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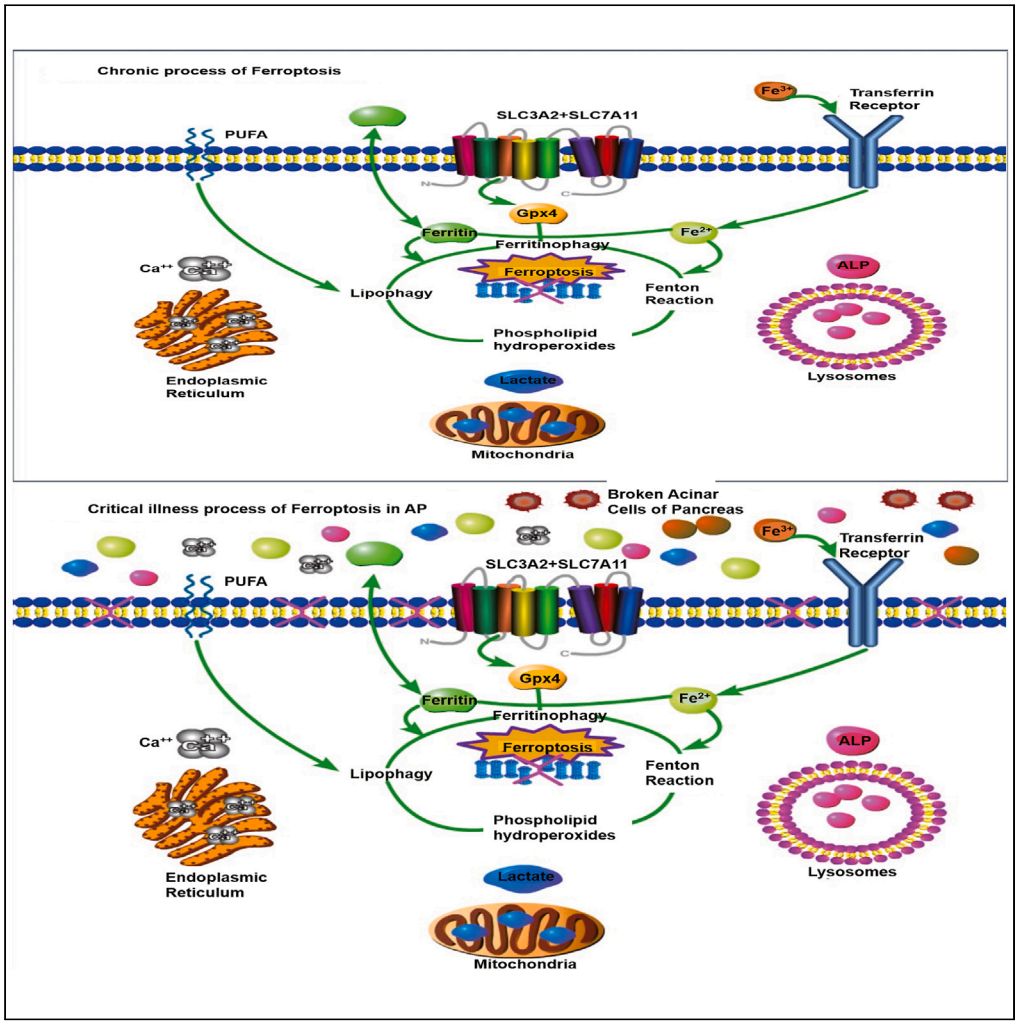

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Highlights

Ferroptosis process affects the prognosis of AP and NAFLD

Serum iron level could reflect the extent of ferroptosis in AP and NAFLD patients

Serum iron is an important marker of the prognosis of AP and NAFLD

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Serum iron fluctuations link ferroptosis process with mortality and prognosis of acute pancreatitis

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SUMMARY

Recently, the existence of ferroptosis has been confirmed in chronic pancreatitis. However, its role in acute pancreatitis (AP) process, especially in critical status, has not yet been mentioned. To verify this hypothesis, we included 873 AP patients (training set) and 1,188 NAFLD patients (internal validation set) selected from MIMIC-III (Medical Information Mark for Intensive Care) database and 218 AP patients (external validation set) in Linshui County People's Hospital ICU data. We analyzed the correlation between mortality and ferroptosis associating factors (such as serum iron, ALP, lactate, etc.) in them through regression analysis. In addition, to test the significance of these factors, the nomogram, AUC, and DCA analysis were applied. The results showed that serum iron, IBC, ALP, and lactate ($p < 0.05$) were independent factors for the mortality and prognosis of these patients. These correlations suggest ferroptosis and follow-up cell programmed death may own an important clinical interference significance among this population.

INTRODUCTION

Iron plays a pivotal role as a critical cofactor in a variety of physiological metabolic reactions,[1] but excessive accumulation of iron also triggers some kind of programmed cell death ferroptosis.[2] As a new type of oxidative process regulating cell death driven by iron-dependent lipid peroxidation, ferroptosis is, indeed, associated with renal failure,[3] cancer,[4] liver steatosis,[5] neurodegeneration,[6] and senescence.[7] Recently, ferroptosis was linked with the pathology of acute pancreatitis (AP) from autophagy[8] and immunology[9] aspects. However, although ferroptosis is iron-dependent, until recently, the exact role of iron in ferroptosis was unclear. Furthermore, an animal study showed that high-iron diets in the pancreas promoted experimental pancreatitis in mice induced by the administration of cerulein.[9] At present, there is little direct evidence to show whether the iron overload in AP (Figure 1A)[10] induces the occurrence of ferroptosis, which is necessary for further study.

