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Evaluation of Alpha-1 Antitrypsin as a Prognostic Biomarker in Severe Influenza: A Prospective Study

Isabel Franco-Burgos [1], Sebastián Menao-Guillén [2,3], Juan-José Perales-Afán [2,3], Almudena García-Gutiérrez [2], Inmaculada Moreno-Gázquez [2], María-Ángel Julián-Ansón [2], Mónica Ramos-Álvarez [2], Susana Olivera-González [4], Susana Torralba-Aznárez [3] and Miguel-Ángel Torralba-Cabeza [1,3,5,6,*]

1 Faculty of Medicine, University of Zaragoza, 50009 Zaragoza, Spain; isabelfranco1008@gmail.com
2 Biochemistry Department, "Lozano Blesa" University Hospital, 50009 Zaragoza, Spain; smenao@hotmail.com (S.M.-G.); juanjo.peafan21@gmail.com (J.-J.P.-A.)
3 Aragon Health Research Institute (IIS Aragón), 50009 Zaragoza, Spain; susana.toaz@gmail.com
4 Department of Internal Medicine, Marina Salud Hospital, 03700 Denia, Spain; susana.olivera@yahoo.es
5 Rare Diseases Unit, Department of Internal Medicine, "Lozano Blesa" University Hospital, 50009 Zaragoza, Spain
6 Working Group on Rare Diseases of the Spanish Society of Internal Medicine (GTEM-SEMI), 3th Pintor Ribera Street, 28016 Madrid, Spain
* Correspondence: mantorra@unizar.es; Tel.: +34-976-768864

Abstract

Background: Influenza is a global health issue involving inflammatory mediators, the imbalances of which contribute to pulmonary and extrapulmonary damage. Objectives: To evaluate whether AAT1 levels are elevated in patients with severe influenza and assess its potential as a prognostic biomarker. Materials and methods: A prospective, analytical, non-interventional study was conducted on 72 patients admitted for severe influenza to the Internal Medicine Department at "Lozano Blesa" University Hospital (Zaragoza, Spain). Blood AAT1 concentrations at admission were analyzed alongside clinical and biochemical variables. Results: The mean patient age was 80.31 years (54.2% female). Overall mortality was 4.17%, one patient required ICU admission, and five had SARS-CoV-2 coinfection. Mean AAT1 concentration was 164.1 mg/dL (normal range: 100–200 mg/dL). AAT1 levels correlated significantly with leukocyte counts and C-reactive protein ($r = 0.582$, $p < 0.001$). Leukocyte counts were significantly higher in the patient requiring intensive care. Notably, AAT1 concentrations were significantly higher in patients with SARS-CoV-2 coinfection compared to non-coinfected influenza cases ($p < 0.05$). Conclusions: Admission AAT1 concentration does not serve as a useful prognostic biomarker in severe influenza. However, elevated AAT1 levels at diagnosis in hospitalized patients should prompt consideration of SARS-CoV-2 coinfection. Further research is warranted to clarify AAT1 involvement in influenza pathophysiology.

Keywords: influenza; Alpha-1 antitrypsin; biomarker; *SERPINA1*

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1. Introduction

Seasonal influenza is a respiratory infection caused by the influenza virus that circulates worldwide. The severity spectrum of this disease is highly variable. While it is true that in most people the symptoms are mild and resolve within a few days, there are cases requiring hospitalization, and others where it can cause death, particularly in high-risk groups such as the elderly, pregnant women, healthcare workers, and patients with severe concomitant pathologies [1]. The etiological agent of influenza is the influenza virus, an RNA virus

