

Optimised Murine Model of Influenza-Induced Respiratory Sepsis for Studying Acute Kidney Injury

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Abstract

Background

Viral respiratory sepsis, driven by influenza and COVID-19, is increasingly prominent clinically. However, there is a lack of preclinical models that reliably replicate its associated acute kidney injury (AKI), a major contributor to morbidity and mortality.

Methods

We refined an established model of influenza-induced respiratory sepsis by administering graded intranasal H1N1 A/PR/8/34 doses (3.7×10^1 , 3.7×10^3 , and 3.7×10^4 TCID₅₀) in male BALB/c mice. We defined humane endpoints to $\geq 30\%$ body weight loss, monitored clinical severity, weight, and glycaemia daily for 14 days, and assessed multi-organ dysfunction via serum biochemistry, histopathology, renal qPCR, and longitudinal serum neutrophil gelatinase-associated lipocalin (NGAL) ELISA.

Results

The 3.7×10^4 TCID₅₀ dose yielded 66.7% mortality by day 8, with a peak clinical score (MSS) of 10, $> 30\%$ weight loss, and hypoglycaemia (blood glucose < 70 mg/dL). Infected mice exhibited dose-dependent multi-organ dysfunction, with substantial elevations in serum creatinine (median: 204 [IQR: 142–600] $\mu\text{mol/L}$), bilirubin (40.62 [29.3–124.9] $\mu\text{mol/L}$), and creatine phosphokinase (CPK) (9822 [1272–11352] U/L). Renal NGAL expression increased 7-fold, aligning with rising serum creatinine levels and histopathological glomerular enlargement. Serum NGAL rose sharply by day 2 (4990 ng/mL) and sustained 5.7–7.6-fold elevation versus sham controls through days 4–8.

Conclusion

This optimised model simulates influenza-induced respiratory sepsis with AKI, highlighting serum NGAL as an early, dynamic biomarker. It provides a valuable preclinical tool for mechanistic and therapeutic studies in respiratory sepsis-associated AKI.

Background

Sepsis affects 49 million people annually and causes 11 million deaths worldwide (1, 2). Acute kidney injury (AKI), characterised by abrupt kidney dysfunction, is one complication of sepsis, closely linked to high mortality (3, 4). Viral respiratory infections, fuelled by escalating community transmission of seasonal influenza and COVID-19, drive an underexplored subset of sepsis-associated AKI.

Conventional AKI diagnosis hinges on serum creatinine, a lagging and nonspecific indicator of renal impairment (5). Neutrophil gelatinase-associated lipocalin (NGAL) is gaining recognition as an early biomarker of tubular injury, rapidly upregulated and secreted from renal epithelium in response to insults (5–7). NGAL rises prior to creatinine alterations, enabling proactive AKI identification (8, 9). Notably, patients with sepsis-associated AKI tend to exhibit higher plasma and serum NGAL levels than those with AKI from non-septic causes (6). Despite its promise, the effectiveness of using NGAL as an AKI marker remains controversial (9–11).

Building on our prior established murine model of influenza-induced respiratory sepsis (12), we further optimised this platform to delineate AKI pathogenesis. Emphasis was placed on renal NGAL expression, inflammation cytokines, and longitudinal tracking of serum NGAL to clarify the role of NGAL as a sensitive, dynamic parameter for early AKI inception and escalation. Integrated with clinical scoring, histopathological examination, and systemic inflammatory analyses, this refined model establishes a dependable preclinical tool for biomarker refinement and therapeutic innovation in viral respiratory sepsis and its associated AKI.

Materials and Methods

Animal Model and Viral Inoculation

Male BALB/c mice (eight weeks old) were used to establish a murine model of viral respiratory sepsis. Mice were intranasally inoculated with the H1N1 influenza A/PR/8/34 strain at predetermined doses of 3.7×10^1 , 3.7×10^3 , 3.7×10^4 median tissue culture infectious dose (TCID₅₀) selected based on our prior work to elicit graded severities of respiratory sepsis (n = 3 per dose) (12). Control animals (sham group, n = 3) received phosphate-buffered saline (PBS) via the same route. This exploratory sample size was expected to attain statistically significant differences of $p < 0.05$ with an ~72 - 75% probability in generating kidney dysfunction as reflected by elevated levels of serum creatinine according to our previous study (12) (Lamorte's Power Calculations). All procedures were conducted under approved institutional animal care protocols. The workflow is shown in Supplementary file 1.

Clinical Monitoring and Endpoint Criteria

Mice were monitored daily for 14 days following viral inoculation. Clinical severity was assessed using the Murine Sepsis Score (MSS). Humane endpoints (HEP) were defined as $\geq 30\%$ body weight loss, a modification from the previously used 20% threshold. In earlier studies, the 20% weight loss criterion may have led to premature euthanasia, potentially precluding the full development of sepsis and associated organ dysfunction. Therefore, this study extended the threshold to 30% weight loss to allow sufficient time for the emergency of clinically relevant sepsis phenotypes. Physiological parameters, including body weight and blood glucose levels, were recorded daily to monitor disease progression.

Tissue Collection and Histological Analysis

Pathological assessment served as the gold standard for confirming organ injury. Animals were euthanised either upon reaching HEP or at the conclusion of the 14-day observation period. Organs including the heart, lungs, spleen, liver, and kidneys were harvested for histological evaluation, as previously described (12, 13). Tissue sections were stained with haematoxylin and eosin (H&E). For each tissue from each mouse, five representative microscopic images were captured at 20× magnification. These images were analysed semi-quantitatively, as detailed in Supplementary file 2. Normal histological features were referenced from educational resources such as Pathweb (National University of Singapore, <https://medicine.nus.edu.sg/pathweb/>).