In addition, infection theory could not totally explain the fluctuation of serum iron concentration and the role of iron in AP, especially in critical status.[11] Thus, we speculate that if serum iron fluctuation is part of the imbalance between intracellular and extracellular iron caused by ferroptosis in AP, that will solve the above-mentioned problem.

AP, for the severe one, accompanied with fluctuation of serum iron, has been reported as one of the most common reasons for critical illness in the United States.[12] Non-alcoholic fatty liver disease (NAFLD) is viewed as the hepatic manifestation of metabolic syndrome and is greatly affected by ferroptosis.[13] We believe that comparing these two diseases under similar conditions can indirectly provide evidence of ferroptosis in AP. More important, until now, rare studies have linked intensive care unit (ICU) mortality and ferroptosis with these two diseases.[14]

Our hypothesis is that, unlike the chronic process suffered with relatively slowly programmed death, cell membranes are suddenly disrupted and a large amount of cellular content will be released into extracellular space under critical status. Marked by damage of organelles, such as lysosomes (alkaline phosphatase (ALP)), endoplasmic reticulum (calcium), and mitochondria (lactate), a large amount of intracellular Fe^{2+} is released into surrounding tissues, causing changes in the concentration of Fe^{2+} in the interstitial spaces of uninjured tissues (Box 1). In such an environment, especially the decrease in pH value, the increase of peripheral iron concentration will continue to affect the concentration of intracellular iron. Through assisted diffusion, this part of iron will be transferred into cells in large quantities with the assistance of ferritin, triggering a more severe signal cascade of ferroptosis. And this process will play a significant role in short-term mortality in severe AP patients.

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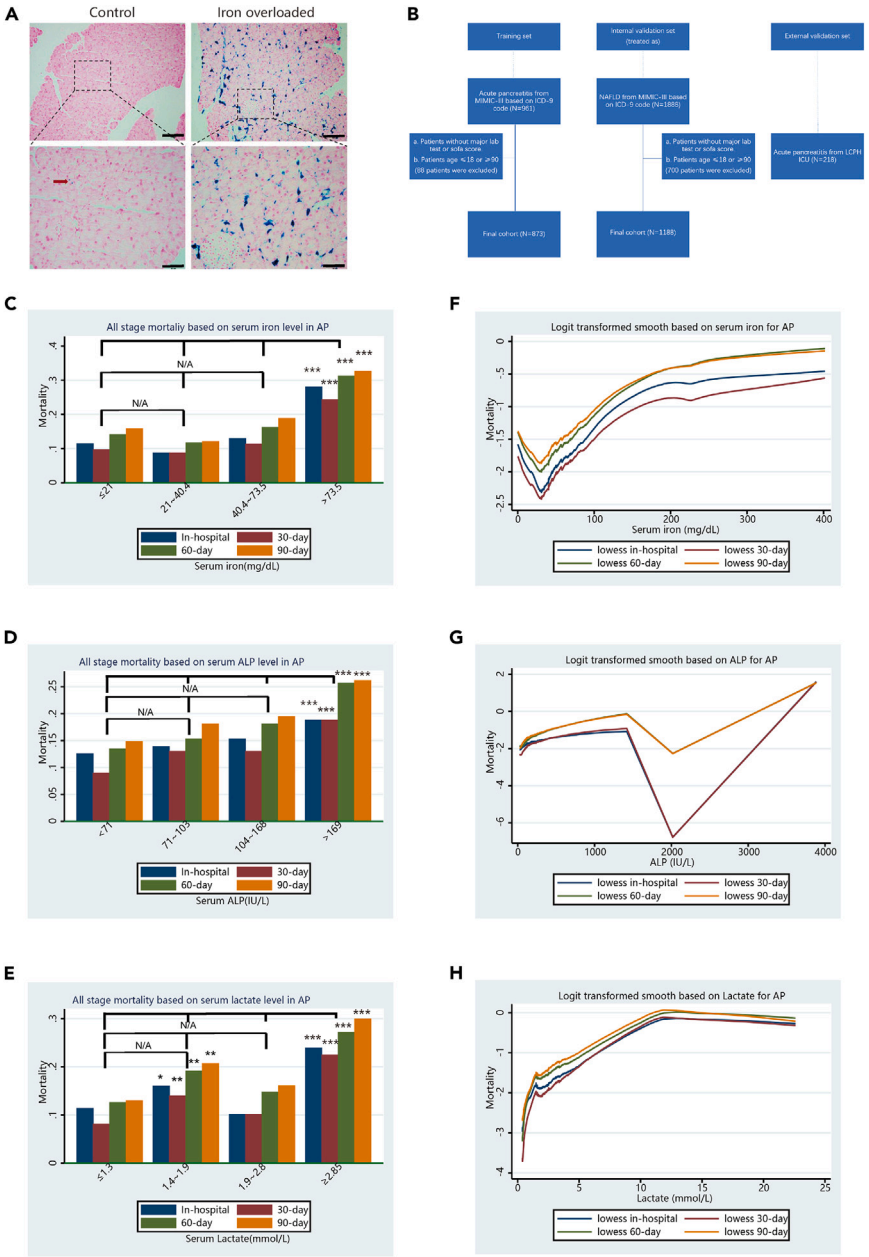

Figure 1. Evidence of AP and ferroptosis

Flowchart of subject screening and the tight relationship between ferroptosis factors (serum iron, ALP, and lactate) values and all-time points mortality in patients with AP.

(A) Male, 8-week-old, C57BL/6 mice were injected intraperitoneally with 120 mg/kg body weight of iron dextran every other week for 12 weeks; Prussian blue staining in the pancreas.[10]

(B) Flowchart of subject screening.

(C–E) Of 873 AP patients admitted to ICU were divided into 4 groups based on theirs' concentration of serum iron (C), ALP (D), and lactate (E) separately. The mortality of different groups in 30, 60, 90-day, and in-hospital were presented.