belonging to the Orthomyxoviridae family. There are different species (Influenza A, B, C) and subtypes, determined by the combination of surface antigens: hemagglutinin and neuraminidase, which confers a significant mutation capacity, enabling it to cause epidemics and pandemics that cyclically affect the global population [2]. Regarding its transmission mechanism, it should be noted that it is a highly transmissible infection, especially in populated areas. The main route is through Pflügge droplets (>5 microns) generated by talking, coughing, or sneezing. It can also occur through direct mucosal contact with the respiratory secretions of an infected person or indirectly via recently contaminated hands or objects. The incubation period ranges from 2 to 8 days, and the period of maximum transmissibility begins 24 h before the onset of symptoms and persists throughout the symptomatic stage [3]. Clinically, influenza is characterized by the sudden onset of a febrile syndrome, which is usually accompanied by myalgia, severe headache with or without photophobia, dry cough, asthenia, odynophagia, and general malaise. After 48 h, the fever frequently begins to subside, and if there are no complications, the clinical picture resolves within a maximum of 7 days. Among the complications, bronchitis and pneumonia stand out, which in risk groups can lead to acute respiratory distress syndrome (ARDS), septic shock, and multiorgan failure, significantly worsening the prognosis [4]. Most cases are diagnosed clinically. However, given the large number of pathogens capable of causing a similar clinical picture, a laboratory test is usually required to establish the definitive diagnosis. Confirmation is commonly performed by direct antigen detection, viral isolation, and, above all, reverse transcription polymerase chain reaction (RT-PCR) [5]. Rapid antigen detection testing is reserved for clinical practice, as it allows for the detection of the virus in a short time without the need to send samples to the laboratory. Nevertheless, it has lower sensitivity, and its reliability largely depends on the conditions in which it is used [1,6]. Pathophysiologically, the influenza virus affects the respiratory tract, as it implants and replicates in the epithelial cells of the upper airways as well as in other cells of the respiratory tract and macrophages. Therefore, it can cause direct viral damage or alter the immune response. In humans, the respiratory epithelium is the only site where hemagglutinin (HA) can be effectively cleaved, giving rise to infectious viral particles [7]. The main mechanism of pathogenicity is the result of pulmonary inflammation caused by direct viral infection of the respiratory epithelium combined with the effects caused by an altered immune response. The latter explains that, although the respiratory system is the primarily affected organ, inflammation can spread systemically, causing the involvement of other organs or even manifesting as multiorgan failure, although this occurs after pulmonary compromise and adult respiratory distress syndrome (ARDS). Influenza is a major health problem, both because of the mortality it can cause directly or indirectly, and because of the complications it can entail and the economic and social costs it generates. The proportion of the population affected during annual epidemics ranges between 5% and 15% in large populations, rising to 50% in specific groups [6]. Although we coexist with it in a controlled manner, the management and treatment of patients remains a challenge, as there is no well-determined pathophysiological mechanism or an adequate biomarker that can help predict patient outcomes.

Alpha-1 antitrypsin (AAT1) is a glycoprotein encoded by the *SERPINA1* gene, located on chromosome 14q32.1, consisting of 7 exons and 6 introns [8]. The transcription and translation of AAT1 are similar to those of other secreted glycoproteins. Numerous factors are involved in their regulation, notably specific hepatic factors and cytokines such as interleukins (IL) IL-1β, IL-6, and tumour necrosis factor-alpha (TNF-α). The end result is the synthesis of a molecule composed of 418 amino acids, which includes a signal peptide that is cleaved in the rough endoplasmic reticulum where glycosylation begins. In the Golgi apparatus, three branched carbohydrate side chains are added, resulting in a

globular protein composed of 394 amino acids arranged in three beta-sheets and a mobile reactive centre loop that rapidly diffuses into the serum. These carbohydrates are what confer its longer half-life and its electrophoretic heterogeneity. This heterogeneity has been demonstrated by electrophoresis techniques, often combined with immunoelectrophoresis, aimed at detecting variants present at very low concentrations [8,9]. AAT1 is primarily synthesized by hepatocytes (75%), although it can also be produced in the intestine, alveolar cells, neutrophils, macrophages, and corneal keratinocytes in supplementary amounts. Under normal conditions, the body can produce 34 mg/kg/day, resulting in concentrations of 100–200 mg/dL. It has a half-life of 3–5 days [10]. The main function of AAT1 is to protect the lung from the activity of serine proteases and neutrophil elastase during inflammation by acting as a potent inhibitor. This inhibition is achieved because the amino acids located in the reactive centre loop of the protein (corresponding to the amino acids serine and methionine) bind to serum elastase forming covalent bonds, thereby inhibiting it, to be subsequently phagocytosed by macrophages and degraded in the hepatocyte. The tissue defence capacity of AAT1 depends on its concentration, the speed at which it traps and inactivates these enzymes, and its phenotype based on the S and Z alleles [11]. This glycoprotein also possesses other properties, notably its anti-inflammatory, immunomodulatory, antimicrobial, and tissue repair characteristics. These secondary qualities are not believed to be related to elastase inhibition, although this assumption has not been confirmed. What has been demonstrated is that AAT1 is primarily activated by products characteristic of pro-inflammatory situations. This is why, in response to infectious or inflammatory stimuli, its plasma concentrations can be notably increased, either through increased synthesis by the cells of the affected tissue or through the recruitment of immune cells to the site, thus allowing it to be considered an acute-phase reactant. Peak concentrations are reached rapidly and can persist for up to 2 weeks [12].

