show promising efficacy among severe H1N1 cases (8).

Despite the above reservations, we believe this study is notable since it suggests that IgG2 deficiency is specifically linked to severe H1N1 disease, more than simply severe noninfluenza CAP *per se*. Whether this association is related to the nature of H1N1 influenza A virus infection or the other clinical features associated with severe H1N1 disease, such as pregnancy, is less clear and requires further investigation in a larger patient cohort.

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REFERENCES

1. **Chan JF, et al.** 2011. Lower serum immunoglobulin G2 level is associated with cytokine dysregulation in severe cases of pandemic H1N1 2009 influenza. Clin. Vaccine Immunol. **18**:305–310.
2. **Charles PG, et al.** 2008. The etiology of community-acquired pneumonia in Australia: why penicillin plus doxycycline or a macrolide is the most appropriate therapy. Clin. Infect. Dis. **46**:1513–1521.
3. **Ekdahl K, Braconier JH, Svanborg C.** 1997. Immunoglobulin deficiencies and impaired immune response to polysaccharide antigens in adult patients with recurrent community-acquired pneumonia. Scand. J. Infect. Dis. **29**:401–407.
4. **Feldman C, et al.** 1996. IgG subclasses in previously healthy adult patients with acute community-acquired pneumonia. S. Afr. Med. J. **86**:600–602.
5. **Gordon CL, et al.** 2010. Association between severe pandemic 2009 influenza A (H1N1) virus infection and immunoglobulin G(2) subclass deficiency. Clin. Infect. Dis. **50**:672–678.
6. **Herer B, et al.** 1990. Selective IgG subclass deficiencies and antibody responses to pneumococcal capsular polysaccharide antigen in adult community-acquired pneumonia. Am. Rev. Respir. Dis. **142**:854–857.
7. **Hewagama S, et al.** 2010. 2009 H1N1 influenza A and pregnancy outcomes in Victoria, Australia. Clin. Infect. Dis. **50**:686–690.
8. **Hung IF, et al.** 2011. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin. Infect. Dis. **52**:447–456.
9. **Kumar A, et al.** 2009. Critically ill patients with 2009 influenza A (H1N1) infection in Canada. JAMA **302**:1872–1879.
10. **Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H.** 1996. Evolution of maternofetal transport of immunoglobulins during human pregnancy. Am. J. Reprod. Immunol. **36**:248–255.
11. **Mandell LA, et al.** 2007. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin. Infect. Dis. **44**(Suppl. 2):S27–S72.
12. **Perez-Padilla R, et al.** 2009. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N. Engl. J. Med. **361**:680–689.