(F–H) Of 873 AP patients admitted to ICU whose mortality changing trend in 30, 60, and 90 days, and in-hospital presented in lowess curves based on serum iron (F), ALP (G), and lactate (H) separately. Abbreviations: NAFLD, Non-alcoholic fatty liver disease; LCPH, Linshui County People Hospital; MIMIC-III, Medical Information Mart for Intensive Care version III. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$(The mortality of each two groups were compared. N/A indicates no statistical difference between the two groups). Scale Bar = 50μM.

Box 1. Mapping of potential markers based on ferroptosis and accompanied organelle impairment among AP and NAFLD.

Organelles or diseases	Potential routine clinical data marker	Biological rationale
Ferroptosis:	Iron ↑	Higher levels of hepatic iron and lipid peroxidation in liver cirrhosis[33]
	Ferritin↑	Autophagy leads to iron-dependent ferroptosis by degradation of ferritin[34]
	Iron-binding capacity↓	Iron-binding capacity was associated with schizophrenia[35]
Acute pancreatitis:	Amylase ↑	Increased in acute pancreatitis[36]
	Lipase↑	
NAFLD:	aspartate transaminase (AST) ↑	Activation of pyroptosis and ferroptosis is involved in the hepatotoxicity induced by polystyrene microplastics in mice[37]
	alanine transaminase (ALT) ↑	
	Cholesterol↑	Dysregulated cholesterol homeostasis results in resistance to ferroptosis in cancer[38]
Renal failure:	Creatinine (Cr)↑	Increased in renal failure and associated with ferroptosis[39]
	Blood Urea Nitrogen (BUN)↑	
Endoplasmic reticulum (ER):	Calcium ↓	ER transmits proper Ca^{2+} signals to mitochondria, which apoptosis[40]
Mitochondria:	Lactate↑	A waste product of anaerobic metabolism, lactate is now known to form continuously under aerobic conditions[18]
Lysosomes:	Alkaline phosphatase (ALP)↑	Responsible for the de-phosphorylation process of small molecules[41]

Therefore, we designed this study to evaluate the relationship between serum iron, ferritin, iron-binding capacity, calcium measurement, lactate, ALP, and the outcomes of ICU patients suffered with AP or NAFLD. At the same time, we hope to explain the relationship between serum iron and ferroptosis from a clinical perspective, thus to better understand the benefit of iron monitoring in AP management.

RESULTS

Patient characteristics

The selected patients are shown in flow chart (Figure 1B). For acute pancreatitis (AP), a total of 873(Medical Information Mart for Intensive Care-III database (MIMIC-III), Training Set), 218 (Linshui County People's Hospital (LCPH), External Validation Set) patients (AP onset within 7 days) were finally included in the study; for NAFLD, a total of 1188(treated as Internal Validation Set) patients were finally included in the study. Among this cohort, the in-hospital mortality was 20.8% (n = 173) in MIMIC-III and 13.8%(n = 30) in LCPH. The whole mortality within30, 60, 90-day were 13.5% (n = 117), 18.8% (n = 158), 20.5% (n = 171) respectively in MIMIC-III. For NAFLD, 1188 patients were included, the in-hospital mortality was 35.7% (n = 376), and the whole mortality within30, 60, and 90 days were 24.8% (n = 277), 20.9% (n = 337), and 35.1% (n = 374) respectively. All these patients were divided into two groups upon the final ending, survival or non-survival. In AP, non-survival group had remarkable differences at baseline data compared with survival one. Most of the patients in non-survival were older with lower pH, SO_2 (oxygen saturation of hemoglobin), and higher SOFA score, accompanied with higher body mass index (BMI), higher low absolute lymphocyte count(L-ALC) ratio,[15] and severity of AP[16] (based on Revised Atlanta Criteria). In terms of complications, non-survival had a stronger association to acute and subacute hepatic necrosis (ASHN), and kidney disease (KD) through all time points. In addition, based on the comparison of common laboratory data in blood, non-survival group appeared to have decreased iron binding capacity (IBC), accompanied with increased ALP, iron, and lactate compared with opposite group. Interestingly, the indicators remarkably related to mortality in AP were almost total overlaid with NAFLD. But there is still a little bit difference between these two diseases. In NAFLD, it was partial pressure of oxygen (PO2), not oxygen saturation of hemoglobin (SO_2) deceased obviously in non-survivals. As for lab test for NAFLD, in addition to remarkable laboratory findings in AP, sodium, chlorine, free calcium, and cholesterol had strong association to mortality as well. Specific baseline characteristics of the patients are presented in Tables 1, S1 and S2. Furthermore, we explored the correlation between all indicators with statistically significant differences in baseline table ($p < 0.05$) and all stages of mortality by univariate logistic regression and multivariate backward logistic regression in AP patients. Age, sequential organ failure assessment (SOFA) score, ALP, iron, lactate. etc., were predictive of mortality when used as the only explanatory variable in a logistic regression model both in MIMIC-III and LCPH. Interestingly, severity of AP did not show strong association in this analysis. We think the main reason is that most patients included in this research were in moderate to high severity of AP. Then, including ferritin, variables with $p < 0.1$ in univariate logistic regression considered potential confounders entered multivariate logistic regression model, and we used backward regression and excluded variables with a likelihood ratio test p-value of at least 0.05. In the logistic regression model finally adjusted for age, SOFA score, ALP, iron, and creatinine has significant predictive value for mortality through all time points in both centers. (Tables 2, S3, and S4).