Influenza A and B viruses are important causes of respiratory disease across all age groups, capable of causing seasonal outbreaks worldwide and, less frequently, pandemics, making them one of the greatest public health concerns globally today. The World Health Organization (WHO) estimates that approximately 1 billion people are infected annually worldwide, of whom between 290,000 and 650,000 die from the disease [1,13]. In Spain, the average incidence of influenza is 2069 cases per 100,000 inhabitants. It not only has a significant impact on mortality and a decline in quality of life, but it also generates a heavy burden every year on primary care, hospital care, pharmaceutical expenditure, and work absenteeism. However, despite the burden this disease causes, its characterization and measurement have ample room for improvement [14].

While AAT1 upregulation and prognostic performance have been extensively documented in COVID-19 cytokine storms [15], its precise dynamics and prognostic utility in severe influenza remain under-investigated. Therefore, the primary objective of this prospective study was to evaluate whether admission serum AAT1 concentrations are elevated in hospitalized severe influenza patients and to assess its prognostic value for clinical severity, ICU admission, and in-hospital mortality.

2. Materials and Methods

2.1. Study Design and Participant Selection

A prospective, analytical, non-interventional study was conducted in adult patients admitted for severe influenza to the Internal Medicine Department of "Lozano Blesa" University Hospital in Zaragoza, Spain, during the seasonal wave of January and February 2026. Severe influenza was defined as laboratory-confirmed viral infection requiring hospital admission. Inclusion criteria were: age ≥ 18 years, laboratory-confirmed influenza virus infection via multiplex real-time RT-PCR or rapid antigen detection testing at presentation,

requirement for inpatient hospital care, and signed informed consent. The study protocol was approved on 24 January 2024 by the Clinical Research Ethics Committee of Aragon (CEICA: C.I. PI23/604) and conducted in strict compliance with the Declaration of Helsinki.

2.2. Diagnostic Protocols and Coinfection Definitions

Influenza diagnosis was established at admission: 58 patients (80.6%) were confirmed via multiplex real-time RT-PCR, and 14 patients (19.4%) were diagnosed initially via rapid immunochromatographic antigen detection testing in the Emergency Department (10 of whom were cross-confirmed by RT-PCR during hospitalization). All presentations represented community-acquired illness at baseline (Day 0). All patients underwent multiplex RT-PCR screening for SARS-CoV-2 co-infection at presentation. Secondary bacterial pneumonia was systematically evaluated at hospital admission using baseline clinical examination, chest radiograph/CT imaging, and microbiological diagnostics (blood cultures × 2 sets, sputum Gram stain and culture, and urinary antigen testing for Streptococcus pneumoniae and Legionella pneumophila).

2.3. Temporal Sequence of Sampling and Interventions

- Diagnostic Testing (Day 0): Nasopharyngeal swabs for viral testing (RT-PCR/Antigen) and microbiological sampling (blood cultures, sputum, urinary antigens) were obtained immediately upon presentation to the Emergency Department (0–6 h).
- Biomarker Sampling (Day 0): Venipuncture for admission serum AAT1, complete blood counts, C-reactive protein (CRP), blood urea, serum creatinine, and arterial blood gas analysis was completed within 12 to 24 h of hospital entry, prior to major therapeutic escalation.
- Pharmacological Timing: Antiviral therapy (Oseltamivir, n = 22, 30.6%) was initiated within 12–24 h of presentation. Empiric broad-spectrum antibiotic therapy (n = 48, 66.7%) was started within 4–12 h of admission following microbiological sampling. Systemic corticosteroid therapy (n = 38, 52.8%) was administered within 24 h for severe respiratory failure, acute bronchospasm, or underlying COPD/asthma exacerbation.

2.4. Biomarker Quantification

Serum AAT1 concentration at diagnosis was quantified by immunonephelometry using BN™ II and BN ProSpec® automated systems (Siemens Healthineers, Erlangen, Germany) with standard reference calibration curves (normal physiological laboratory range: 100–200 mg/dL).