Assessment of Organ Function and Inflammatory Markers

Blood samples were collected for biochemical analysis of systemic organ function using the Sysmex BX-3010 chemistry analyser (Sysmex, Norderstedt, Germany). Parameters measured included serum creatinine, creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), and total bilirubin. Platelet counts were determined using the Sysmex XN-1000V haematology analyser. Kidney injury and inflammation were further evaluated by quantifying the expression of NGAL and inflammatory cytokines (IL-1 β , IL-6, TNF, and IL-10) via quantitative polymerase chain reaction (qPCR) using the Roche LightCycler 480 system. The primer information was shown in Supplementary file 3. Serum NGAL levels were assessed from tail-vein blood samples collected at two-day intervals to track longitudinal changes and quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Fisher Scientific, Cat# EMLCN2).

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (v.10 GraphPad Software, USA). Data were expressed as median with 95% confidence intervals (CI) or mean $\pm$ standard deviation (SD), as appropriate. Group comparisons were conducted using the Man-Whitney U test, Student's t-test, or Kruskal-Wallis Test. Correlation analyses were performed using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

Results

Temporal profiles of sepsis following H1N1 infection.

Survival rate: Mice challenged with the highest viral dose (3.7×10^4 TCID₅₀) exhibited 66.7% mortality by day 8 (Figure 1A), whereas no fatalities occurred in the lower-dose groups, indicating a threshold for lethality at higher viral loads.

Clinical severity (MSS): MSS increased in a dose-response manner, peaking on day 8 post-infection. An MSS of 10 was recorded in mice exposed to the highest viral dose (3.7×10^4 TCID₅₀), characterised by extensive back piloerection and suppressed activity (Figure 1B), indicative of severe systemic distress.

Glycaemia: Hypoglycaemia (blood glucose < 70 mg/dL) was evident from day 3, particularly in the higher dose groups, indicating metabolic dysregulation (Figure 1C).

Body weight loss: Progressive weight loss was observed across all infected groups. In the 3.7×10^4 TCID₅₀ group, two mice reached the humane endpoint threshold of 30% loss (Figure 1D).

Correlation analysis revealed significant associations between MSS and body weight loss (Spearman $r = -0.5469$ [95% CI -0.6523 to -0.4208]), as well as MSS and glycaemia ($r = -0.4687$ [95% CI: -0.6263 to -0.2741]) (Figure 2), supporting the utility of these parameters in tracking viral respiratory sepsis severity.

Biochemical assessment of organ dysfunction

Kidney function: Serum creatinine levels remained low and stable in sham mice (median [IQR]: 38 [32-38] $\mu\text{mol/L}$). In infected mice, levels increased in a dose-dependent manner, with values of 54 [50 – 67], 148 [10 – 166], and 204 [142 – 600] $\mu\text{mol/L}$ across ascending H1N1 doses (Figure 3A; Supplementary file 4). Based on the proposed murine-SOFA creatinine criteria (Supplementary file 5), individual mice in the 3.7×10^3 and 3.7×10^4 TCID₅₀ groups scored from 1 to 4 for renal dysfunction, whereas all sham mice scored 0 (Supplementary file 6).

Liver function: Serum bilirubin levels also rose progressively with viral doses. Sham mice exhibited a median of 8.15 [6.86 – 8.67] $\mu\text{mol/L}$. Infected mice showed elevations to 15.93 [11.62 – 18.18], 31.38 [19.68 – 35.84] and 40.62 [29.3 – 124.9] $\mu\text{mol/L}$ for the 3.7×10^1 , 3.7×10^3 , and 3.7×10^4 TCID₅₀ groups, respectively (Figure 3B; Supplementary file 4). Four mice scored 1 and one scored 2 on the murine-SOFA bilirubin criterion, indicating liver dysfunction (Supplementary file 6).

Coagulation: Platelet counts declined slightly only in the highest dose group (1554 [1477-2226] $\times 10^3/\mu\text{L}$) compared with sham (2066 [1786 – 2520] $\times 10^3/\mu\text{L}$) (Figure 3C; Supplementary file 4). All mice scored 0 on the murine-SOFA platelet criterion, suggesting no significant coagulopathy (Supplementary file 6).

Cardiac and systemic injury: Serum CPK levels increased markedly in response to escalating viral doses (Figure 3D; Supplementary file 4), compared with low levels in sham mice (336.0 [130 – 465] U/L). In the 3.7×10^4 TCID₅₀ group, hyperCPKemia (> 10,000 U/L) was observed, suggesting substantial cardiac muscle injury. LDH levels followed a similar trend (Figure 3E; Supplementary file 4).

Overall, biochemistry parameters of creatinine, bilirubin, platelets, CPK, and LDH reflected the onset of multiple organ dysfunction after influenza H1N1 infection.

Histopathological findings of tissue damage

Lung: Lung tissues from sham mice exhibited classic thin alveolar septa (Figure 4), indicative of normal pulmonary architecture and efficient respiratory gas exchange. In contrast, mice infected with H1N1 at doses of 3.7×10^3 TCID₅₀ and 3.7×10^4 TCID₅₀ showed extensive inflammatory cellular infiltration, reflecting severe lung inflammation and injury following H1N1 invasion.

Kidney: Sham mice displayed normal glomeruli with clear Bowman's spaces, whereas infected mice particularly those receiving the highest viral dose (3.7×10^4 TCID₅₀) exhibited enlarged glomeruli, suggesting kidney injury and impaired renal function.