Table 1. Baseline characteristics of the patients with AP grouped by in-hospital mortality (MIMIC-III database & LCPH)

MIMIC-III database				LCPH			
Overall population	Survivors N = 741	Non-survivors N = 132	p-value	Overall population	Survivors N = 188	Non-survivors N = 30	p-value
Age (years)	57.65 ± 0.60	64.21 ± 1.38	**p < 0.001**	Age (years)	58.67 ± 1.17	66.45 ± 2.71	**p = 0.013**
Gender, n (%)			p = 0.024	Gender, n (%)			p = 0.902
Male	414(47.4)	81(9.3)		Male	98(45.0)	16(7.3)	
Female	327(37.5)	51(5.8)		Female	90(41.3)	14(6.4)	
BMI (kg/m2)	29.46 ± 0.31	31.92 ± 0.86	**p < 0.001**	BMI (kg/m2)	26.06 ± 0.36	27.96 ± 0.70	**p = 0.043**
pH	7.36 ± 0.004	7.31 ± 0.013	**p < 0.001**	pH	7.37 ± 0.007	7.26 ± 0.032	**p < 0.001**
PO2 (mmHg)	130.42 ± 3.61	125.06 ± 8.06	p = 0.560	PO2 (mmHg)	125.93 ± 7.26	145.20 ± 19.55	p = 0.331
PCO2 (mmHg)	40.5 ± 0.49	39.8 ± 1.32	p = 0.637	PCO2 (mmHg)	40.6 ± 1.14	40.4 ± 2.07	p = 0.946
SO2 (%)	91.16 ± 0.44	86.76 ± 1.33	**p < 0.001**	SO2 (%)	91.47 ± 0.92	84.80 ± 2.72	**p = 0.010**
SOFA Score	4.41 ± 0.12	9.16 ± 0.42	**p < 0.001**	SOFA Score	3.90 ± 0.19	9.57 ± 1.00	**p < 0.001**
Severity of AP(%)			**p = 0.028**	Severity of AP(%)			p = 0.235
Mild	13(1.5)	4(0.5)		Mild	4(1.8)	2(0.9)	
Moderate	234(26.8)	27(3.1)		Moderate	70(32.1)	8(3.7)	
Severe	494(56.6)	101(11.6)		Severe	114(52.3)	20(9.2)	
Medical History, n(%)				Medical History, n(%)			
Anemia	164(18.8)	19(2.2)	**p = 0.044**	Anemia	38(17.4)	2(0.9)	p = 0.075
CHD	107(12.3)	21(2.4)	p = 0.660	CHD	26(11.9)	7(3.2)	p = 0.177
ALD	38(4.4)	15(1.7)	**p = 0.006**	ALD	11(5.0)	2(0.9)	p = 0.861
CHF	184(21.1)	39(4.5)	p = 0.253	Diabetes	60(27.5)	10(4.6)	p = 0.877
Diabetes	235(26.9)	41(4.7)	p = 0.882	Hypertension	97(44.5)	13(6.0)	p = 0.401
Hypertension	365(41.8)	56(6.4)	p = 0.148	Malignant tumor	22(10.1)	5(2.3)	p = 0.443
Malignant tumor	95(10.9)	21(2.4)	p = 0.335	NAFLD	15(6.9)	3(1.4)	p = 0.709
NAFLD	44(5.0)	8(0.9)	p = 0.956	PCD	7(3.2)	1(0.5)	p = 0.916
PCD	19(2.2)	8(0.9)	**p = 0.033**	ASHN	12(5.5)	8(3.7)	**p < 0.001**
ASHN	47(5.4)	34(3.9)	**p < 0.001**	Kidney disease	81(37.2)	25(11.5)	**p < 0.001**
Kidney disease	365(41.8)	108(12.4)	**p < 0.001**	**Laboratory Data at admission**			
Laboratory Data				RBC(10^{12}/L)	3.88 ± 0.06	3.65 ± 0.14	p = 0.160
RBC(10^{12}/L)	3.91 ± 0.03	3.74 ± 0.07	**p = 0.027**	WBC(10^9/L)	13.01 ± 0.49	13.83 ± 1.51	p = 0.553
WBC(10^9/L)	13.52 ± 0.40	15.02 ± 0.86	p = 0.138	Platelets(10^9/L)	268.26 ± 10.25	215.07 ± 26.40	p = 0.056
Platelets(10^9/L)	252.88 ± 5.21	209.88 ± 11.57	**p = 0.001**	Neutrophils(%)	78.25 ± 0.86	76.80 ± 2.97	p = 0.557
Neutrophils(%)	77.44 ± 0.51	76.10 ± 1.66	p = 0.340	Lymphocytes(%)	11.91 ± 0.60	10.09 ± 1.35	p = 0.257
Lymphocytes(%)	12.41 ± 0.37	10.13 ± 0.80	**p = 0.016**	Monocytes(%)	4.68 ± 0.21	4.53 ± 0.45	p = 0.789
Monocytes(%)	4.48 ± 0.10	4.43 ± 0.33	p = 0.866	Eosinophils(%)	1.21 ± 0.14	0.59 ± 0.13	p = 0.077
Eosinophils(%)	1.23 ± 0.09	0.78 ± 0.13	**p = 0.048**	Basophils(%)	0.30 ± 0.03	0.28 ± 0.07	p = 0.768
Basophils(%)	0.31 ± 0.01	0.29 ± 0.06	p = 0.814	Hemoglobin(g/dL)	11.79 ± 0.18	11.25 ± 0.46	p = 0.263
Hemoglobin(g/dL)	11.93 ± 0.09	11.43 ± 0.22	**p = 0.032**	Calcium(mg/dL)	8.37 ± 0.09	7.84 ± 0.16	**p = 0.019**
Calcium(mg/dL)	8.30 ± 0.04	8.08 ± 0.11	p = 0.063	Sodium(mmol/L)	138.36 ± 0.38	138.10 ± 1.80	p = 0.826
Sodium(mmol/L)	138.08 ± 0.20	137.41 ± 0.65	p = 0.225	Potassium(mmol/L)	4.22 ± 0.06	4.39 ± 0.18	p = 0.304
Potassium(mmol/L)	4.17 ± 0.03	4.40 ± 0.09	**p = 0.007**	Chlorine(mmol/L)	102.88 ± 0.49	105.03 ± 1.54	p = 0.117
Chlorine(mmol/L)	102.76 ± 0.28	103.09 ± 0.71	p = 0.679	Anion gap(mmol/L)	16.59 ± 0.42	19.10 ± 1.44	**p = 0.036**
Anion gap(mmol/L)	16.71 ± 0.22	18.35 ± 0.61	**p = 0.004**	Bicarbonate(mmol/L)	23.09 ± 0.38	20.03 ± 1.32	**p = 0.006**
Bicarbonate(mmol/L)	22.96 ± 0.20	21.28 ± 0.56	**p = 0.002**	Phosphosate(mg/dL)	3.45 ± 0.12	4.52 ± 0.46	**p = 0.002**

