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ORIGINAL ARTICLE

Impact of severe hematological abnormalities in the outcome of hospitalized patients with influenza virus infection

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Abstract Although hematological abnormalities have been described among patients with influenza virus infection, little is known about their impact on the outcome of the patients. The aim of this study was to assess the frequency and clinical impact of severe hematological abnormalities in patients with confirmed influenza virus infection. This was an observational retrospective study including all adult patients with diagnosis of influenza virus infection hospitalized from January to May 2016 in our institution. Influenza virus infection was diagnosed by means of rRT-PCR assay performed on respiratory samples. Poor outcome was defined as a composite endpoint in which at least one of the following criteria had to be fulfilled: (a) respiratory failure, (b) SOFA ≥2, or (c) death. Two hundred thirty-nine patients were included. Applying the HLH-04 criteria for the diagnosis of hemophagocytic syndrome, cytopenias (hemoglobin ≤9 g/dl, platelets <100,000/μl or neutrophils <1,000/μl) were present in 51 patients (21%). Patients with hematological abnormalities showed higher SOFA scores, respiratory failure, septic shock and in-hospital mortality than the remaining patients. The composite endpoint was present in 33.3% in the cytopenias group vs. 13.3% in the group without cytopenias (p=0.001). In a multivariate analysis, variables associated with the composite endpoint were: use of steroids prior to present admission (OR: 0.12; 95% CI: 0.015–0.96, p=0.046), presence of any hematological abnormality (OR: 3.54; 95% CI:1.66–7.51, p=0.001), and LDH>225 U/l (OR:4.45; CI:1–19.71, p=0.049). Hematological abnormalities are not uncommon among hospitalized patients with influenza virus infection, and they are associated with a poorer outcome.

Keywords Influenza · Hemophagocytic syndrome · Hematological · Mortality · Steroids

Introduction

Hematological abnormalities have been previously described among patients with influenza virus infection [1, 2]. Moderate thrombocytopenia [1, 3, 4] and lymphopenia [1–6] are the most common findings, whilst anemia and neutropenia are rarely described [4]. As an example, the incidence of leukopenia and thrombocytopenia in patients who were hospitalized for the treatment of influenza A (H1N1) pdm09 early in the U.S. epidemic was 20% and 14%, respectively [7].

Most studies directed toward identifying mortality risk factors in influenza virus infection mainly take into consideration clinical variables, especially those related to comorbidities [2, 3]. Little is known about the impact of hematological abnormalities in the prognosis of this infection [1, 2]. Lymphopenia and thrombocytopenia are more frequently seen in patients with respiratory failure and shock [7, 8]. Additionally, a tendency toward increased mortality has been observed in the

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presence of lymphopenia or thrombocytopenia. However, these findings have not been confirmed in large multivariate analyses [3].

Moreover, hemophagocytic syndrome (HPS) secondary to influenza virus infection has been rarely described in immunocompetent and immunocompromised patients, with a high mortality rate [1, 9–17]. Nevertheless, the real incidence of this severe complication in patients with influenza is not known. To the best of our knowledge, there is only one prospective observational study carried out in 25 critically ill patients with respiratory failure and HPS secondary to influenza A (H1N1) pdm09 infection. HPS was found in 9 patients (36%), with a high mortality rate (89%) compared to those without HPS (25%) [18].

The aim of the present study was to assess the frequency and clinical impact of hematological abnormalities in the range of those accepted by the Histyocite Society for the suspicion of HPS [19] in patients who were admitted to the hospital with a confirmed influenza virus infection.

Materials and methods

Study population

We conducted an observational retrospective study including all adult patients with a diagnosis of influenza virus infection hospitalized from January to May 2016 in a 1300-bed tertiary teaching hospital in Madrid, Spain. The study protocol was approved by the University Hospital 12 de Octubre Review Board.

Microbiological methods

A confirmed case was defined by a positive result of a real-time reverse-transcriptase–polymerase-chain-reaction (rRT-PCR) assay performed at the local laboratory performed on respiratory samples [nasopharyngeal swabs (flocked swabs in UTM™ viral transport medium, Copan, Brescia, Italy)] from adult patients with respiratory tract symptoms. For the molecular diagnosis, RNA was extracted from 200 μl of the specimen using NucliSENS®easyMAG instrument (bioMérieux Diagnostics, Marcy l'Etoile, France) and eluted in 50 μl. Five μl of the elution were employed to perform each RT-PCR reaction. The modular duplex rRT-PCR for influenza A/influenza B detection (Influenza A/B r-gene ™, bioMérieux) was run in the LightCycler 480 instrument (Roche) [20]. All samples testing positive for influenza A were subtyped using rRT-PCR previously described [21] to detect specific regions of subtypes H1 and H3 hemagglutinin. For the detection of influenza A (H1N1) pdm09 subtype commercially available primers and probe (RealTime ready infA/H1N1 Detection set, Roche) [22] were used.

Study definitions

Hematological abnormalities secondary to influenza virus infection were only considered when they are in the range of the HLH-04 updated criteria proposed by the Histiocyte Society [19] for the diagnosis of hematophagocytic syndrome (hemoglobin ≤9 g/dl, platelets <100,000/μl, neutrophils <1000/μl).

A diagnosis of hemophagocytic syndrome needed to fulfill at least five of the clinical and non-clinical findings included in the previously mentioned HLH-04 updated criteria [19]: (a) fever of 38.5 °C or more, (b) splenomegaly, (c) cytopenias affecting at least two of three cell lineages in peripheral blood (hemoglobin ≤9 g/dl, platelets <100,000/μl, neutrophils <1000/μl), (d) hypertriglyceridemia (≥265 mg/dl) and/or hypofibrinogenemia (≤150 mg/dl), (e) hyperferritinemia (≥500 ng/ml), (f) hemophagocytosis in bone marrow, spleen, lymph nodes, or liver, (g) low or absent NK cell activity or (h) increased soluble IL-2 receptor concentration.