Liver: Hepatic tissues from sham mice demonstrated abundant glycogen accumulation within hepatocytes. In mice infected with 3.7×10^4 TCID₅₀, there is a notable depletion of glycogen within hepatocytes, accompanied by cytoplasmic vacuolisation, indicative of hepatocellular stress and metabolic disruption.

Heart: Cardiac tissues from infected mice (3.7×10^3 TCID₅₀ and 3.7×10^4 TCID₅₀) revealed wavy cardiac muscle fibres, a hallmark of myocardial infarction which may be secondary to hypoxia resulting from severe lung inflammation.

Spleen: In sham mice, spleen architecture was well-preserved, with clearly defined white pulps (WP) and surrounding red pulps (RP). In H1N1 infected mice, the white pulp was expanded by increased numbers of lymphocytes, and the boundary between WP and RP appeared blurred, likely reflecting an active immune response to viral infection.

Kidney injury and inflammatory response

NGAL expression: Kidney NGAL mRNA levels were significantly elevated in influenza-infected mice, with 2-fold and 7-fold increases observed in non-surviving mice at the highest dose (3.7×10^4 TCID₅₀; $P = 0.0483$) (Figure 5A). This upregulation positively correlated with serum creatinine levels, supporting virus-induced kidney injury.

IL-1 β expression: Kidney IL-1 β mRNA levels were also significantly increased in the highest dose group (Median [IQR]: 1.72 [1.64 – 1.95]; $P = 0.0315$) (Figure 5B). As IL-1 β is a known driver of renal NGAL production (16), its elevation at day 8 post-infection supports ongoing injury and repair processes.

Other cytokines: IL-6, TNF- α , and IL-10 were undetectable in kidney tissue across all groups, possibly due to tissue-specific immune responses or the timing of sample collection. The kidney, not being a primary target of influenza A virus, may exhibit minimal systemic cytokine expression in the absence of secondary inflammation.

Temporal Changes in Serum NGAL Levels

Serum NGAL levels, initially low at baseline (median 191 ng/mL; IQR 169 – 212), rose sharply to 4990 ng/mL by day 2 post-infection in the most severely affected mouse (peak MSS: 9; 33% body weight loss) (Figure 6). Levels then declined to 1438 ng/mL by day 4, yet remained markedly elevated (5.7 – 7.6-fold higher) compared to sham controls through days 4 - 8 (Figure 6). These dynamics highlight serum NGAL as a sensitive early and dynamic biomarker of AKI in influenza-induced sepsis.

Discussion

We have developed and optimised a murine model of influenza-induced respiratory sepsis that induces kidney injury. This model provides a valuable preclinical platform for studying respiratory sepsis-associated AKI, supporting biomarker discovery and therapeutic development. Using this model, we further demonstrate that serum NGAL serves as a sensitive and dynamic biomarker, exhibiting a marked elevation as early as day 2 post-infection, with sustained increases aligning with disease progression.

The murine model effectively recapitulated key clinical features of severe respiratory sepsis. The overall mortality rate among patients with severe respiratory sepsis ranges from 30% to 70% (14, 15). At the highest viral dose (3.7×10^4 TCID₅₀), the high mortality rate of 66.7% in mice mirrors this clinical reality. Variability in disease severity observed at the same dose may be explained by individual genetic susceptibility to sepsis. Alongside significant weight loss, severe clinical scores and hypoglycaemia which provide useful complementary parameters for assessing disease progression and severity, biochemical analyses and histopathological observations confirmed organ injury across the lung, kidney, heart, liver and spleen, reflecting the systemic nature of influenza-associated sepsis.

One refinement in humane endpoints, extending the weight loss criteria from 20% to 30%, allowed fuller manifestation of organ dysfunction and sepsis phenotypes before euthanasia, thereby enhancing model validity. This refinement also enabled detection of a dose-dependent effect, contrasting with our previous study using 20% weight loss as the HEP in which no dose-response relationships were observed between organ dysfunction and virus doses (12). This dose-dependent severity further highlights the model's utility for studying various stages of systemic illness, including progressive multi-organ dysfunction.

Histopathological and molecular analyses confirmed prominent AKI in severely infected mice, with glomerular enlargement, 7-fold upregulation of renal NGAL mRNA, and increased IL-1 β expression. IL-1 β is a known driver of renal NGAL production, and its elevation indicates ongoing local inflammation and tissue repair processes (16). Notably, other inflammatory cytokines (IL-6, TNF, and IL-10) were undetectable in kidney tissue at day 8 post-infection. This absence likely reflects the temporal dynamics that IL-6 and TNF typically peak within hours of stimulation and decline rapidly due to short half-lives and tight regulation (17–20). IL-1 β can persist longer due to continuous processing and release via the inflammasome in response to ongoing cellular stress or damage, thus contributing to more sustained tissue responses in the kidney microenvironment (21). Additionally, influenza A virus primarily targets the respiratory tract, so renal cytokine expression may remain minimal without direct viral invasion. AKI in this model therefore probably arises indirectly via hypoxaemia from severe lung inflammation, cardiac compromise, oxidative stress, or systemic metabolic dysregulation (22). The observed multi-organ pathology including lung infiltration, myocardial wavy fibre, hepatic glycogen depletion, and splenic lymphoid hyperplasia, further supports interconnected mechanisms that amplify kidney injury.