(*Continued on next page*)

Table 1. *Continued*

MIMIC-III database				LCPH			
Overall population	Survivors N = 741	Non-survivors N = 132	p-value	Overall population	Survivors N = 188	Non-survivors N = 30	p-value
Phosphosate (mg/dL)	3.45 ± 0.06	4.28 ± 0.19	**p < 0.001**	ALT(IU/L)	183.99 ± 53.97	302.50 ± 106.89	p = 0.404
ALT(IU/L)	229.39 ± 34.08	239.28 ± 57.74	p = 0.907	AST(IU/L)	337.42 ± 114.08	854.93 ± 579.43	p = 0.159
AST(IU/L)	339.36 ± 58.29	525.77 ± 181.44	p = 0.238	ALP(IU/L)	148.66 ± 10.58	220.63	**p = 0.018**
ALP(IU/L)	151.85 ± 5.98	208.61 ± 31.68	**p = 0.004**	Amylase(IU/L)	432.88 ± 50.43	255.48 ± 89.68	p = 0.178
Amylase(IU/L)	384.66 ± 23.54	368.36 ± 47.45	p = 0.783	Lipase(IU/L)	999.37 ± 147.51	667.33 ± 201.02	p = 0.381
Lipase(IU/L)	834.32 ± 68.41	834.73 ± 180.80	p = 0.998	Magnesium(mmol/L)	1.86 ± 0.03	2.03 ± 0.10	**p = 0.046**
Magnesium (mmol/L)	1.86 ± 0.01	1.98 ± 0.04	**p = 0.001**	Iron(ug/dL)	52.07 ± 3.87	111.53 ± 16.41	**p < 0.001**
Iron(ug/dL)	49.61 ± 1.96	80.56 ± 6.17	**p < 0.001**	Glucose(mg/dL)	159.11 ± 8.33	195.78 ± 26.45	p = 0.118
Glucose(mg/dL)	158.15 ± 3.84	178.36 ± 9.95	**p = 0.044**	Free calcium(mmol/L)	1.11 ± 0.01	1.07 ± 0.02	p = 0.095
Free calcium(mmol/L)	1.09 ± 0.005	1.07 ± 0.013	p = 0.181	IBC(ug/dL)	198.29 ± 5.61	207.23 ± 17.05	p = 0.567
IBC(ug/dL)	204.94 ± 2.92	187.63 ± 8.14	**p = 0.025**	Ferritin(ug/L)	538.55 ± 37.81	1159.10 ± 381.86	**p < 0.001**
Ferritin(ug/L)	1826.68 ± 918.03	5584.84 ± 1994.37	p = 0.108	PT(s)	16.22 ± 0.63	18.18 ± 1.07	p = 0.233
PT(s)	15.93 ± 0.29	17.80 ± 0.60	**p = 0.011**	APTT(s)	31.50 ± 0.99	44.78 ± 4.67	**p < 0.001**
APTT(s)	31.72 ± 0.49	39.23 ± 1.63	**p < 0.001**	INR-PT	1.51 ± 0.09	1.84 ± 0.24	p = 0.180
INR-PT	1.52 ± 0.05	1.86 ± 0.12	**p = 0.008**	BUN(mmol/L)	25.40 ± 1.33	39.03 ± 5.23	**p < 0.001**
BUN(mmol/L)	26.98 ± 0.83	41.14 ± 2.60	**p < 0.001**	Creatinine(umol/L)	1.40 ± 0.10	2.07 ± 0.35	**p = 0.019**
Creatinine(umol/L)	1.65 ± 0.07	2.19 ± 0.18	**p = 0.002**	Lactate(mmol/L)	2.32 ± 0.14	4.86 ± 0.69	**p < 0.001**
Lactate(mmol/L)	2.46 ± 0.08	3.74 ± 0.32	**p < 0.001**	L-ALC at admission (<0.8x10^9/L) (%)	48(22.1)	22(10.1)	**p = 0.004**
Hyperchloremia(%)	200(22.9)	43(4.9)	**p = 0.187**	Hyperchloremia at admission (%)	44(20.1)	10(4.6)	**p = 0.242**
L-ALC (<0.8x10^9/L) (%)	248(28.4)	50(5.7)	**p = 0.325**				

BMI, body mass index; SOFA, Sequential Organ Failure Assessment; CHD, coronary heart disease; ALD, alcohol liver disease; NAFLD, non-alcoholic fatty liver disease; ASHN, acute &subacute hepatic necrosis; PCD, pulmonary circulation disease; CHF, congestive heart failure; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PT, prothrombin time; APTT, activated partial thromboplastin time; INR-PT, international normalized ratio-PT; BUN, blood urea nitrogen; Cr, creatinine; L-ALC, Low-Absolute Lymphocytes count. Data are n/N (%) or mean ± SE. Bold font means $p < 0.05$.