Comorbidity was defined using the Charlson index. Immunosuppression was defined as the presence of any the following: active malignant neoplasia, autoimmune disease, solid organ transplantation, HIV infection, use of steroids or chemotherapy. Use of steroids was defined as: (1) more than 20 mg/day of oral prednisone for 7 days or longer or (2) less than 20 mg/day over a minimum of 3 months [23].

Respiratory failure was defined as the need for mechanical ventilation, either non-invasive positive pressure ventilation or invasive mechanical ventilation, including those patients who had a clinical indication for ventilatory support but were finally not ventilated.

Sepsis, septic shock and organ dysfunction were defined according to the terms proposed recently by the Third International Consensus Definitions for Sepsis and Septic Shock [24] using for this purpose the SOFA score and the qSOFA score. Acute respiratory distress syndrome (ARDS) was defined according to the American-European Consensus Conference on ARDS [25, 26].

Poor outcome was defined as a composite endpoint in which at least one of the following criteria had to be fulfilled: (a) respiratory failure, (b) SOFA ≥2, or (c) death (related or not related to influenza infection).

Statistical methods

A descriptive analysis of patients was initially performed, comparing those who had cytopenias with those who did not have cytopenias. Descriptive analysis was performed using means (±SD) or medians with interquartile ranges (IQR). Student's t-test for independent samples was used to compare continuous variables. Mann–Whitney U test was used to compare continuous variables with a non-normal distribution, and the Fisher exact test to compare proportions. We further analyzed the risk factors associated with poor outcome by means

of a logistic regression model that included those variables found to be statistically significant at the univariate level or those deemed clinically relevant. Associations were expressed as odds ratios (ORs) with 95% confidence intervals (95% CI). Seventeen independent variables were initially selected based on clinical judgment and published literature. Prior to model fitting, cluster analysis was used to reduce the number of candidate variables. Backward selection with a type I error rate of 0.05 was used to reach a final reduced model containing three predictor variables. Discrimination of the final model was quantified via a C Index (ROC area). All statistical tests were 2-tailed and the threshold of statistical significance was $p < 0.05$. Statistical analysis was performed with computer software (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp and SAS/STAT 10.1; SAS Institute Inc., Cary, NC).

Results

During the study period 401 cases of influenza virus infection were confirmed by RT-PCR at our institution, of which 239 were included in the present study (Fig. 1). All cases were diagnosed between January and May 2016.

Clinical characteristics

Patient's demographic and clinical characteristics are shown in Table 1. Only 4 (1.7%) patients were living in a nursing home at the time of admission, and three patients were HIV positive. Only 40% of the cases were vaccinated against influenza with a mean time between vaccination and confirmed infection of 117.8 ± 26.3 days.

Symptoms initiated a median of 3 days (range 2–5 days) before influenza diagnosis. Dyspnea was present in 59.4% of the patients, and bronchospasm was described in 43% of them.

Hematological abnormalities

Laboratory parameters of the study population are presented in Table 2. Both baseline and nadir values were significantly lower in all cell lineages in patients who developed cytopenias during admission (Fig. 2). The median time to nadir of leukocytes, neutrophils, lymphocytes, platelets and hemoglobin was 1 day (range, 0–4), 2 days (range, 0–4), 0 days (range, −1 to 0), 0 days (range, −1 to 1), and 1 day (range, 0–3), respectively. When applying the HLH-04 criteria, we observed that 21% ($n = 51$) of the patients had at least one hematological abnormality, while 7.5% ($n = 18$) had cytopenias affecting at least two of three cell lineages. As is shown in Table 2, neutropenia (<1000 neutrophils/μl) was present in 5.9% of the cases ($n = 14$), thrombocytopenia (<100,000 platelets/μl) in 15.1% of the cases ($n = 36$), and anemia (Hb <9 g/dL) in 10.5% ($n = 25$) of the cases.

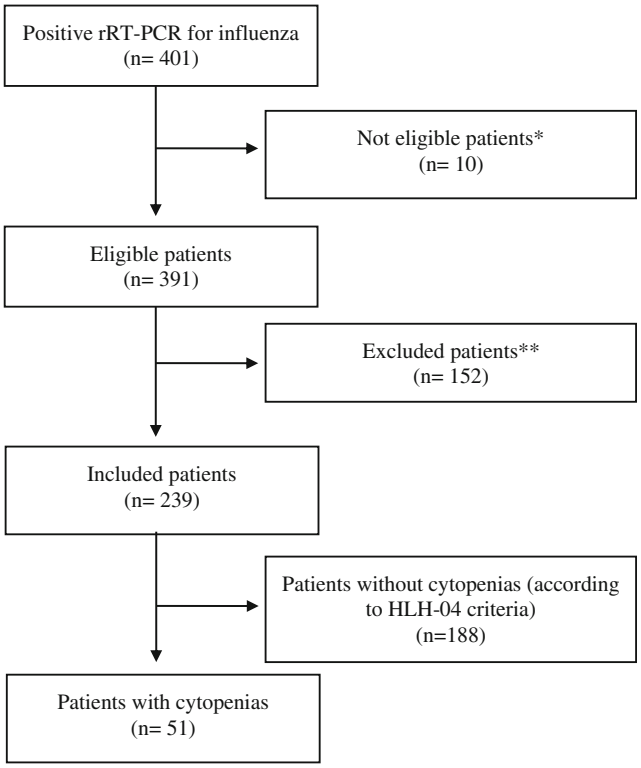

Fig. 1 Flow chart of included patients (Footnote of the figure: rRT-PCR= Real-time reverse-transcriptase–polymerase-chain-reaction. *Not eligible patients: 2 cases=invalid clinical history number, 2 cases=hematologic abnormalities not associated with influenza virus infection (present >30 days before influenza infection diagnosis), 5 cases=severe hematologic disease (2 cases of Leukemia, 1 case of Myelodysplastic Syndrome, 1 case of Immune Thrombocytopenic Purpura, 1 case of Megaloblastic Anemia), 1 case= multiorgan failure of abdominal origin. ** Excluded patients: 152 patients= no hospital admission)