2.5. Statistical Analysis

Sample size was established by consecutive sampling of all eligible admissions during the two-month seasonal epidemic wave (N = 72). Post hoc power analysis indicated that N = 72 provided >80% power at alpha = 0.05 to detect a Cohen's d = 0.65 difference in serum AAT1 levels between coinfected and non-coinfected subgroups. Continuous variables were evaluated for distributional normality using the Shapiro–Wilk test and visual inspection of Q-Q plots. Normally distributed continuous variables are reported as Mean ± Standard Deviation (SD) and compared using Student's independent samples *t*-test. Non-normally distributed continuous variables are presented as Median [Interquartile Range, IQR] and compared using the Mann–Whitney U test. Categorical variables are presented as counts and percentages (n, %) and compared using Pearson's Chi-square test or Fisher's exact test. Bivariate correlation analyses were evaluated using the Pearson correlation coefficient (r) for normally distributed variables and Spearman rank correlation coefficient (rho) for non-parametric continuous or ordinal variables; *p*-values were adjusted using

the Benjamini–Hochberg False Discovery Rate (FDR) procedure for secondary analyses. Receiver Operating Characteristic (ROC) curve analysis evaluated Area Under the Curve (AUC), optimal cutoff selection (Youden's J index), sensitivity, and specificity for mortality prediction. Statistical analyses were executed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA), with two-tailed $p < 0.05$ considered statistically significant.

3. Results

3.1. Baseline Clinical and Demographic Characteristics

A total of 72 adult hospitalized patients were included (54.2% female, mean age 80.31 ± 13.90 years). Hospital length of stay had a median of 8.0 [5.0–11.0] days (mean 8.63 ± 4.63 days). Overall in-hospital mortality was 4.17% (n = 3), and 1 patient required ICU admission (1.39%). Five patients (6.94%) presented with SARS-CoV-2 coinfection. Baseline demographics, comorbidities, microbiological findings, clinical severity scores, admission laboratory parameters, and early pharmacological treatments are comprehensively summarized in Table 1. To verify that the diagnostic testing method did not introduce selection bias, a sensitivity analysis restricting the cohort to RT-PCR-confirmed cases (n = 58) demonstrated identical baseline clinical and biomarker characteristics (Supplementary Table S1).

Table 1. Baseline Demographic, Clinical, Diagnostic, Laboratory, and Outcome Characteristics of Hospitalized Patients with Severe Influenza (N = 72).

Characteristic/Variable	Overall Cohort (N = 72)
Demographics and Health Behaviours	
Age (years), Mean ± SD	80.31 ± 13.90
Female Sex, n (%)	39 (54.2%)
Male Sex, n (%)	33 (45.8%)
Active Tobacco Smoking, n (%)	14 (19.4%)
Seasonal Influenza Vaccination, n (%)	46 (63.9%)
Diagnostic Method for Influenza	
Multiplex RT-PCR Confirmed, n (%)	58 (80.6%)
Rapid Antigen Test Only, n (%)	4 (5.6%)
Rapid Antigen + RT-PCR Confirmed, n (%)	10 (13.9%)
Comorbidities	
Presence of ≥1 Comorbidity, n (%)	58 (80.6%)
Heart Failure, n (%)	31 (43.1%)
Diabetes Mellitus, n (%)	25 (34.7%)
Chronic Obstructive Pulmonary Disease (COPD), n (%)	17 (23.6%)
Asthma, n (%)	15 (20.8%)
Chronic Kidney Disease, n (%)	11 (15.3%)
Active Malignancy/Cancer, n (%)	5 (6.9%)
Autoimmune Disease, n (%)	2 (2.8%)
Coinfections	
SARS-CoV-2 Coinfection (RT-PCR), n (%)	5 (6.9%)
Suspected/Confirmed Bacterial Coinfection, n (%)	48 (66.7%)
- Microbiologically Confirmed Bacterial Pathogen, n (%)	18 (25.0%)
Streptococcus pneumoniae	9 (12.5%)
Haemophilus influenzae	5 (6.9%)
Staphylococcus aureus	3 (4.2%)
Moraxella catarrhalis	1 (1.4%)

Table 1. *Cont.*

Characteristic/Variable	Overall Cohort (N = 72)
Clinical Severity Scores and Outcomes	
FLU-IV Score, Median [IQR]	3.0 [2.0–5.0]
FLU-IV Score > 5 (Ventilatory Support Required), n (%)	9 (12.5%)
Hospital Length of Stay (days), Median [IQR]	8.0 [5.0–11.0]
Intensive Care Unit (ICU) Admission, n (%)	1 (1.39%)
In-Hospital Mortality (Exitus), n (%)	3 (4.17%)
Admission Laboratory Parameters	
Alpha-1 Antitrypsin (AAT1) (mg/dL), Mean ± SD	164.10 ± 52.58
- Male AAT1 (mg/dL), Mean ± SD	171.28 ± 54.10
- Female AAT1 (mg/dL), Mean ± SD	159.13 ± 51.20
C-Reactive Protein (CRP) (mg/L), Mean ± SD	71.05 ± 41.20
Total Leukocyte Count ($\times 10^3$/uL), Mean ± SD	8.65 ± 3.42
Absolute Lymphocyte Count ($\times 10^3$/uL), Median [IQR]	0.95 [0.60–1.40]
Blood Urea (mg/dL), Median [IQR]	48.50 [34.00–68.00]
Serum Creatinine (mg/dL), Median [IQR]	1.15 [0.85–1.60]
PaO2/FiO2 Ratio (mmHg), Median [IQR]	265.0 [210.0–310.0]
Arterial pH, Mean ± SD	7.39 ± 0.06
Early Treatments Initiated (<24 h)	
Any Early Pharmacological Treatment, n (%)	56 (77.8%)
Oseltamivir Antiviral Therapy, n (%)	22 (30.6%)
Systemic Corticosteroids, n (%)	38 (52.8%)
Empiric Antibiotic Therapy, n (%)	48 (66.7%)

