

Immune Biomarkers Mediate the Effect of Rib Fractures on Acute Lung Injury: A Compositional Mediation Analysis



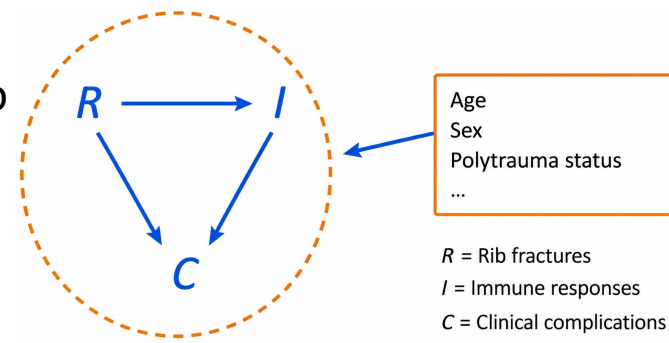
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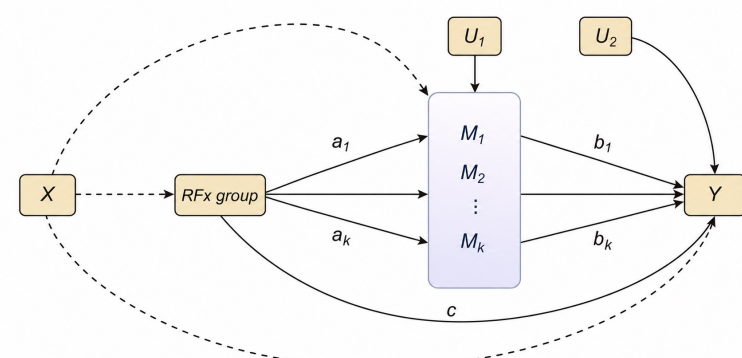
INTRODUCTION

- Rib fractures (Rfx or R) can lead to severe clinical complications (C), such as acute lung injury (ALI), which contribute to increased morbidity and mortality.
- Prior research used machine learning to identify 12 biomarkers that predict multiple outcomes such as ALI.
- The total effect of R on C is decomposed into two distinct pathways: direct effect ($R \rightarrow C$), and indirect effect ($R \rightarrow I \rightarrow C$).
- We hypothesized that these biomarkers act as compositional mediators of the Rfx-ALI relationship within the RIC triad.
- Our goals are to test whether the mediation effect of the post-Rfx immune response (I), as measured by the 12 biomarkers, is significantly different from zero and to identify the major individual mediators.



METHODS

- The Rfx cohort includes 142 patients who were treated for Rfx at Duke University Hospital, Grady Memorial Hospital, and Walter Reed Medical Center during 2014-2020.
- We used the Abbreviated Injury Scale to identify polytrauma patients ($N = 44$).
- We analyzed 34 proteins from 431 serum samples using the MesoScale Discovery platform.
- Partial correlation analysis was applied to investigate the I - C and R - C relationships based on the RIC triad. The I - C correlations were calculated with adjustment for Rfx by location, age, sex, and polytrauma status (PS); the R - C correlations were adjusted for immune responses, age, sex, and PS.
- Cluster analysis was based on Gower's distance metric and Ward's method.
- To estimate the biomarkers' mediation effects and the direct effect of Rfx group on ALI, we employed a compositional mediation model (CMM) shown below.
- By contrasting the two states (with or without fractures) in each Rfx group, the CMM estimates direct and indirect effects using a generalized linear model with a probit link for the binary outcome (ALI), yielding effects on the probability difference (PD) scale. The total effect is reported as an odds ratio (OR) from logistic regression.
- Sensitivity to unmeasured mediator-outcome confounding was assessed by varying ρ , a hypothetical correlation parameter representing the magnitude of confounding between disturbance variables U_1 and U_2 , across a plausible range.



X denotes covariates (e.g. age, sex, and polytrauma status);
 Y is a clinical outcome (e.g. ALI);
 M indicates biomarkers as compositional mediators.

RESULTS

Figure 1. Partial correlations reveal three Rfx groups. Here C represents the post-Rfx complications: ALI, acute kidney injury (AKI), sepsis, pneumonia (PNM), and ventilator-associated pneumonia (VAP). **A** and **B**: the I - C and R - C correlations, respectively.