In the group of patients with at least one hematological abnormality, neutropenia was present in 27.5%, thrombocytopenia in 70.6%, and anemia in 49%, respectively. Only one patient fulfilled the five criteria required for the diagnosis of HPS. One patient met three criteria for the diagnosis of HPS, six patients met two criteria, and 23 cases met one criterion. Overall, 20 of the 51 patients with at least one hematological abnormality did not meet any of the HLH-04 criteria. However, it is noteworthy that fibrinogen levels were only available in eight patients (>150 mg/dl in all cases), triglyceride levels in 20 patients (≥265 mg/dl in one case), and ferritin levels in 20 patients (>500 ng/dl in 8 cases). NK cell activity was not available in any case, while increased soluble IL-2 receptor concentration was observed in one of the two patients from whom it was available. Bone marrow biopsy was performed in one patient, which showed 26% of hemophagocytosis. Splenomegaly was not reported in any patient.

Table 1 Baseline demographic and clinical characteristics of patients with influenza infection

Characteristics	All patients (N = 239)	Patients with cytopenias (N = 51)	Patients without cytopenias (N = 188)	p-value
Age (years)	67.08 ± 16.09	64.59 ± 15.33	67.76 ± 16.26	0.12
Sex (male)	142 (59.7%)	33 (64.7%)	109 (58.3%)	0.408
Charlson comorbidity index score	2 (1–4)	2.5 (2–4)	2 (1–4)	**0.042**
Previous comorbid conditions				
Pregnancy	2 (0.8%)	0	2 (1.1%)	1
Active smoker	48 (20.1%)	7 (13.7%)	41 (21.8%)	0.2
Ex-smoker	56 (23.4%)	6 (11.8%)	50 (26.6%)	**0.027**
Obesity	43 (18%)	2 (3.9%)	41 (21.8%)	**0.003**
Diabetes mellitus	52 (21.8%)	7 (13.7%)	45 (23.9%)	0.11
Asthma	15 (6.3%)	1 (2%)	14 (7.4%)	0.15
Chronic obstructive pulmonary disease	51 (21.3%)	10 (19.6%)	41 (21.8%)	0.73
Cardiovascular disease	68 (28.5%)	10 (19.6%)	58 (30.9%)	0.11
Chronic liver disease	10 (4.2%)	4 (7.8%)	6 (3.2%)	0.22
Chronic kidney disease	36 (15.1%)	9 (17.6%)	27 (14.4%)	0.56
Neurologic disorder	23 (9.6%)	3 (5.9%)	20 (10.6%)	0.42
Immunosuppression (including steroids)	53 (22.2%)	20 (39.2%)	33 (17.6%)	**0.001**
Immunosuppression (without steroids)	25 (10.5%)	12 (23.5%)	13 (6.9%)	**0.001**
Steroids	28 (11.7%)	8 (15.7%)	20 (10.6%)	0.32
Influenza vaccination in the last year	96 (40.3%)	19 (37.3%)	77 (41.2%)	0.61
Pneumococcal vaccination[a]	76 (35%)	15 (31.3%)	61 (36.1%)	0.53
Influenza A (vs influenza B)	207 (86.6%)	44 (86.3%)	163 (86.7%)	0.93
Clinical findings at admission				
Fever[b]	28 (12.7%)	9 (20.5%)	19 (10.7%)	0.083
Dyspnea	142 (59.4%)	24 (47.1%)	118 (62.8%)	**0.043**
Bronchospasm	102 (42.7%)	13 (25.5%)	89 (47.3%)	**0.005**
Heart rate	95.56 ± 22.08	95.04 ± 21.65	95.7 ± 22.24	0.85
Systolic blood pressure	123.92 ± 21.8	122.8 ± 22.7	124.2 ± 21.62	0.69
Respiratory rate	19.95 ± 7.97	22.27 ± 11.4	19.56 ± 7.28	0.71
PaO2	62.74 ± 14.78	64.04 ± 14.77	62.5 ± 14.82	0.64
SpO2 (%)	91.28 ± 5.05	92.31 ± 5.79	91.02 ± 4.84	0.14

Results are expressed as mean ± standard deviation or median with interquartile range (IQR) or as absolute value (percentage). Values in bold text are considered statistically significant.

[a] In the last 5 years

[b] Fever of 38.5 °C or more

Image techniques

Of 225 patients in which chest X-ray was performed, 50.2% (n = 113) had abnormal findings (56.8% in the cytopenias group vs. 48.6% in the group without cytopenias, p = 0.32). The most common radiologic findings were patchy infiltrates (22.2%) and interstitial infiltrates (8.4%). Thoracic computed tomography was performed in 18 cases, of which 83.3% (n = 15) had abnormal findings (66.7% in the cytopenias group vs. 86.7% in the group without cytopenias, p = 0.44). The most common abnormalities were patchy infiltrates (39.8%) and ground-glass opacities (16.7%).