One key limitation of this study is the small blood volume obtainable via serial tail-vein sampling (~ 10 μ L, yielding ~ 4 μ L serum). This volume was sufficient for sensitive assays such as NGAL ELISA but insufficient for serum creatinine measurement, which typically requires > 100 μ L serum and thus

necessitates terminal bleeding. This constraint precluded longitudinal monitoring of serum creatinine in individual animals, preventing direct within-subject comparisons of creatinine and NGAL dynamics. Nevertheless, the early and marked elevations of serum NGAL, as early as day 2 post-infection, with sustained increases through day 8, supports its potential as a sensitive and dynamic biomarker of AKI in influenza-induced sepsis. Future work should explore methods that enable simultaneous serial measurement of NGAL and creatinine in this viral sepsis model. Such combined monitoring could provide a more nuanced picture of AKI progression (23–26).

Other limitations of this study include the relatively small sample sizes per group ($n = 3$) and the focus on a single viral strain and host species, which may limit generalisability. As this was an exploratory dose-ranging study, the use of $n = 3$ per group, is consistent with the Reduction principle of the 3Rs and institutional minimum guidelines. Future studies could benefit from larger group sizes (e.g., $n = 5$ per group for $\sim 90\%$ power) to enhance detection of significant effects. Additionally, the absence of more detailed cytokine profiling and mechanistic studies on NGAL regulation during viral infection calls for further investigation. Future work should include validation of the NGAL in clinical cohorts of viral respiratory infections, exploration of serum NGAL measures, and the potential impact of therapeutic interventions on biomarker dynamics.

Conclusions

This work provides a preclinical tool for studying AKI the context of viral respiratory sepsis. Our data also demonstrate that serum NGAL is a promising early and sensitive biomarker, offering potential to improve timely diagnosis and management of sepsis-associated AKI in patients with influenza and other viral respiratory infections.

Abbreviations

AKI: Acute Kidney Injury

BALB/c: Bagg Albino Laboratory-bred/c

CI: Confidence Interval

CPK: Creatine Phosphokinase

H&E: Haematoxylin and Eosin

HEP: Humane Endpoint

IL-1 β : Interleukin-1 Beta

IL-6: Interleukin-6

IL-10: Interleukin-10

LDH: Lactate Dehydrogenase

MSS: Murine Sepsis Score

NGAL: Neutrophil Gelatinase-Associated Lipocalin

PBS: Phosphate-Buffered Saline

qPCR: Quantitative Polymerase Chain Reaction

SD: Standard Deviation

SOFA: Sequential Organ Failure Assessment

TCID₅₀: Median Tissue Culture Infectious Dose

TNF: Tumour Necrosis Factor

Declarations

Ethics approval and consent to participate

This study was conducted under the license of the Committee on the Use of Live Animals in Teaching and Research (approval number: 23-507). This study was carried out by persons holding valid Cap. 340 licenses issued by the Department of Health, and the principles of laboratory animal care were followed along the study.

Consent for publication

Not applicable.

Availability of data and materials

All datasets, on which the conclusions of the manuscript rely on, are presented in the paper.

Competing interests

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors' contributions

YJ and TR conceived the study and designed the study. YJ, LC, YC, CL and YZ performed the experiments. YJ, JN and TR analysed the data. YJ drafted the manuscript. TR revised the manuscript for critical content and approved the submitted version of the manuscript.