Table 1. Characteristics of Rfx patients with and without complications.			
Characteristic	With n = 73	Without n = 69	P
Age, mean (SD)	46.4 (17.7)	43.9 (15.9)	0.378
Sex, n (%)			0.789
Female	27 (37.0)	28 (40.6)	
Male	46 (63.0)	41 (59.4)	
Injury severity score, mean (SD)	34.4 (14.6)	24.54 (10.2)	<0.001
Rib fracture patterns			
Rfx-G1, mean (SD)	0.6 (0.8)	0.4 (0.6)	0.136
Rfx-G2, mean (SD)	1.5 (1.3)	1.3 (1.1)	0.478
Rfx-G3, mean (SD)	2.2 (2.0)	2.1 (1.7)	0.836
Count of rib fractures, mean (SD)	4.2 (3.0)	3.8 (2.5)	0.396
Complications			
PNM, n (%)	6 (8.2)		
ALI, n (%)	28 (38.4)		
AKI, n (%)	22 (30.1)		
VAP, n (%)	17 (23.3)		
Sepsis, n (%)	13 (17.8)		
Count of complications, mean (SD)	2.7 (2.2)		
Total hospital days, mean (SD)	25.1 (17.0)	9.1 (8.2)	<0.001

Figure 2. Total serum samples of polytrauma (A) vs. isolated Rfx (B). The number of fractures in each group (e.g. Rfx-G1 is the Rfx count in G1) is associated with specific immunological signatures that are similar between polytrauma and isolated Rfx patients.

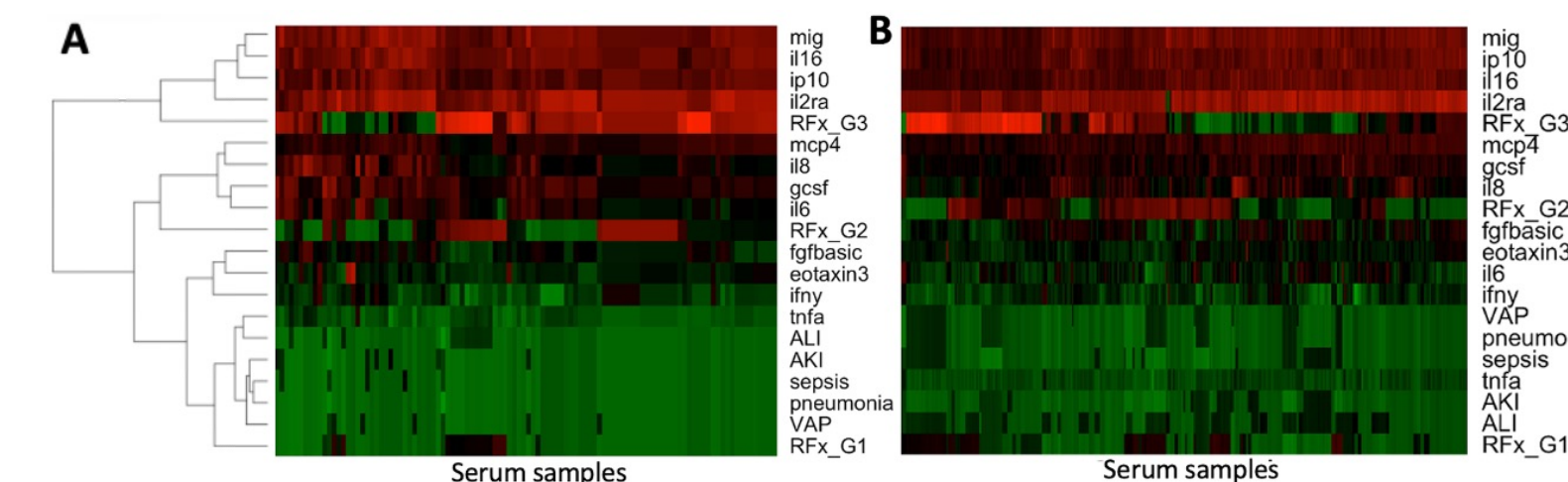


Table 2. Patients' severe and non-severe injuries by body region.

Injury Region	Severe	Not severe
Chest	44	98
Skin	2	140
Extremities	26	116
Face	1	141
Head and neck	16	126
Pelvis	43	99