Note: Normally distributed continuous variables are presented as Mean ± Standard Deviation (SD). Non-normally distributed continuous variables are presented as Median [Interquartile Range, IQR]. Categorical variables are reported as counts and percentages (n, %).

3.2. Admission Serum AAT1 Levels, Bivariate Correlations, and Subgroup Comparisons

Mean admission serum AAT1 concentration across the entire cohort was 164.10 ± 52.58 mg/dL, remaining within normal physiological reference limits (100–200 mg/dL). Bivariate correlation analysis demonstrated statistically significant positive relationships between admission serum AAT1 and major systemic acute-phase markers: CRP (Pearson $r = 0.582$, $p < 0.001$; Figure 1A) and total leukocyte counts (Pearson $r = 0.441$, $p < 0.01$; Figure 1B). Arterial blood pH showed a significant inverse correlation with the FLU-IV clinical severity scale score (Spearman rho = −0.532, $p < 0.001$; Figure 1C).

Comparative subgroup analyses revealed crucial biological differences: (1) Patients with SARS-CoV-2 coinfection exhibited significantly higher mean admission serum AAT1 levels compared to patients with isolated influenza (228.40 ± 34.10 mg/dL vs. 159.30 ± 48.20 mg/dL, $t = 3.12$, $p = 0.008$); (2) Patients prescribed antibiotic therapy for suspected secondary bacterial coinfection had significantly higher admission CRP concentrations than non-treated patients (89.40 ± 42.10 mg/L vs. 34.20 ± 18.60 mg/L, $t = 5.84$, $p < 0.001$) (Supplementary Table S2); (3) The single patient requiring ICU transfer presented with marked leukocytosis (18,400/µL) compared to ward care patients (8250 ± 3100/µL, Mann–Whitney U, $p = 0.038$). All correlation metrics, subgroup comparisons, and test statistics are detailed in Table 2.