Management and outcome

As is shown in Table 3, 28 patients (11.7%) developed an associated bacterial pneumonia in the course of admission (p = NS between groups) with *Staphylococcus aureus* being the causative microorganism in two cases (0.8%), both in the cytopenias group.

Antiviral treatment with oseltamivir was started in 96.7% (n = 231) of the patients and it was always initiated early upon confirmation of influenza diagnosis. Empiric antibiotic treatment was started in 72.8% (n = 174) of the cases, with a median duration of treatment of 6 days (range 4.7–9). In

Table 2 Laboratory parameters of patients with influenza infection

Variable	All patients (N = 239)	Patients with cytopenias (N = 51)	Patients without cytopenias (N = 188)	p-value
Baseline laboratory findings				
Leukocytes (cells/μl)	8100 (5550–10,950)	5900 (3200–9800)	8500 (6300–11,125)	**0.002**
Neutrophils (cells/μl)	6100 (3850–9150)	4900 (2100–8000)	6350 (4375–9613)	**0.011**
Neutrophils (%)	76.71 ± 13.69	74.20 ± 16.89	77.4 ± 12.63	0.21
Lymphocytes (cells/μl)	800 (500–1200)	600 (400–1100)	800 (600–1300)	**0.047**
Lymphocytes (%)	11 (6.02–18)	12 (7.8–24)	10.3 (5.8–17.3)	0.108
Platelets ($\times 10^3$ /μl)	187 (146–245)	134 (84–193)	206 (158.75–253)	**<0.0001**
Hemoglobin (g/dl)	13.06 ± 2.13	11.95 ± 2.48	13.36 ± 1.92	**<0.0001**
LDH (U/l)	274.5 (223.7–347.7)	329 (241–440.5)	266 (222–334.5)	**0.006**
Hypertransaminasemia	48 (20.1%)	11 (21.6%)	37 (19.7%)	0.76
C-reactive protein (mg/dl)	5.31 (2.44–11.55)	4.67 (1.66–15.44)	5.4 (2.66–11.19)	0.38
Peak laboratory values				
LDH (U/l)	292 (247.5–394.5)	377 (276–493)	281 (242–361)	**0.032**
LDH >225U/l[a]	196 (83.8%)	45 (88.2%)	151 (82.5%)	0.39
AST (mg/dl)	30 (23–51)	38 (26–71)	29 (22–45)	**0.01**
Creatinine (mg/dl)	0.98 (0.74–1.33)	1.2 (0.8–1.95)	0.94 (0.72–1.23)	**0.019**
C-reactive protein (mg/dl)	8 (4–15)	9 (4–17.5)	8 (4–14.5)	0.36
Nadir blood cell count				
Leukocytes (cells/μl)	5800 (4150–8100)	3600 (2100–5500)	6350 (4800–8400)	**<0.0001**
Neutrophils (cells/μl)	3700 (2400–5650)	2300 (1000–3700)	4350 (2800–6200)	**<0.0001**
Neutrophils (%)	64.09 ± 16.39	59.22 ± 19.35	65.46 ± 15.23	0.037
Lymphocytes (cells/μl)	700 (400–1000)	400 (200–700)	700 (500–1000)	**<0.0001**
Lymphocytes (%)	9.4 (5.1–16.5)	12 (4.9–22.25)	9.1 (5.15–15.42)	0.24
Platelets ($\times 10^3$ /μl)	176 (119–227.5)	84 (60–116)	190 (149–237.5)	**<0.0001**
Hemoglobin (g/dl)	11.96 ± 2.35	9.56 ± 2.32	12.62 ± 1.89	**<0.0001**
Other criteria from HLH-04[b]				
Fibrinogen levels (mg/dl)	704 (477–833.7)	708.5 (377.7–1050)	675.5 (477–822.2)	0.41
Fibrinogen ≤150 mg/dl	0	0	0	0.29
Triglyceride levels (mg/dl)	129.5 (96.25–176.5)	115 (74.5–191.75)	135 (100–168.75)	0.58
Triglyceride ≥265 mg/dl	1 (1.1%)	0	1 (1.5%)	0.061
Ferritin levels (ng/ml)	231 (127–500)	414.5 (161.5–775.5)	217 (125–374)	0.22
Ferritin ≥500 ng/ml	19 (24.1%)	7 (35%)	12 (20.3%)	NA
Soluble IL-2 receptor concentration (pg/ml)	7718	15,100	336	0.083
Fever ≥38.5 °C	28 (12.7%)	9 (20.5%)	19 (10.7%)	
Diagnosis and treatment of hemphagocytic syndrome				
Clinical suspicion	2 (0.8%)	1 (2%)	1 (0.5%)	0.32
Specific immunomodulatory treatment[c]	1 (0.4%)	1 (2%)	0	0.054

NA: not applicable

Results are expressed as mean ± standard deviation or median with interquartile range (IQR) or as absolute value (percentage). Values in bold text are considered statistically significant [a] Upper limit of LDH in the local laboratory is 225 mg/dl. [b] NK cell activity was not determined in any case, splenomegaly was not reported in any patient, bone marrow biopsy was performed in only one patient, and soluble IL-2 receptor concentration was determined in only two patients. [c] Dexamethasone (10 mg/m^2), observing a prompt clinical improvement

7.1% (n = 17) of cases, pathogen directed antibiotic treatment was used, with a median duration of treatment of 6 days (range 2–9). No differences were observed between groups in the use of antimicrobial therapy.