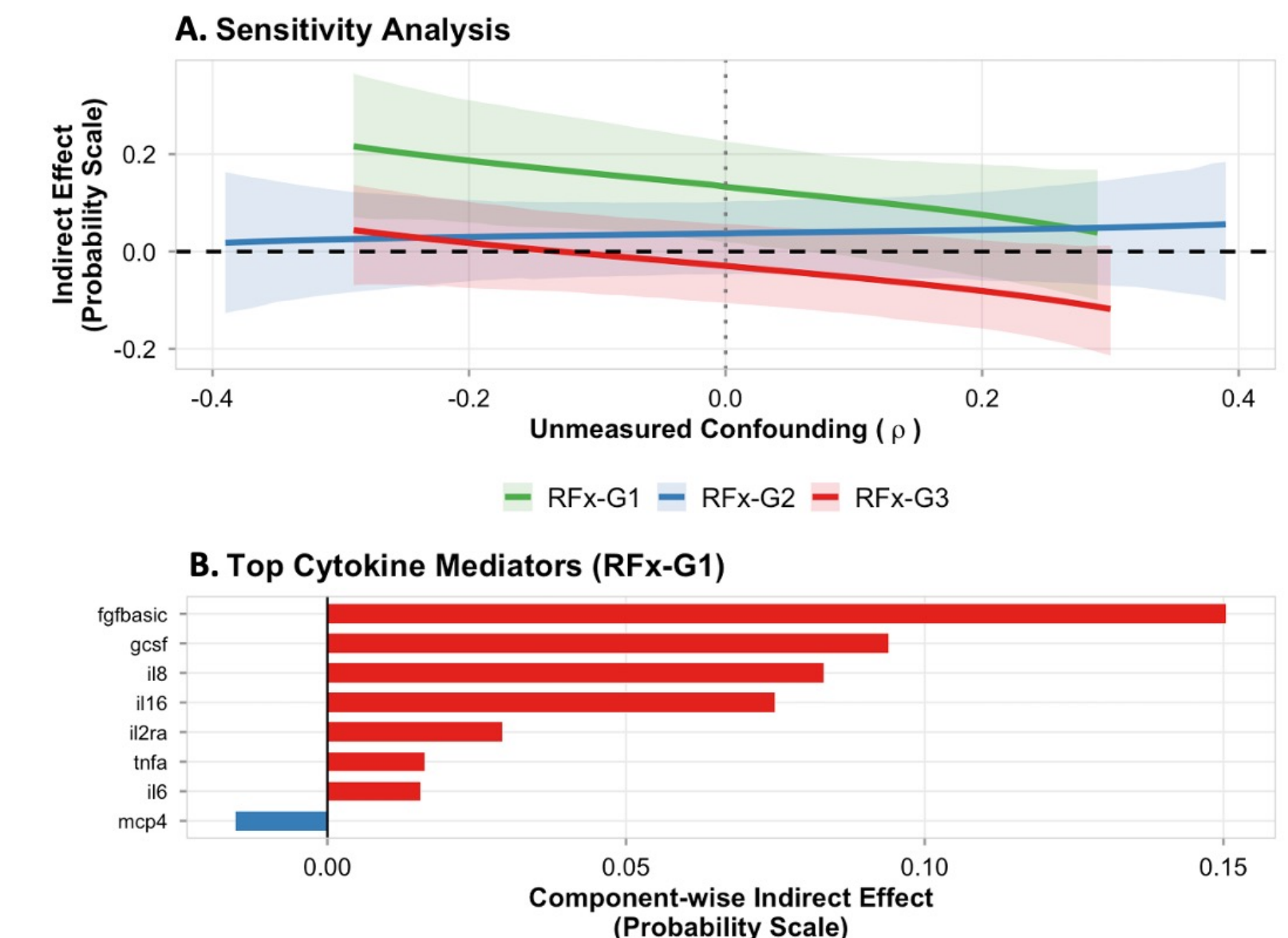
Non-Severe: An AIS score of 1 or 2. Severe: An AIS score ≥ 3 .

Table 3. Decomposing the total Rfx by group effect on ALI. The indirect or direct effect is significant if its 95% CI does not include zero.

Rib Group	Total Effect OR (95% CI)	p-value	Indirect Effect (95% CI) (PD)	Direct Effect (95% CI) (PD)
Rfx-G1	3.01 (1.66-5.66)	0.000	0.13 (0.02, 0.23)	0.10 (-0.04, 0.21)
Rfx-G2	0.94 (0.59-1.49)	0.797	0.04 (-0.04, 0.10)	0.04 (-0.07, 0.13)
Rfx-G3	1.01 (0.78-1.31)	0.938	-0.03 (-0.10, 0.06)	-0.05 (-0.14, 0.07)

Here Rfx-G1 is a binary variable, with or without fractures in G1, as are Rfx-G2 and Rfx-G3 in G2 and G3, respectively.

Figure 3. The compositional mediation analysis. The 12 biomarkers shown in Figure 2 were used as compositional mediators for the relationships between Rfx groups and ALI. The total, direct, and indirect effects are shown in Table 3. Rfx-G1 showed a significant total effect on ALI risk (OR = 3.01) with a significant indirect effect through the 12 biomarkers, while the direct effect was non-significant. Rfx-G2 and Rfx-G3 showed no significant total, indirect, or direct effects. Sensitivity analysis confirmed robustness of the Rfx-G1 indirect effect across $\rho \approx -0.3$ to 0.1 (**A**), with FGF-basic, G-CSF, IL-8, and IL-16 as the dominant positive mediators and MCP-4 as the negative mediator (**B**).



CONCLUSION

- Three Rfx groups were identified, each of which clustered with specific immunological biomarkers that were similar between polytrauma and isolated Rfx patients.
- Patients with fractures of ribs 1-2 had 3.01 times the odds of developing ALI compared to patients without Rfx-G1 fractures (OR = 3.01, 95% CI: 1.66-5.66, $p < 0.001$), adjusting for age, sex, and polytrauma status.
- The mediation effect of the 12 main biomarkers is significant.
- Both pro-inflammatory cyto/chemokines (e.g., IL-8, IL-16, TNF- α , IL-6) and growth factors/immune modulators (e.g., FGF-basic, GCSF) demonstrated positive mediation, with FGF-basic exhibiting the strongest effect.
- FGF-basic is involved in tissue repair, angiogenesis, and inflammatory responses, suggesting that it may contribute to ALI via increased vascular permeability, inflammatory cell recruitment, and endothelial dysfunction.