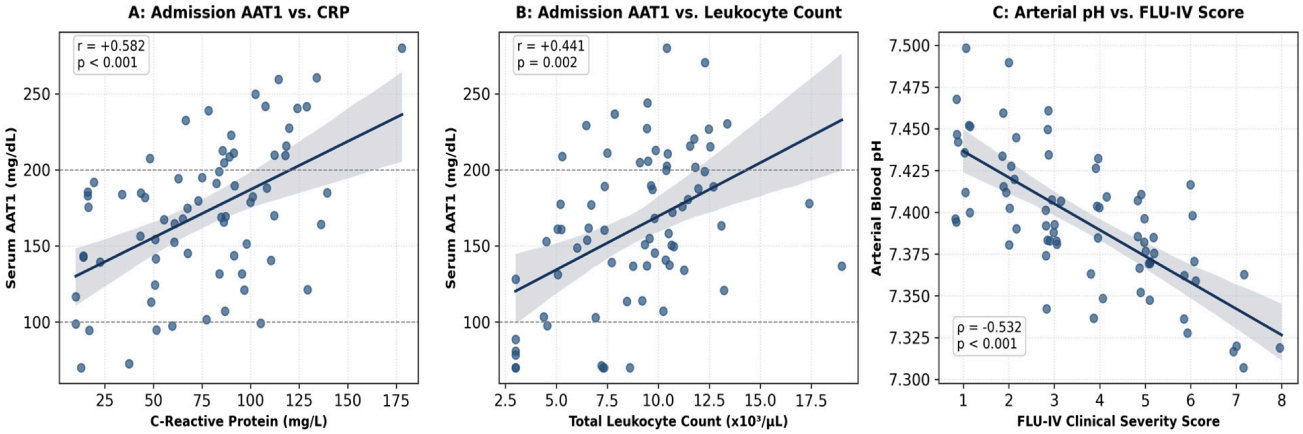


Figure 1. Bivariate correlation scatter plots of primary admission biomarkers in hospitalized severe influenza patients (N = 72). (**A**) Scatter plot showing the significant positive correlation between admission serum Alpha-1 Antitrypsin (AAT1, mg/dL) and C-reactive protein (CRP, mg/L) (Pearson $r = +0.582$, $p < 0.001$). Solid line represents the linear regression trendline; shaded band denotes the 95% confidence interval. Dashed lines indicate normal laboratory reference range for AAT1 (100–200 mg/dL). (**B**) Scatter plot depicting the positive correlation between admission serum AAT1 (mg/dL) and total leukocyte count ($\times 10^3/\mu L$) (Pearson $r = +0.441$, $p = 0.002$). (**C**) Scatter plot showing the significant inverse correlation between arterial blood pH at presentation and the FLU-IV clinical severity score (Spearman $\rho = -0.532$, $p < 0.001$).

Table 2. Bivariate Biomarker Correlations, Subgroup Comparative Statistics, and Prognostic ROC Performance for In-Hospital Mortality.

PART A: Subgroup Comparative Analyses				
Comparison Subgroup	**Group 1 Metric**	**Group 2 Metric**	**Test Statistic**	***p*-Value**
SARS-CoV-2 Coinfection vs. Isolated Influenza	Coinfected AAT1: 228.4 ± 34.1	Isolated AAT1: 159.3 ± 48.2	$t = 3.12$	0.008
Empiric Antibiotics vs. No Antibiotic Tx	Antibiotic CRP: 89.4 ± 42.1	No Antibiotic CRP: 34.2 ± 18.6	$t = 5.84$	<0.001
ICU Admission vs. Ward Care	ICU Leukocytes: 18.40	Ward Leukocytes: 8.25 ± 3.10	Mann–Whitney U	0.038
PART B: Prognostic ROC Performance for In-Hospital Mortality				
Biomarker Evaluated	**AUC (95% CI)**	**Optimal Cutoff Value**	**Sensitivity (%)**	**Specificity (%)**
Admission Serum AAT1 (mg/dL)	0.512 (0.178–0.846)	168.5 mg/dL	66.7%	52.2% ($p = 0.892$)
Blood Urea (mg/dL)	0.874 (0.667–1.000)	67.5 mg/dL	100.0%	62.3% ($p = 0.024$)

Note: FDR = False Discovery Rate (Benjamini–Hochberg procedure). ROC AUC = Area Under the Receiver Operating Characteristic Curve. Optimal cutoffs established via maximization of Youden's J index.

3.3. Diagnostic and Prognostic ROC Performance for Mortality and Severity

Receiver Operating Characteristic (ROC) curve analysis demonstrated that admission serum AAT1 possessed no predictive capacity for in-hospital mortality (AUC = 0.512, 95% CI: 0.178–0.846, $p = 0.892$) or ICU transfer. In contrast, markers of acute kidney injury exhibited strong prognostic performance for in-hospital mortality: Blood Urea achieved an AUC of 0.874 (95% CI: 0.667–1.000, $p = 0.024$; optimal cutoff 67.5 mg/dL yielding 100% sensitivity and 62.3% specificity), while Serum Creatinine achieved an AUC of 0.862

(95% CI: 0.658–1.000, $p = 0.031$; optimal cutoff 2.27 mg/dL yielding 66.7% sensitivity and 98.6% specificity). Complete prognostic performance metrics are detailed in Table 2.

3.4. Proposed Pathophysiological Model of Host Protease Processing

Based on these comparative subgroup observations and recent biochemical literature, we synthesized an illustrative pathophysiological model (Figure 2) delineating differential host protease engagement between severe influenza monoinfection and SARS-CoV-2 coinfection.