Steroids were used in 56.9% (n = 136) of the patients for different clinical indications (39.2% in the group with hematological abnormalities vs. 61.7% in the group without them, p = 0.004). In 85% (n = 116) of the cases steroids were used for COPD exacerbations, asthma exacerbations or bronchospasm. Other indications included septic shock in three cases and secondary HPS in one case. Median duration of steroid treatment was 7 days (range, 4–11.25) (8 days [range, 5.5–15.5] in the cytopenias group vs. 6 days [range, 4–11] in the group without cytopenias, p = 0.15).

Overall in-hospital mortality was 5.2%, with 4.6% influenza related mortality, and a 30-day in-hospital mortality of 4.6%. As is shown in Table 3, patients with hematological abnormalities presented with higher SOFA and qSOFA scores, had longer hospital and ICU stays, and more frequently presented respiratory failure and septic shock that required

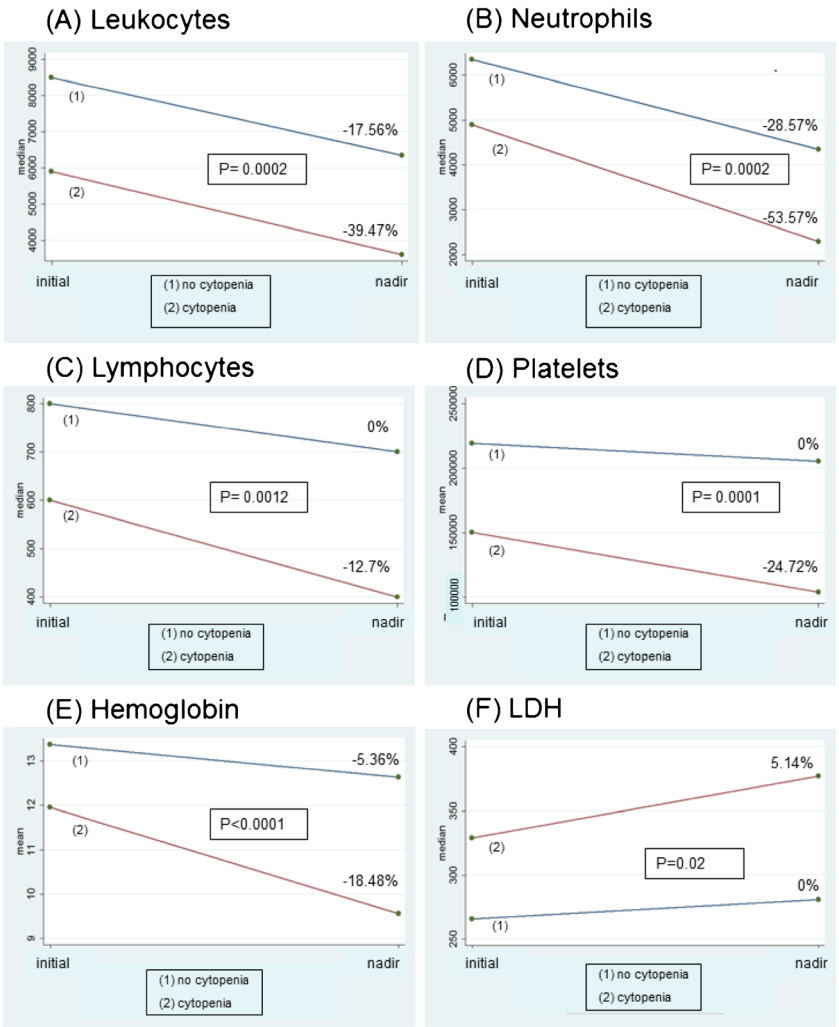

Fig. 2 Analytical percentage difference between laboratory values at admission and at nadir time (Footnote of the figure: Values are expressed as the mean percentage difference (or median) in both groups (cytopenias vs no cytopenias) between initial laboratory findings and nadir. The mean had been used for the hemoglobin and the median for the rest of the variables. (1) group with cytopenias, (2) group without cytopenias)

ICU admission. As a consequence, we did notice significant differences between the two groups in the poor outcome composite endpoint (33.3% vs. 13.3%, $p = 0.001$).

Risk-factor analysis

Patient characteristics with respect to the poor outcome composite endpoint are presented in Table 4. No statistically significant differences between active smokers (18.8% in the good outcome group vs. 26.2% in the poor outcome group) or ex-smokers (23.4% in the good outcome group vs. 23.8% in the poor outcome group) were observed. In the favorable outcome group, 181 of the cases (95.3%) presented complete recovery of the episode and nine patients (4.7%) presented sequelae, while in the poor outcome group only 25 of the cases (59.5%) presented complete recovery and five cases (11.9%) presented sequelae. Twelve patients died during hospitalization. Thirty-day related mortality was 0.5% in the good outcome group vs. 23.8% in the poor outcome group ($p < 0.0001$).

A reduced model to identify factors associated with poor outcome was generated. The three following variables remained significantly associated with the presence of the composite endpoint: use of steroids prior to present admission (OR: 0.12; 95% CI: 0.015–0.96, $p = 0.046$), presence of any hematological abnormality (OR: 3.54; 95% CI: 1.66–7.51, $p = 0.001$), and LDH > 225 U/l (OR: 4.45; CI:1–19.71, $p = 0.049$) (Table 5).

Discussion

This observational retrospective study seeks to address mainly two issues. On the one hand, we seek to identify the frequency of hematological abnormalities (cytopenias) secondary to influenza virus infection in patients who required in-hospital treatment. On the other hand, we seek to determine if these hematological abnormalities are associated with a poor outcome defined as a composite endpoint that included the presence of respiratory failure, SOFA score ≥ 2, and mortality.