High level of serum iron in patients has strong association with mortality in all stages

We had tested the association of iron, ALP, and lactate and mortalities among all stage by lowess curve. Given such results, based on the quantity of patients, we further stratified iron, ALP, and lactate into 4 levels. Finally, we selected cut-off points of 21, 40.4 and 73.5 mg/dL (ALP and lactate shown in Figure S1). After adjust, we found, whether it is univariate analysis or multivariate analysis, when the serum iron value is greater than 73.5 mg/dL, it is closely related to the mortality of patients at various stages. And more important, this cut-off points predict value was also verified in LCPH data(The above-mentioned results were presented in Table2; Figures 1C–1H). We further used an ROC curve to compare the predictive ability of serum iron, SOFA score, and others ferroptosis-indicators in all stages of mortality. After combined serum iron, other indicators (ALP, lactate, anion gap, creatinine, phosphatase, BUN, hemoglobin (HGB), International Normalized Ratio_Prothrombin Time (INR_PT), lymphocytes, magnesium, SO2, PH, iron binding capacity (IBC), free calcium), and SOFA score, it improved the prediction ability obviously compared with sofa score only in all stage mortality. In-hospital (iron: AUC = 0.634, 95% CI 0.576–0.692; SOFA score: AUC = 0.7943, 95% CI 0.749–0.839, combination: AUC = 0.8419, 95% CI 0.805–0.879, Chi2 = 0.000) and 30, 60, 90-day results was shown in Figure 2A, 2C-2E. In LCPH, (iron: AUC = 0.743; sofa score: AUC = 0.812; combination: AUC = 0.959, Chi2 = 0.000) in Figure 2B, the ROC curve is almost overlaid with MIMIC-III one. When serum iron is combined with others ferroptosis indicators and sofa score, the value of predicting mortality can be remarkably improved.

Variables with significant differences ($p < 0.05$) in Table 1 were included in univariate Cox regression analysis also. The results showed that unadjusted iron was significantly associated with prognosis among all stages. Variables with $p < 0.10$ in the univariate Cox analysis were entered in the univariate Cox regression analysis. Except for ferritin and free calcium, other candidate variables showed a $p < 0.10$. Then,

Table 2. Results of univariate and multivariate logistic regression analysis for AP patients (in-hospital mortality)–(MIMIC-III database & LCPH)

	MIMIC-III database							LCPH					
	Univariable LR			Multivariable LR				Univariable LR			Multivariable LR		
Variable	OR	95% CI	p-value	OR	95% CI	p-value	Variable	OR	95% CI	p-value	OR	95% CI	p-value
Age	1.026	1.014–1.039	**0.000**	1.042	1.025–1.060	**0.000**	Age	1.034	1.006–1.062	**0.015**	1.052	1.010–1.095	**0.014**
BMI	1.034	1.011–1.056	**0.003**	1.039	1.010–1.068	**0.008**	BMI	1.091	1.003–1.186	**0.043**			
ICU-lasting	1.049	1.032–1.066	**0.000**	1.037	1.017–1.058	**0.000**	SO2	0.966	0.941–0.992	**0.012**	0.954	0.919–0.991	**0.014**
PO2	0.999	0.997–1.001	0.560				pH	0.001	0.000–0.026	**0.000**			
PCO2	0.995	0.981–1.010	0.526				SOFA	1.452	1.278–1.648	**0.000**	1.565	1.314–1.863	**0.000**
SO2	0.976	0.963–0.989	**0.000**	0.976	0.959–0.994	**0.010**	ASHN	5.333	1.965–14.474	**0.001**			
pH	0.048	0.012–0.192	**0.000**				Kidney disease	6.605	2.423–18.001	**0.000**			
SOFA	1.343	1.274–1.416	**0.000**	1.352	1.262–1.449	**0.000**	Calcium	0.644	0.446–0.929	**0.019**			
Severity of AP	1.422	0.961–2.103	0.078				Anion gap	1.057	1.002–1.115	**0.042**			
ALD	2.372	1.265–4.448	**0.007**				Bicarbonate	0.912	0.853–0.975	**0.007**			
ASHN	5.123	3.140–8.357	**0.000**				Phosphosate	1.308	1.087–1.573	**0.004**	1.656	1.221–2.246	**0.001**
Kidney disease	4.636	2.911–7.381	**0.000**				ALP	1.002	1.000–1.004	**0.027**	1.003	1.000–1.006	**0.023**
RBC	0.772	0.614–0.971	**0.027**				Magnesium	2.327	0.999–5.422	**0.050**			
WBC	1.010	0.996–1.025	0.176				Iron	1.011	1.006–1.016	**0.000**			
Lymphocytes	0.972	0.949–0.995	**0.017**				Ferritin	1.000	1.000–1.001	**0.024**			
Monocytes	0.995	0.933–1.060	0.866				APTT	1.035	1.012–1.059	**0.002**			
Neutrophils	0.994	0.983–1.006	0.340				BUN	1.025	1.009–1.042	**0.002**			
Eosinophils	0.846	0.726–0.985	**0.031**				Cr	1.260	1.025–1.549	**0.028**	0.522	0.325–0.838	**0.007**
Basophils	0.951	0.628–1.442	0.814				Lactate	1.363	1.171–1.586	**0.000**			
Platelet	0.997	0.996–0.999	**0.001**				Iron≤21				1		
Hemoglobin	0.920	0.853–0.993	**0.033**				21<Iron≤40.4				1.667	0.380–7.311	0.498
Sodium	0.981	0.950–1.012	0.225				40.4<Iron≤73.5				2.171	0.491–9.588	0.307
Potassium	1.292	1.070–1.561	**0.008**				Iron>73.5				9.616	2.619–35.304	**0.001**
Chlorine	1.006	0.982–1.031	0.642				L-ALC at admission	2.625	1.332–5.171	0.005	2.619	1.284–5.340	0.008
Calcium	0.866	0.745–1.007	0.063										
Anion gap	1.039	1.011–1.067	**0.005**										
Bicarbonate	0.950	0.920–0.981	**0.002**										
Phosphosate	1.228	1.125–1.341	**0.000**										
ALT	1.000	0.999–1.000	0.907										
AST	1.000	0.999–1.000	0.248										
ALP	1.000	1.000–1.002	**0.018**	1.001	1.001–1.002	**0.003**							