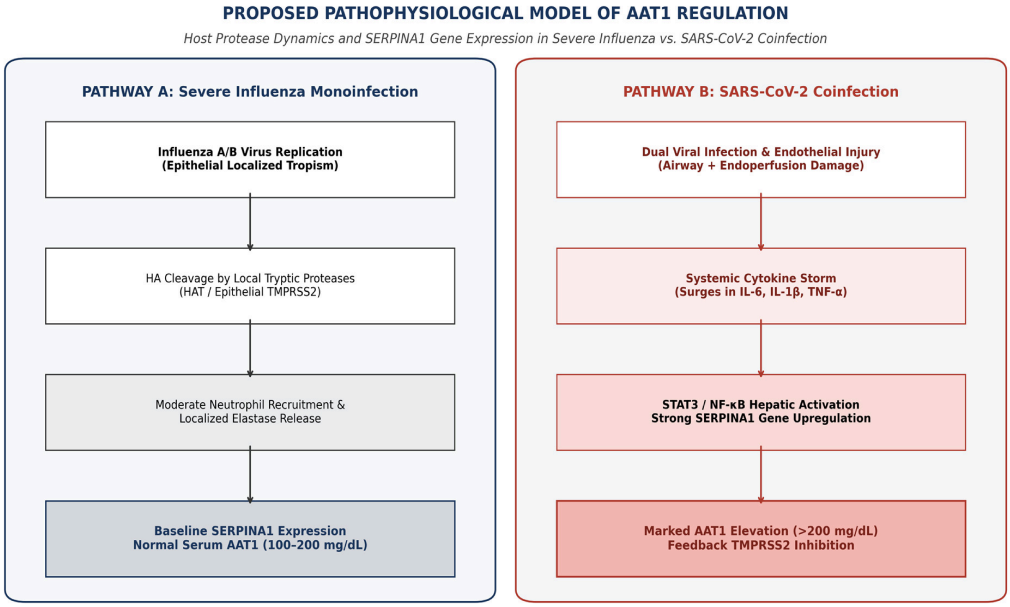


Figure 2. Proposed illustrative pathophysiological model of host protease regulation and SERPINA1 gene expression during severe influenza monoinfection versus SARS-CoV-2 coinfection. (Pathway A) In severe influenza monoinfection, viral replication is restricted to respiratory epithelial cells, where hemagglutinin (HA) cleavage relies on localized mucosal tryptic proteases (HAT/TMPRSS2). Local neutrophil recruitment and elastase release are counterbalanced by baseline physiological AAT1 levels. In vaccinated elderly hosts with pre-existing Th1/memory B cell immunity, immune memory contains viral burden without triggering systemic hyper-cytokinemia; consequently, hepatic SERPINA1 transcription remains unaugmented, maintaining normal circulating AAT1 (100–200 mg/dL). (Pathway B) In SARS-CoV-2 coinfection, dual viral stimulation triggers widespread endothelial damage and systemic hyper-cytokinemia (IL-6, IL-1β, TNF-α). Cytokines bind to hepatic receptors, activating STAT3/NF-κB transcription factors that drive intense SERPINA1 gene expression, causing circulating AAT1 to surge (>200 mg/dL). Elevated AAT1 binds to cell-surface TMPRSS2, serving as an endogenous feedback mechanism to limit viral entry and attenuate systemic endothelial damage.

4. Discussion

This prospective analytical study evaluated the clinical utility and biological dynamics of admission serum Alpha-1 Antitrypsin (AAT1) in adult patients hospitalized with severe influenza. Our primary findings demonstrate that while admission serum AAT1 correlates moderately with classic acute-phase inflammatory markers (C-reactive protein and total leukocyte count), absolute AAT1 concentrations remain within physiological reference limits (100–200 mg/dL) and fail to predict clinical severity, ICU admission, or in-hospital mortality. However, marked AAT1 elevation (>200 mg/dL) at presentation serves as a distinct clinical signal alerting physicians to concurrent SARS-CoV-2 coinfection or severe secondary inflammatory recruitment.

4.1. Pathophysiological Insights: Influenza vs. SARS-CoV-2 Host Protease Processing

The absence of marked AAT1 elevation in isolated severe influenza can be explained by fundamentally distinct host–pathogen interactions and protease activation pathways, as illustrated in our pathophysiological model (Figure 2). In influenza virus infection, viral hemagglutinin (HA) cleavage and cell entry rely primarily on localized trypsin-like proteases—such as human airway trypsin-like protease (HAT) and transmembrane protease serine 2 (TMPRSS2)—which are strictly confined to apical surfaces of the respiratory epithelium [7,16–18]. Influenza viral replication remains largely localized to the respiratory tree. Neutrophil recruitment to the lungs, while present, does not generate the massive systemic endothelial destruction or systemic cytokine storm observed in other viral pneumonias [16,19]. Consequently, neutrophil elastase release remains localized, and hepatic *SERPINA1* transcription is not maximally stimulated.