Table 3 Management and outcome of patients with influenza infection

Variable	All patients (N = 239)	Patients with cytopenias (N = 51)	Patients without cytopenias (N = 188)	p-value
Associated bacterial pneumonia	28 (11.7%)	7 (13.7%)	21 (11.2%)	0.61
Microbiological study	174 (72.8%)	43 (84.3%)	131 (69.7%)	**0.037**
Treatment				
Antivirals	231 (96.7%)	48 (94.1%)	183 (97.3%)	0.37
Empiric antibiotic therapy	174(72.8%)	40 (78.4%)	134 (71.3%)	0.3
Pathogen directed antibiotic therapy	17 (7.1%)	6 (11.8%)	11 (5.9%)	0.21
Steroids	136 (56.9%)	20 (39.2%)	116 (61.7%)	**0.004**
Management				
Length of hospital stay (days)	7 (5–12)	12 (6–27)	7 (5–9)	**0.001**
ICU admission	13 (5.4%)	6 (11.8%)	7 (3.7%)	**0.036**
Length of ICU stay (days)	21 (6.5–37.-5)	33.5 (18.25–45)	13 (3–31)	**0.054**
Respiratory failure	19 (7.9%)	7 (13.7%)	12 (6.4%)	0.086
Invasive mechanical ventilation	9 (3.8%)	6 (11.8%)	3 (1.6%)	**0.004**
Non-invasive mechanical ventilation	8 (3.3%)	1 (2%)	7 (3.7%)	1
Duration of invasive mechanical ventilation (days)	15.5 (8–28.5)	22 (13.25–30)	9 (2–16.75)	0.07
ARDS	3 (1.3%)	0	3 (1.6%)	1
Vasoactive drugs	10 (4.2%)	8 (15.7%)	2 (1.1%)	**<0.0001**
Septic shock	13 (5.4%)	7 (13.7%)	6 (3.2%)	**0.008**
SOFA score at ICU admission	1 (0–3.75)	3.5 (0–7.75)	0.5 (0–2.75)	0.084
SOFA ≥2	30 (15.1%)	15 (31.9%)	15 (9.9%)	**<0.0001**
qSOFA score	0.5 ± 0.71	0.62 ± 0.86	0.46 ± 0.65	0.31
qSOFA ≥1	58 (39.5%)	16 (43.2%)	42 (38.2%)	0.58
Outcome				
Recovery without sequelae	206 (88.8%)	39 (79.6%)	167 (91.3%)	0.13
Recovery with sequelae	14 (6%)	5 (10.2%)	9 (4.9%)	
Overall in-hospital mortality	12 (5.2%)	5 (10.2%)	7 (3.8%)	
Non-related mortality	2 (0.9%)	1 (2%)	1 (0.5%)	
Related mortality	10 (4.3%)	4 (8.2%)	6 (3.3%)	
30-day in-hospital mortality	11 (4.6%)	5 (9.8%)	6 (3.2%)	0.06
Poor outcome combined end point[a]	42 (17.6%)	17 (33.3%)	25 (13.3%)	**0.001**

Results are expressed as mean ± standard deviation or median with interquartile range (IQR) or as absolute value (percentage). Values in bold text are considered statistically significant. *ICU* intensive care unit, *ARDS* acute respiratory distress syndrome. (1) Taking as reference the date of positivity of the microbiological test. [a] Poor outcome was defined as a combined end point in which at least one of the following criteria had to be fulfilled: (a) respiratory failure, (b) SOFA ≥2, or (c) death (related or not related to influenza infection)

In the present study, hematological abnormalities were defined according to the criteria proposed by the HLH-04 for the diagnosis of HPS [19]. One theoretical disadvantage of these criteria is that lymphocyte count is not considered as part of the diagnosis and lymphopenia is frequently seen in patients with influenza virus infection. Additionally, more severe cytopenias are considered in the HLH-04 definition than those frequently reported in patients with influenza virus infection [1–6]. However, it is noticeable that in the present study we observed that 21.3% of the patients presented with at least one cytopenia in the HLH-04 range, while 7.5% of the cases presented with cytopenias affecting at least two of three cell lineages. Thrombocytopenia (15% of all patients) was the most common hematologic abnormality.

As is shown in Table 2, these hematological abnormalities were already present in baseline laboratory data at admission. Additionally, the median time to nadir was between 0 and 1 day depending on the affected cell lineage. These

