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Short communication

# IgM levels in plasma predict outcome in severe pandemic influenza

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ABSTRACT

*Background:* Little is known on the participation of immunoglobulin isotypes and subclasses in the pathogenesis of the severe disease caused by the pandemic influenza virus (influenza A(H1N1)pdm09).
*Objectives:* (1) To evaluate the association between plasma levels of IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE and outcome in patients with severe pandemic influenza. (2) To evaluate the association between immunoglobulin and cytokine levels in these patients.
*Study design:* 40 critically ill patients with community acquired pneumonia and influenza A(H1N1)pdm09 infection were recruited from November 2010 to February 2011. Plasma samples were collected during the first 24 h following admission to the ICU. Immunoglobulins and 17 major cytokines were profiled in plasma.
*Results:* 15 patients died (37.5%). When the association between clinical variables and prognosis was assessed, prior immunosuppression, APACHE II score, levels of IgG2 and levels of IgM were associated with outcome in a univariate Cox regression analysis. Kaplan Meier analysis showed that patients with

*Abbreviations:* ICU, intensive care unit; CAP, community acquired pneumonia; COPD, chronic obstructive pulmonary disease; HBP, high blood pressure.

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levels of IgG2 and IgM < 59 and < 58 mg/dl respectively died earlier. Multivariate Cox regression analysis showed that APACHE II score and levels of IgM were the best predictors of outcome, being levels of IgM a protective factor against mortality. IgM was the immunoglobulin showing the largest number of negative correlations with cytokine levels.

*Conclusions:* Our results support a central role of IgM in preventing uncontrolled inflammatory response and mortality in severe pandemic influenza. Early assessment of IgM could contribute to guide clinical decisions in these patients.



## 1. Background

Pandemics generated by 2009 influenza A/H1N1 virus (influenza A(H1N1)pdm09) caused a great concern in the society. Little is known on the participation of immunoglobulin isotypes in the pathogenesis of the severe disease caused by this virus. Gordon et al. [1] described lower levels of the IgG2 subclass in severe cases of pandemic influenza. Chan et al. reported that the lower IgG2 levels in these cases are associated with cytokine dysregulation [2]. To the present date, there is no any available information on the involvement of immunoglobulins in the prognosis of patients with critical respiratory illness caused by this virus.

## 2. Objectives

(1) To evaluate the association between plasma levels of IgG1, IgG2, IgG3, IgG4, IgA, IgM, IgE and outcome in patients with severe pandemic influenza. (2) To evaluate the association between immunoglobulin and cytokine levels in these patients.

## 3. Study design

*Patients*: 40 critically ill patients with community acquired pneumonia (CAP) with influenza A(H1N1)pdm09 infection were prospectively recruited from November 2010 to February 2011. Definition of CAP was based on current American Thoracic Society and Infectious Disease Society of America guidelines [3]. Patients with HIV infection or those receiving radiotherapy, immunosuppressive drugs, including chemotherapy or systemic steroids in the last three months previous to admission to the ICU were considered as immunosuppressed. Informed consent was obtained directly from each patient and control before enrolment. Approval of the study protocol for both the scientific and the ethical aspects was obtained from the Scientific Committee for Clinical Research of our hospital. The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

*Samples*: A single blood sample was obtained from each patient in tubes containing ethylenediaminetetraacetic acid within the first 24 h following admission to the ICU. Cytokine levels in plasma were evaluated using the Biorad© 17 plex assay (Hercules, CA, USA). This system allows for quantitative measurement of IL-1β; IL-6; IL-8; IL-7; IL-17; G-CSF; MCP-1; MIP-1β; IL-2; IL-4; IL-5; IL-10; IL-12(p70); IL-13; GM-CSF; IFN-γ; TNF-α. Levels of IgG1, IgG2, IgG3, IgG4, IgA, IgM, and IgE in plasma were measured by using a multiplex Immunoglobulin Isotyping kit purchased to Biorad on the same Luminex platform. Microbiological identification was performed by standard diagnostic methods in each participating Hospital using respiratory cultures, blood cultures, and/or positive urinary antigen test to *Legionella pneumophila* or *Streptococcus pneumoniae*. Molecular diagnosis was used for influenza detection in respiratory samples, using the *pandemic Influenza A/H1N1 Detection Set* (*Roche*®) on a *Roche 2.0* Light Cycler platform. Presence of other viruses was excluded by using the *Respiratory Viral Panel-XTAG RVP* (*Abott*®) on the Luminex platform.