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Figures

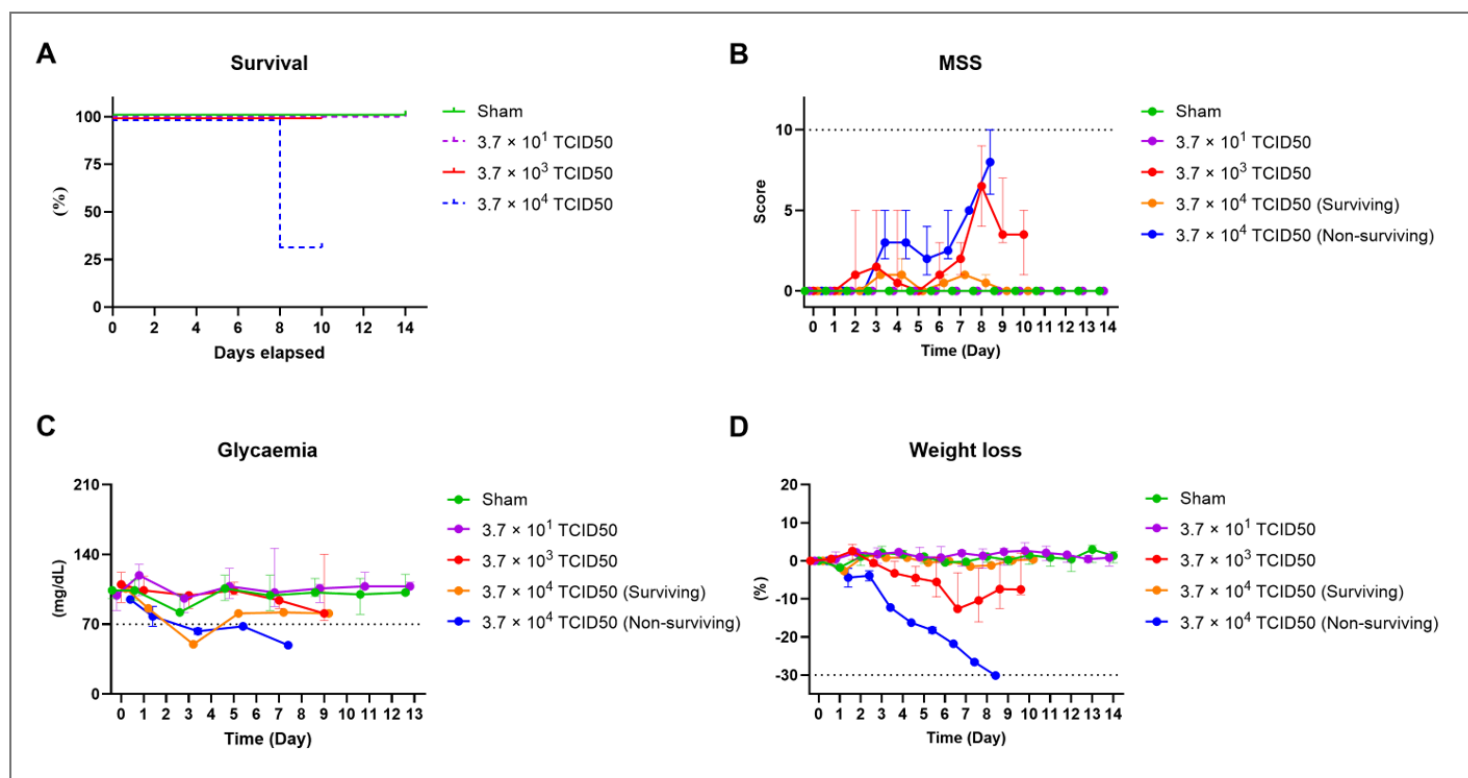


Figure 1

Physical conditions of 8-week-old male BALB/c mice after intranasally inoculated with influenza virus H1N1 strain A/PR/8/34. Doses of 3.7×10^1 , 3.7×10^3 and 3.7×10^4 median tissue culture infectious dose (TCID₅₀) were used. Survival curve (A), Murine Sepsis Score (MSS) (B), glycemia (C), and body weight loss (D) were evaluated over 14 days. Sham: PBS control. n = 3 per group. Data are presented as median with 95% confidence interval (CI).

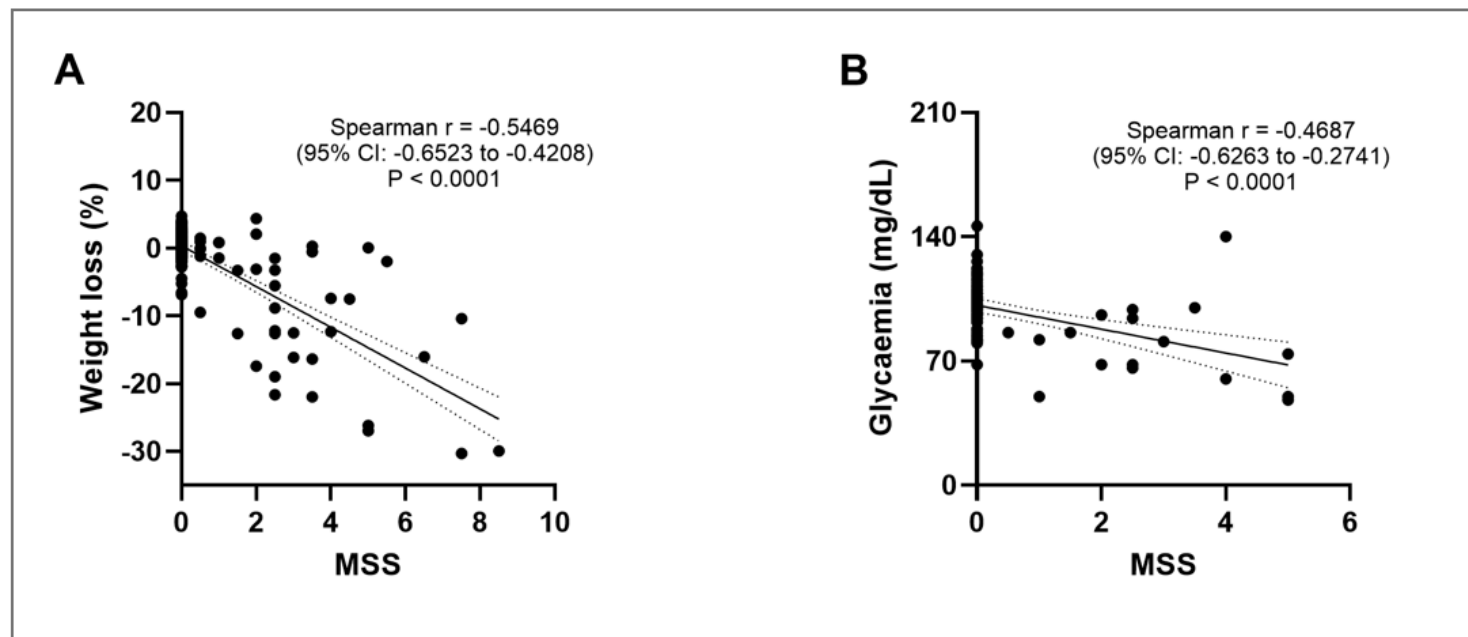


Figure 2

Correlation between MSS, body weight loss, and glycaemia. Correlation analyses were performed using Spearman's rank correlation coefficient. 95% CI: 95% confidence interval.