Table 4 Poor outcome vs good outcome in patients with influenza infection

Variable	Good outcome (N = 197)	Poor outcome (N = 42)	p-value
Age (years)	68.13 ± 15.97	62.18 ± 15.91	**0.019**
Charlson comorbidity index score at admission	2 (1–4)	2 (1–3)	0.36
Previous comorbid conditions			
Obesity	34 (17.3%)	9 (21.4%)	0.52
Diabetes mellitus	46 (23.4%)	6 (14.3%)	0.19
Asthma	14 (7.1%)	1 (2.4%)	0.48
Chronic obstructive pulmonary disease	41 (20.8%)	10 (23.8%)	0.66
Immunosuppression (including steroids)	45 (22.8%)	8 (19%)	0.59
Immunosuppression (without steroids)	18 (9.1%)	7 (16.7%)	0.16
Previous use of steroids	27 (13.7%)	1 (2.4%)	**0.036**
Influenza vaccination in the last year	80 (40.6%)	16 (39%)	0.85
Clinical findings			
Dyspnea at admission	115 (58.4%)	27 (64.3%)	0.47
Bronchospasm at admission	87 (44.2%)	15 (35.7%)	0.31
Chest X-ray abnormal findings	88 (47.6%)	25 (62.5%)	0.087
Thoracic CT abnormal findings	14 (82.4%)	1 (100%)	1
Associated bacterial pneumonia	20 (10.2%)	8 (19%)	0.11
Treatment with steroids	114 (57.9%)	22 (52.4%)	0.51
Management			
Length of hospital stay (days)	7 (5–10)	13 (6.75–33.25)	**<0.0001**
ICU admission	0	13 (31%)	**<0.0001**
Respiratory failure	0	19 (45.2%)	**<0.0001**
ARDS	0	3 (7.1%)	**0.005**
Vasoactive drugs	1 (0.5%)	9 (21.4%)	**<0.0001**
Septic shock	2 (1%)	11 (26.2%)	**<0.0001**
SOFA score at ICU admission	0.22 ± 0.42	5.71 ± 3.83	**<0.0001**
SOFA ≥2	0	30 (75%)	**<0.0001**
qSOFA score	0.33 ± 0.525	1.09 ± 0.933	**<0.0001**
qSOFA ≥1	34 (30.1%)	24 (70.6%)	**<0.0001**
Overall in-hospital mortality	0	12 (28.6%)	**<0.0001**
Baseline laboratory findings			
Leukocytes (cells/μl)	8100 (5700–10,500)	8150 (4900–13,675)	0.395
Neutrophils (cells/μl)	6000 (3800–8700)	6800 (3825–12,000)	0.194
Lymphocytes (cells/μl)	800 (500–1300)	600 (500–1100)	0.096
Platelets ($\times 10^3$ /μl)	193 (145–248)	180 (143.7–230)	0.55
Hemoglobin (g/dl)	13.03 ± 2.05	13.17 ± 2.46	0.51
LDH (U/l)	261 (220–337)	324 (275–433.5)	**0.001**
Peak laboratory values			
LDH (U/l)	282 (241.5–363)	401 (285–534.5)	**<0.0001**
LDH > 225U/l[a]	157 (81.3%)	39 (95.1%)	**0.034**
Creatinine (mg/dl)	0.98 (0.74–1.33)	0.97 (0.72–1.35)	0.95
Nadir blood cell count			
Leukocytes (cells/μl)	5800 (300–8000)	5050 (3125–8275)	0.48
Neutrophils (cells/μl)	3600 (2400–5600)	3750 (2175–6100)	0.641
Lymphocytes (cells/μl)	700 (500–1100)	300 (200–700)	**<0.0001**
Platelets ($\times 10^3$ /μl)	179 (132–228)	130.5 (86.5–218.2)	**0.016**
Hemoglobin (g/dl)	12.17 ± 2.2	10.99 ± 2.79	**0.003**
Hematologic abnormalities[b]			
At least one cytopenia	34 (17.3%)	17 (40.5%)	**0.001**
At least two cytopenias	9 (4.6%)	9 (21.4%)	**0.001**

Table 4 (continued)

Variable	Good outcome (N = 197)	Poor outcome (N = 42)	p-value
<1000 neutrophils (cells/μl)	9 (4.6%)	5 (11.9%)	0.07
<100,000 platelets (cells/μl)	23 (11.7%)	13 (31%)	**0.002**
Hb < 9 g/dl	14 (7.1%)	11 (26.2%)	**0.001**
Other criteria from HLH-04[b]			
Fibrinogen levels (mg/dl) (1)	806 (498.2–838)	660.5 (469.7–790.7)	0.74
Triglyceride levels (mg/dl) (2)	125 (95.5–154)	192 (118–214)	**0.015**
Ferritin levels (ng/ml)	222 (124–390.5)	414.5 (167.5–810.5)	0.057
Ferritin ≥500 ng/ml	12 (19.7%)	7 (38.9%)	0.12
Soluble IL-2 receptor concentration (pg/ml) (3)	15,100	336	NA
Fever ≥38.5 °C	20 (10.9%)	8 (21.6%)	0.1
Diagnosis and treatment of HPS			
Clinical suspicion	1 (0.5%)	1 (2.4%)	0.32

Results are expressed as mean ± standard deviation or median with interquartile range (IQR) or as absolute value (percentage).). ICU: Intensive Care Unit. ARDS: Acute respiratory distress syndrome. [a] Upper limit of LDH in the local laboratory is 225 mg/dl. [b] According to the HLH-04 criteria. (1) No patient presented fibrinogen levels ≤150 mg/dl. (2) Only one patient in the good outcome group presented with triglyceride levels ≥265 mg/dl. (3): SD was not calculated because soluble IL-2 receptor concentration was determined in only two patients (one in each group).

observations suggest that cytopenias present early in the course of the infection. We therefore believe that the presence of hematological abnormalities is an early predictive marker of poor outcome in patients with influenza virus infection who required hospitalization. Interestingly, we observed a tendency of a decrease in peripheral blood cell values even in patients without cytopenias, with a median time to nadir similar to those patients with cytopenias.

Table 5 Univariate and multivariate analysis of patients with poor outcome

Variable	Univariate		Multivariate	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Previous medical history				
Charlson index score	0.94 (0.787–1.132)	0.53		
Immunosuppression (including steroids)	0.79 (0.34–1.83)	0.59		
Previous use of steroids	0.15 (0.02–1.16)	0.07	**0.12 (0.015–0.96)**	**0.046**
Immunosuppression (without steroids)	1.98 (0.77–5.11)	0.15		
Obesity	1.3 (0.57–2.98)	0.52		
Active smoker	1.53 (0.707–3.331)	0.27		
Clinical findings				
Dyspnea at admission	1.28 (0.643–2.56)	0.48		
Bronchospasm at admission	0.7 (0.352–1.4)	0.31		
Associated bacterial pneumonia	2.08 (0.848–5.113)	0.11		
Use of steroids	0.8 (0.41–1.56)	0.515		
Laboratory findings				
Any hematologic abnormality	3.26 (1.58–6.68)	**0.001**	**3.54 (1.66–7.51)**	**0.001**
Hematologic abnormality compatible with HPS	5.69 (2.106–15.41)	**0.001**		
<1000 neutrophils (cells/μl)	2.82 (0.89–8.9)	0.077		
<100,000 platelets (10^3 /μl)	3.39 (1.54–7.43)	**0.002**		
Hb < 9 g/dl	4.63 (1.93–11.14)	0.001		
Ferritin ≥500 ng/ml	2.59 (0.83–8.11)	0.1		
LDH > 225 U/l	4.47 (1.03–19.37)	**0.045**	**4.45 (1–19.71)**	**0.049**