In stark contrast, SARS-CoV-2 infection triggers widespread pulmonary endothelial cell injury, endoperfusion impairment, and systemic hyper-cytokinemia driven by massive surges of IL-6, IL-1β, and TNF-α [15,19,20]. These circulating cytokines bind to hepatic receptors, activating STAT3 and NF-κB signalling pathways that drive intense transcription of the *SERPINA1* promoter, resulting in circulating AAT1 levels exceeding 200–300 mg/dL [8,20]. Furthermore, recent molecular research demonstrates that AAT1 acts as a potent endogenous inhibitor of TMPRSS2 [21]. In SARS-CoV-2 coinfection (Figure 2, Pathway B), dual viral activation produces a synergistic cytokine response that sharply upregulates hepatic AAT1 as an acute host defensive mechanism to inhibit viral spike priming and attenuate endothelial damage [22]. This mechanism explains why coinfected patients in our cohort exhibited significantly elevated AAT1 levels (228.40 ± 34.10 mg/dL vs. 159.30 ± 48.20 mg/dL, $p = 0.008$).

4.2. Impact of Host Age and High Seasonal Vaccination Rates

Our study population comprised predominantly elderly hospitalized patients (mean age 80.31 years) with a high rate of seasonal influenza vaccination (63.9%). Prior research indicates that seasonal influenza vaccination induces robust memory T helper (Th1/Th17) and memory B cell responses that rapidly contain viral replication upon re-exposure [23]. In vaccinated elderly hosts, immune memory restricts influenza viral load without triggering unchecked neutrophilic degranulation or massive acute-phase hepatic serpin synthesis [12,23]. This host immune attenuation explains why baseline AAT1 concentrations remained within normal limits across most of our cohort.

4.3. Prognostic Performance of Renal Biomarkers vs. AAT1

While AAT1 showed no prognostic value for mortality (AUC = 0.512), markers of acute renal dysfunction—blood urea (AUC = 0.874) and serum creatinine (AUC = 0.862)—demonstrated strong prognostic accuracy for in-hospital death. In elderly patients hospitalized with severe influenza, acute mortality is driven predominantly by multiorgan dysfunction, cardiorenal syndrome, and acute kidney injury secondary to systemic hypoperfusion and dehydration rather than isolated primary lung destruction. Renal function monitoring upon admission is therefore far superior to acute-phase protein measurement for stratifying mortality risk in severe geriatric influenza.

4.4. Study Limitations and Generalizability

Several limitations must be considered. First, the single-centre design at Lozano Blesa University Hospital and two-month temporal window (January–February 2026) during a single epidemic wave may introduce selection biases. Second, the high mean age (80.31 years), elevated comorbidity rate (80.6%), and high vaccination rate (63.9%) mean these findings cannot be directly extrapolated to younger adult or pediatric cohorts,

non-hospitalized outpatient populations, unvaccinated demographic groups, or seasons dominated by different circulating influenza strains. Third, the low overall frequency of adverse outcomes (1 ICU transfer [1.39%] and 3 deaths [4.17%]) restricted multivariable risk adjustments.

5. Conclusions

Admission serum Alpha-1 Antitrypsin (AAT1) concentration does not serve as a useful prognostic biomarker for predicting clinical severity, Intensive Care Unit admission, or in-hospital mortality in adult patients hospitalized with severe influenza. Although AAT1 concentrations correlate moderately with standard systemic inflammatory markers such as C-reactive protein and leukocyte counts, physiological AAT1 levels remain largely unaugmented in isolated influenza infection due to localized respiratory tropism and memory immune containment in vaccinated elderly hosts. However, markedly elevated admission AAT1 concentrations (>200 mg/dL) represent a clinically valuable signal that should alert physicians to suspected SARS-CoV-2 coinfection or severe secondary systemic inflammatory recruitment, reflecting distinct host protease processing and cytokine-driven hepatic SERPINA1 transcription.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biom16111675/s1, Table S1. Sensitivity Analysis: Comparison of Baseline Characteristics and Primary Outcomes Restricted to RT-PCR-Confirmed Cases vs. Overall Cohort; Table S2. Diagnostic Characterization of Secondary Bacterial and Viral Coinfections at Presentation.

Author Contributions: I.F.-B.: Data collection, fieldwork, and manuscript drafting. S.M.-G.: Project conception and design, data analysis, manuscript review and editing. J.-J.P.-A., A.G.-G., I.M.-G., M.-Á.J.-A., M.R.-Á.: Laboratory assays and manuscript review. S.O.-G.: Statistical analysis collaboration. S.T.-A.: Translation and manuscript review. M.-Á.T.-C.: Supervision, project conceptualization, data interpretation, manuscript editing and final approval. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved on 24 January 2024 by the Clinical Research Ethics Committee of Aragon (CEICA: C.I. PI23/604).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to participant confidentiality.

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