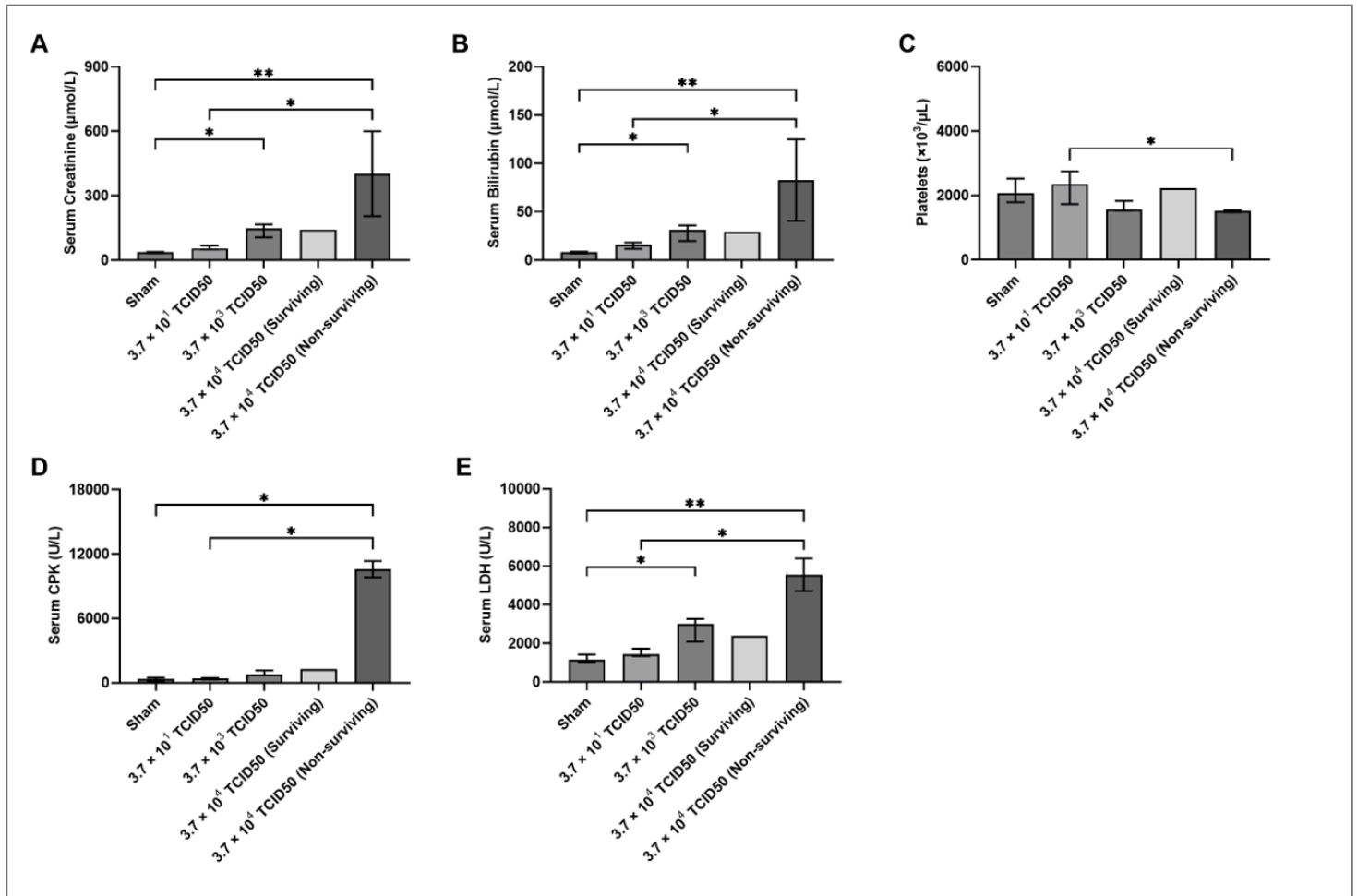


Figure 3

Influenza virus-induced organ dysfunction assessed by serum biomarkers. Mice were intranasally administrated influenza A virus H1N1 strain A/PR/8/34 at doses of 3.7×10^1 , 3.7×10^3 , or 3.7×10^4 median tissue culture infectious dose (TCID₅₀), or sham-treated with PBS (control). Shown are serum levels of (A) creatinine (renal function), (B) bilirubin (liver function), (C) platelet counts (coagulopathy), (D) creatinine phosphokinase (CPK; cardiac muscle injury), and (E) lactate dehydrogenase (LDH; systemic tissue injury/inflammation). Data are presented as medians with 95% confidence intervals (CI); n = 3 per group. Statistical analysis: Kruskal-Wallis test with uncorrected Dunn's multiple comparisons (asterisks indicate significant comparisons only: * P < 0.05; ** P < 0.01).