OR odds ratio, *CI* confidence interval

Values in bold text are considered statistically significant. Although non-invasive mechanical ventilation, septic shock and use of vasoactive drugs were statistically significant in the univariate analysis, they were excluded from the final multivariate model because they were indirectly included in the poor outcome combined end point

We did not randomly choose the HLH-04 criteria for defining the hematological abnormalities in the present study. We suspect that hemophagocytic syndrome triggered by influenza virus infection was more frequent than previously reported. Unfortunately however, no specific disease markers were ordered in most cases, making it impossible for us to retrospectively know the exact incidence of this disorder in our present cohort. In fact, albeit 51 patients presented with at least one cytopenia according to the HLH-04 criteria [19], fibrinogen levels were available in only eight cases, triglycerides and ferritin levels in 20 cases, and soluble IL-2 receptor concentration in only two cases. Although rarely, hemophagocytic syndrome has been described in patients with severe influenza virus infection, usually associated with a poor outcome and a high mortality rate [3, 27]. In Beutel's study of 25 critically ill patients with influenza A (H1N1) pdm09 virus associated hemophagocytic syndrome, the absence of steroid therapy in the early phase of the infection might have contributed to the high incidence of HPS (9 out of 25 patients) and the rather poor outcomes [18]. We consider that a low clinical index of suspicion for influenza associated hemophagocytic syndrome is the main factor for delayed initiation of immunomodulatory therapy, which could have improved the outcome in these patients [28].

In the present study, a multivariate model was conducted to identify risk factors associated with a poor outcome. Presence of hematological abnormalities and LDH levels >225 U/l remained significantly associated with a worse outcome in patients with influenza virus infection, while previous use of steroids was identified as a favorable prognostic factor. Although no conclusive evidence has been reached, thrombocytopenia has been associated with a worse outcome in previous studies [3, 4]. In our univariate analysis, both thrombocytopenia and anemia were significantly associated with poor outcome. To our knowledge, anemia as a poor prognostic marker in patients with influenza virus infection had not been previously described. Indeed, the two most notable aspects of our model are the presence of any hematological abnormality as a marker of poor outcome and the potential beneficial role of steroids in these patients.

An interesting finding in our study was the better outcome observed in patients who were already using steroids before admission, while the use of steroids during admission was associated with poor outcome. This paradox could be explained by two facts. First, as in other series [4, 18], steroids were mostly prescribed for COPD exacerbations, asthma exacerbations or bronchospasms, but not as immunomodulatory therapy; therefore, time to treatment initiation, duration of treatment, and prescribed doses, were not always optimal. In fact, only one patient in our series received high.dose steroids (dexamethasone 10 mg/m^2) and that was for the treatment of hemophagocytic syndrome triggered by influenza virus infection with marked hemophagocytosis in bone marrow. Second, it is known that onset of influenza A viruses infections is very acute by triggering a cascade of immune responses and switching on almost all parts of the immune defense system [29, 30]. In fact, in the present study, hematological abnormalities presented early in the course of the infection in a large number of patients. We believe that steroid therapy before admission could have modulated the acute inflammatory response triggered by influenza virus infection in the early course of the disease, and with this, prevented an uncontrolled immune response [28]. This finding, if confirmed in properly prospective designed studies, may establish the utility of early use of steroids in some subgroup of patients with influenza virus who required hospitalization.

Limitations of the study

By using more restrictive criteria for the definition of cytopenias as compared to other series, our study might have underestimated the percentage of patients who presented with hematological abnormalities. Additionally, the observational retrospective nature of the study complicates the accurate assessment of the role of steroid therapy during admission, especially since it was mostly prescribed for other indications rather than the infection itself. This wide range of indications, although similar to other series, precludes a correct assessment of steroid therapy as a poor prognostic factor. The potential benefit of steroid therapy in patients with influenza virus infection with risk factors for poor outcome should be further studied.

Unfortunately, an active pursuit of hemophagocytic syndrome secondary to influenza virus infection was conducted only in a few cases, which precluded us to confirm the real incidence of this severe complication in the subgroup of patients with hematological abnormalities. Since a complete blood count is an easily accessible test, we believe that it should be the cornerstone in the screening of a possible underlying HPS secondary to influenza virus infection. If severe cytopenias were observed, we consider that a systematic determination of ferritin, triglycerides and fibrinogen levels would be beneficial. In the case of abnormal levels of any of these three HPS markers, determination of NK cell activity and soluble IL-2 receptor concentration should be ordered to confirm the diagnosis.

Conclusions

Significant hematological abnormalities are frequently seen in patients with influenza virus infection who required hospital admission and are associated with a poor outcome. The ongoing use of steroids upon the start of influenza was associated

with a better prognosis suggesting that an early immunomodulatory therapy could improve the outcome of these patients.

Compliance with ethical standards

Funding information This study has not received any funding.

Conflict of interest The authors declare no conflicts of interest.

Ethical approval The study protocol was approved by the University Hospital 12 de Octubre Review Board.

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