(*Continued on next page*)

Table 2. *Continued*

	MIMIC-III database							LCPH					
	Univariable LR			Multivariable LR				Univariable LR			Multivariable LR		
Variable	OR	95% CI	p-value	OR	95% CI	p-value	Variable	OR	95% CI	p-value	OR	95% CI	p-value
Magnesium	2.036	1.318–3.147	**0.001**										
Iron	1.008	1.005–1.011	**0.000**										
Glucose	1.002	1.000–1.003	**0.050**										
Amylase	0.999	0.999–1.000	0.783										
Lipase	1	0.999–1.000	0.998										
Free calcium	0.405	0.108–1.517	0.180										
IBC	0.997	0.995–0.999	**0.026**										
Ferritin	1.000	0.999–1.000	0.152	1.000	0.999–1.000	**0.027**							
PT	1.023	1.005–1.043	**0.015**										
APTT	1.026	1.015–1.037	**0.000**	1.018	1.004–1.032	**0.013**							
INR-PT	1.150	1.030–1.284	**0.013**										
BUN	1.019	1.012–1.025	**0.000**	1.016	1.005–1.028	**0.006**							
Cr	1.137	1.046–1.236	**0.003**	0.790	0.657–0.951	**0.013**							
Lactate	1.163	1.094–1.235	**0.000**										
Iron ≤ 21				1									
21<Iron ≤ 40.4				0.593	0.237–1.482	0.263							
40.4<Iron ≤ 73.5				1.018	0.460–2.251	0.965							
Iron>73.5				3.528	2.076–5.997	**0.000**							
L-ALC(<0.8)	1.212	0.826–1.778	0.325										

BMI, body mass index; SOFA, Sequential Organ Failure Assessment; ALD, alcohol liver disease; ASHN, acute &subacute hepatic necrosis; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PT, prothrombin time; IBC, Iron-binding capacity; APTT, activated partial thromboplastin time; INR-PT, international normalized ratio-PT; BUN, blood urea nitrogen; Cr, creatinine; L-ALC, Low Absolute Lymphocytes count. Data are n/N (%) or mean ± SE. Bold font means P.

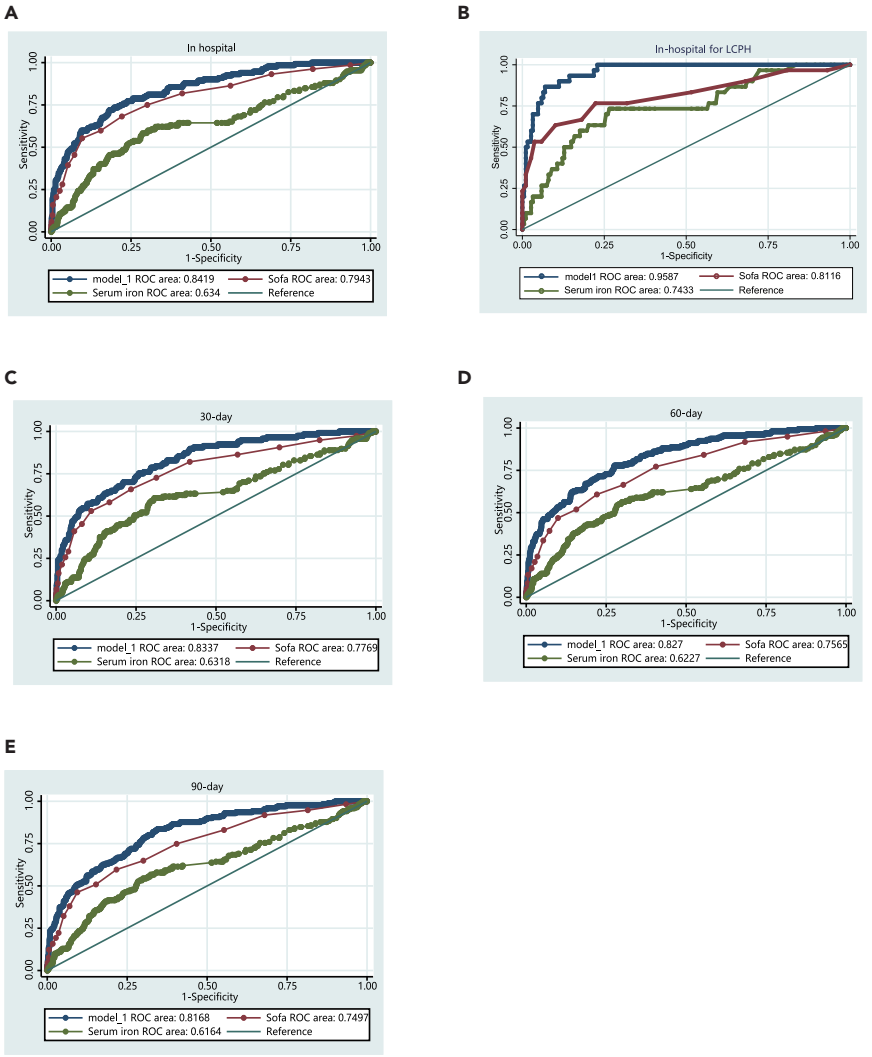

Figure 2. The prediction capacity of serum iron alone and combination of ferroptosis factors, compared with sofa score in AP patients