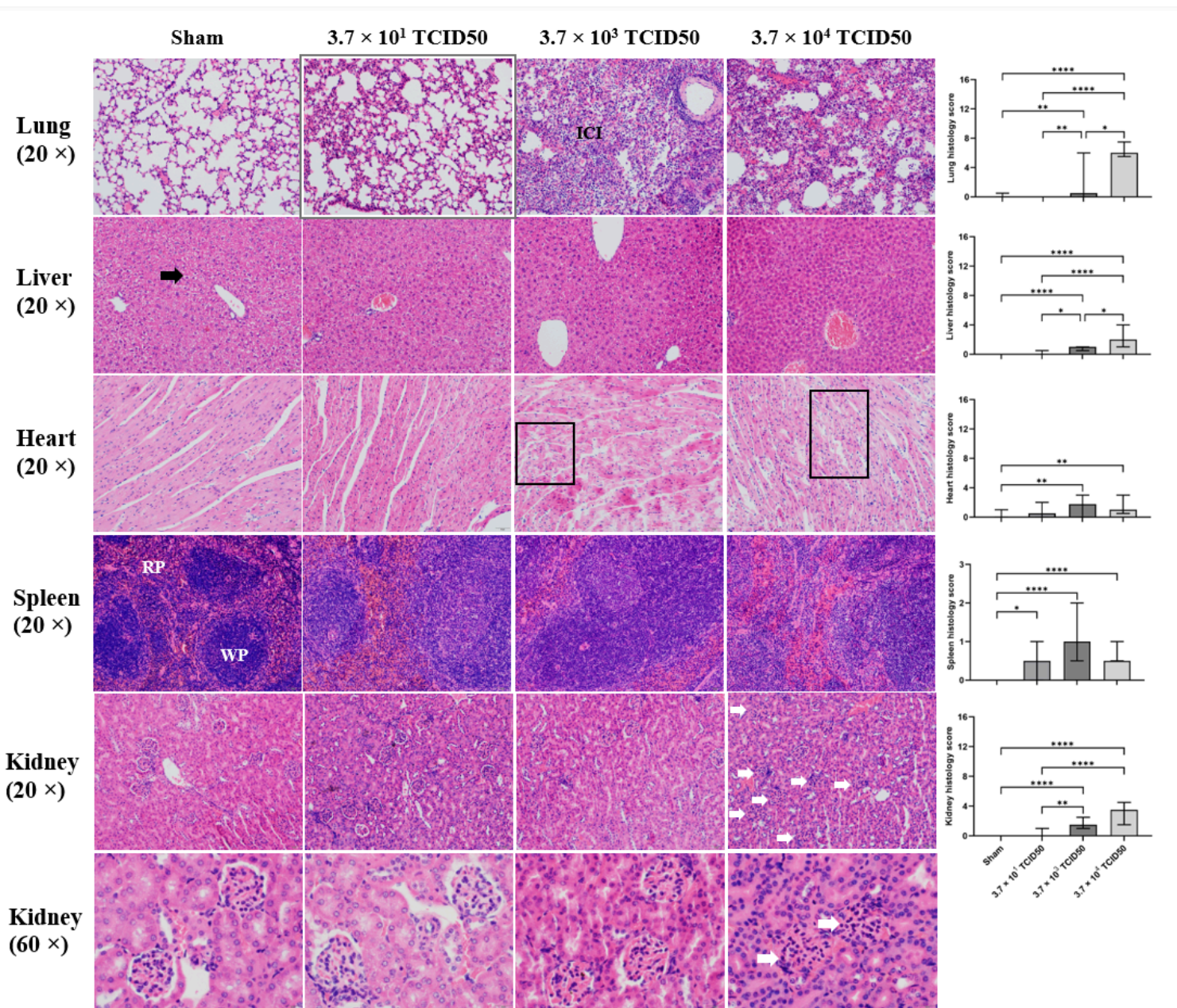


Figure 4

Histopathological changes in multiple organs of sham (PBS control) and influenza A/PR/8/34 (H1N1) infected mice. Representative hematoxylin and eosin (H&E)-stained sections of lung, liver, heart, spleen, and kidney are shown. Key features are indicated: ICI, inflammatory cellular infiltration; white arrow, enlarged glomeruli; black arrow, hepatocytes containing abundant glycogen; black frame, wavy myocardial fibres; RP, red pulp; WP, white pulp. All images were acquired at 20× magnification. For the kidney, additional images at 60× magnification are provided to highlight glomerular enlargement. Histology scores for each organ were compared using the Kruskal-Wallis test followed by uncorrected Dunn’s post hoc test. Statistical significance is indicated only for comparisons reaching significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