*Statistical analysis*: The impact of clinical variables and immunoglobulin levels on mortality over time was assessed by using univariate Cox regression analysis. Those variables yielding $p$ values < 0.1 in the univariate analysis were further introduced in a multivariate analysis using the Wald test for forward selection. The accuracy of immunoglobulins for diagnosis of mortality was studied by calculating areas under the receiver operating characteristic (AUROC) curve. Correlation between immunoglobulin and cytokine levels was assessed by using the Spearman Karber test. Those correlations that were significant at the level of $p < 0.05$ were represented in a heat map by using the JColorGrid software (University of California San Francisco and University of California Berkeley) [4]. All statistical tests were 2 sided, and $p < 0.05$ was considered significant. Data analysis was performed using SPSS 20.0 software (IBM-SPSS, Chicago, IL).

**Table 1**
Clinical characteristics and immunoglobulin levels of the patients. Differences between groups were assessed using the $\chi^2$ test for categorical variables and the Mann Whitney *U* test for continuous variables when appropriate. Continuous variables were expressed as median [interquartile rank]. Categorical variables are shown as *n* (% over column). COPD, chronic obstructive pulmonary disease; HBP, high blood pressure.

| Characteristics | Survivors ($n = 25$) | Non survivors ($n = 15$) | $p$ |
|---|---|---|---|
| Age (years) | 52 [27] | 58 [9] | n.s. |
| Sex | | | |
| Female | 7 (28) | 5 (33) | n.s. |
| Male | 18 (72) | 10 (66) | n.s. |
| Comorbidities | | | |
| Digestive disease | 2 (8) | 1 (6) | n.s. |
| Cardiovascular disease | 4 (16) | 3 (20) | n.s. |
| Neurological disease | 3 (12) | 0 (0) | n.s. |
| Renal disease | 2 (8) | 0 (0) | n.s. |
| Respiratory disease | 8 (32) | 3 (20) | n.s. |
| Immunosuppression | 6 (24) | 9 (60) | 0.023 |
| Diabetes | 4 (16) | 2 (13) | n.s. |
| HBP | 8 (32) | 4 (26) | n.s. |
| COPD | 5 (20) | 2 (13) | n.s. |
| Smoker (ever) | 11 (44) | 7 (46) | n.s. |
| Alcohol abuse | 2 (8) | 1 (6) | n.s. |
| IV drugs abuse | 1 (4) | 0 (0) | n.s. |
| Steroids | 5 (20) | 3 (20) | n.s. |
| Obesity | 5 (20) | 3 (20) | n.s. |
| Bacterial infection | | | |
| At ICU admission | 5 (20) | 5 (33) | n.s. |
| At any time | 6 (24) | 8 (53) | n.s. |
| IMV | 14 (56) | 15 (100) | 0.004 |
| NMV | 10 (40) | 1 (6) | n.s. |
| APACHE | 13 [8] | 21 [9] | 0.001 |
| Death time (days) | – | 8 [24] | n.s. |
| PAFI | 115 [139] | 86 [71] | n.s. |
| Creatinine level (mg/dl) | 1.05 [0] | 1.16 [6] | n.s. |
| AST (U/l) | 60 [56] | 50 [104] | n.s. |
| ALT (U/l) | 34 [33] | 41 [68] | n.s. |
| WBC count ($\times 10^3/\mu l$) | 10.9 [9.9] | 3.9 [18.1] | n.s. |
| Neutrophils ($\times 10^3/\mu l$) | 8.8 [8.7] | 3.6 [8.4] | n.s. |
| IgG1 (mg/dl) | 856 [674] | 816 [2057] | n.s. |
| IgG2 (mg/dl) | 69 [85] | 34 [150] | n.s. |
| IgG3 (mg/dl) | 102 [202] | 66 [172] | n.s. |
| IgG4 (mg/dl) | 43 [91] | 23 [51] | n.s. |
| IgM (mg/dl) | 91 [93] | 50 [86] | 0.049 |
| IgA (mg/dl) | 135 [84] | 130 [136] | n.s. |