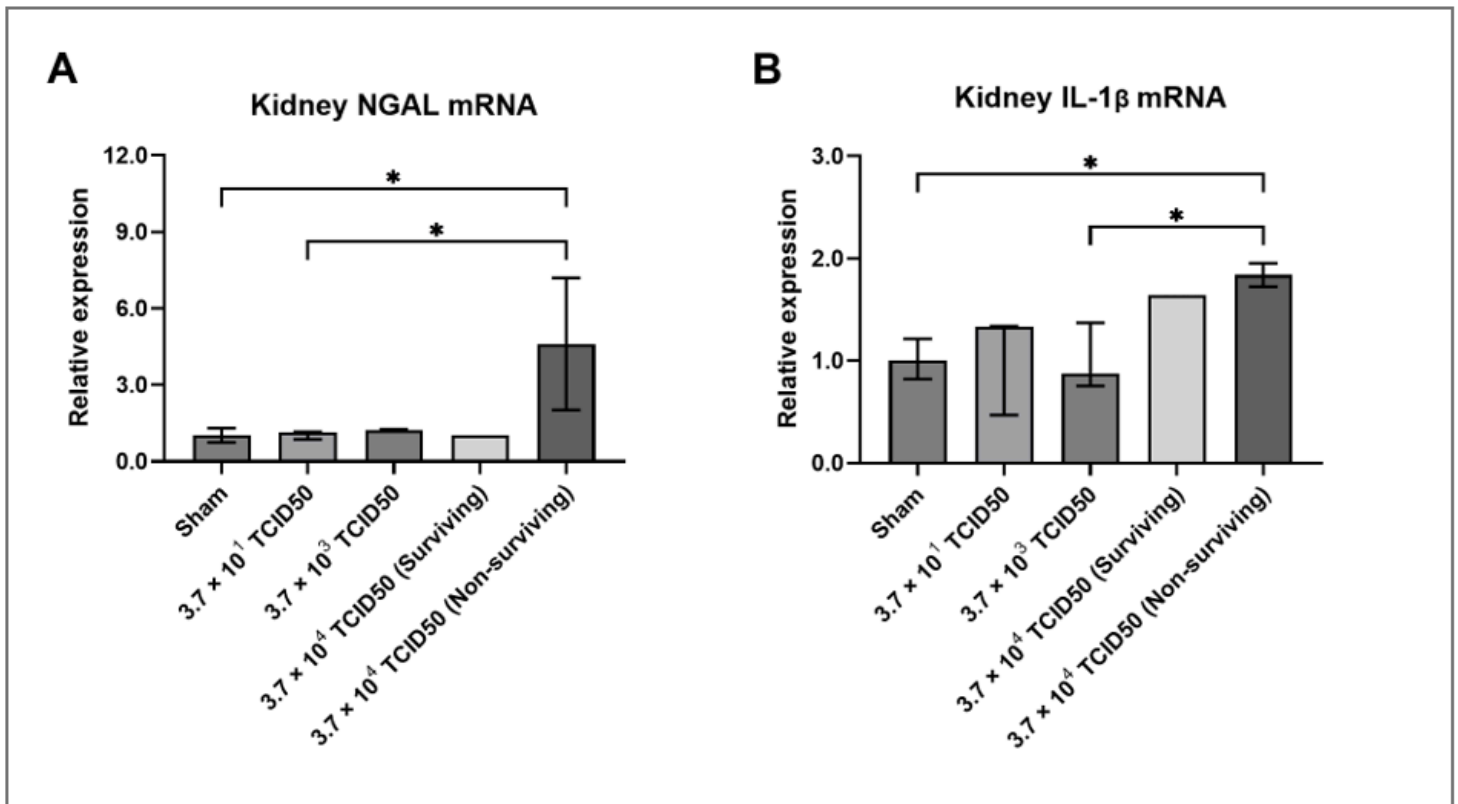


Figure 5

Quantitative real-time PCR (qPCR) analysis of NGAL (A) and IL-1β (B) mRNA expression in mouse kidneys following influenza A/PR/8/34 (H1N1) infection. Mice were intranasally inoculated with influenza A/PR/8/34 (H1N1) virus at doses of 3.7×10^1 , 3.7×10^3 , and 3.7×10^4 TCID₅₀. Kidney tissues were collected at the humane endpoint (HEP) or on day 14 post-infection. mRNA levels were normalised to GAPDH and relative expression was calculated using the $\Delta\Delta C_t$ method. Data are shown as median with 95% confidence interval (CI; n = 3 per group). Statistical analysis: Kruskal-Wallis test with uncorrected Dunn's multiple comparisons (asterisks indicate significant comparisons only: * P < 0.05)

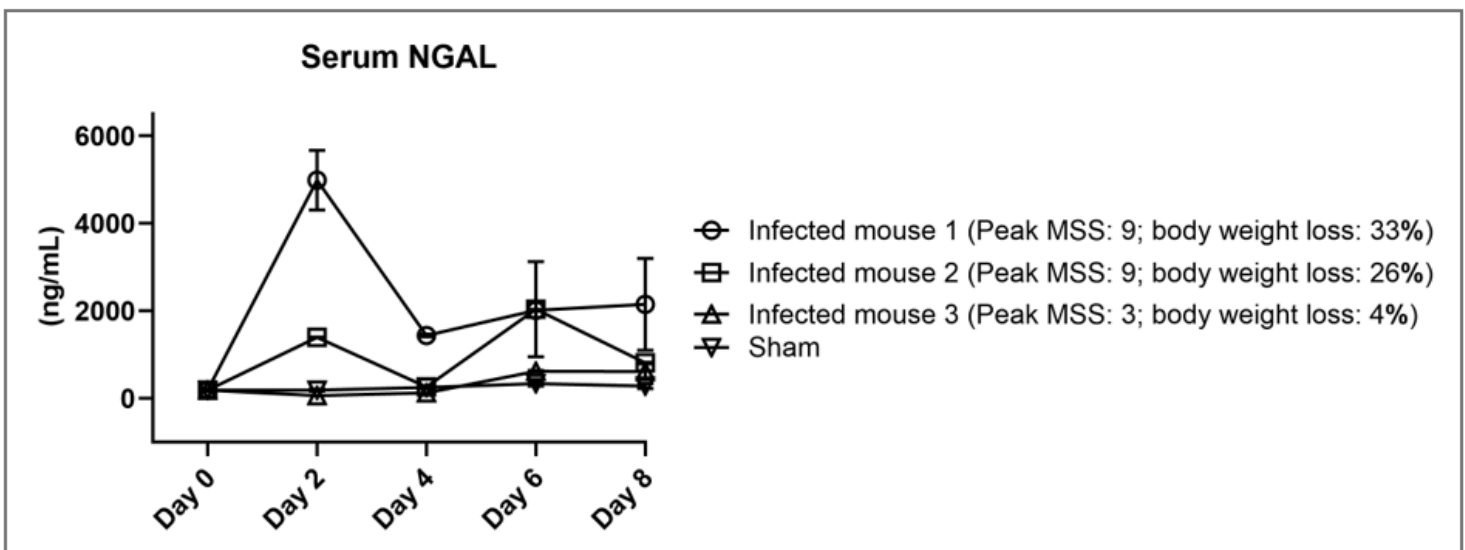


Figure 6

Dynamic changes in serum NGAL levels following influenza A/PR/8/34 (H1N1) infection. Mice (n = 3) were intranasally inoculated with 3.7×10^4 TCID₅₀ of influenza A/PR/8/34 (H1N1) virus. Infected mice exhibited variable disease severity, with peak Mouse Sepsis Score (MSS) of 9 (mouse 1; 33% body weight loss), 9 (mouse 2; 26% body weight loss), and 3 (mouse 3; 4% body weight loss). Serum NGAL levels were measured in duplicate by ELISA using tail vein blood samples collected serially at 2-day intervals post-infection. Data are presented as median with 95% confidence interval (CI).

Supplementary Files

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