(A–E) ROC curves comparing serum iron and combination of ferroptosis factors with SOFA score for 30 (C), 60 (D), 90-day (E), and in-hospital (A,B) mortality; ROC, receiver operating characteristic; SOFA, Sequential Organ Failure Assessment. Green curve presented the serum iron, red curve presented sofa score, and blue curve presented the combination of ferroptosis factors (model 1). Model 1 means: combination of ALP, lactate, anion gap, creatinine, phosphatase, BUN, HGB, PT-INR, lymphocytes, magnesium, SpO_2, pH, iron binding capacity, free calcium.

they were admitted backward multivariable Cox regression analysis and excluded variables with a likelihood ratio test p-value of at least 0.05. In the final Cox regression model, same to logistic regression, iron (when iron>73.5 mg/dL) was an independent risk factor for mortality in patients with AP in all stage mortality. And this result was tested by LCPH data too (Results are summarized in Tables 3, S7, and S8). Survival curves were plotted using the Kaplan–Meier method for the four different iron levels in MIMIC-III data. The mortality rates of the four iron levels were, in-hospital for MIMIC-III (11.4%, 8.7%, 12.9%, and 27.3%; log-rank test $p < 0.0001$); (5.1%, 8.2%, 10.4%, and 34.0%; log-rank test $p < 0.0001$) for LCPH. The differences among the last group and the first three groups survival curves were statistically significant among all stages, as shown in Figures 3A–3E. Further analysis found that after ALP and lactate stratification, the last groups of them were significantly associated with AP patients' mortality for MIMIC-III (Figures S1A–S1H). However, we analyzed these three indicators in pairs and found that the iron-induced mortality was closely related to the concentration of lactate, not to ALP (Figures 3F and 3G).

Organelle markers, ALP (lysosome) and lactate (mitochondria); and ferroptosis indicators, serum iron, and iron binding capacity, has a solid association with prognosis of AP patients in critical illness.

The results of the multivariate regression model presented in Table 3 were used to establish a nomogram for MIMIC-III(Figures 4A and 4B). The nomogram contained all important independent factors predicting in-hospital, 30-day, 60-day, and 90-day mortality in the cohort. The nomogram indicates that ALP, sofa score, and age are the most important factors affecting prognosis, and it also includes lactate, BUN, iron

Table 3. Results of univariate and multivariate Cox regression analysis for AP patients (in-hospital mortality)—(MIMIC-III database & LCPH)

	MIMIC-III database							LCPH					
	Univariable CR			Multivariable CR				Univariable CR			Multivariable CR		
Variable	HR	95% CI	p-value	HR	95% CI	p-value	Variable	HR	95% CI	p-value	HR	95% CI	p-value
Age	1.025	1.013–1.036	**0.000**	1.034	1.021–1.048	**0.000**	Age	1.014	1.005–1.023	**0.014**	1.040	1.003–1.078	**0.035**
BMI	1.032	1.011–1.056	**0.002**	1.027	1.007–1.048	**0.008**	BMI	1.083	1.006–1.166	**0.033**			
ICU-lasting	1.031	1.021–1.041	**0.000**	1.016	1.003–1.030	**0.018**	SO2	0.972	0.951–0.994	**0.012**	0.962	0.927–0.998	**0.040**
PO2	0.999	0.997–1.001	0.347				pH	0.002	0.002–0.017	**0.000**			
PCO2	0.996	0.982–1.010	0.536				SOFA	1.350	1.255–1.451	**0.000**	1.195	1.039–1.374	**0.012**
SO2	0.977	0.966–0.988	**0.000**	0.987	0.975–0.999	**0.000**	ASHN	4.507	2.003–10.142	**0.000**			
pH	0.045	0.013–0.148	**0.000**				KD	6.027	2.306–15.749	**0.000**			
SOFA	1.285	1.239–1.332	**0.000**	1.233	1.176–1.293	**0.000**	Calcium	0.687	0.504–0.937	**0.018**			
ALD	2.279	1.330–3.904	**0.003**				Anion gap	1.057	1.009–1.107	**0.018**			
ASHN	4.101	2.762–6.091	**0.000**				Bicarbonate	0.912	0.859–0.968	**0.002**			
KD	4.105	2.636–6.394	**0.000**				Phosphosate	1.271	1.102–1.467	**0.001**			
RBC	0.795	0.645–0.981	**0.033**				ALP	1.002	1.000–1.003	**0.015**	1.003	1.001–1.005	**0.009**
WBC	1.007	0.997–1.017	0.150				Magnesium	2.366	1.143–4.897	**0.020**			
Lymphocytes	0.974	0.953–0.996	**0.019**				Iron	1.009	1.005–1.012	**0.000**			
Monocytes	0.999	0.942–1.059	0.963				Ferritin	1.000	1.000–1.001	**0.000**			
Neutrophils	0.995	0.984–1.005	0.329				APTT	1.024	1.013–1.034	**0.000**			
Eosinophils	0.852	0.737–0.986	**0.031**				BUN	1.023	1.010–1.035	**0.000**			
Basophils	0.947	0.640–1.400	0.785				Creatinine	1.234	1.054–1.444	**0.009**			
Platelet	0.998	0.996–0.999	**0.001**				Lactate	1.205	1.127–1.289	**0.000**	1.219	1.016–1.463	**0.034**
Hemoglobin	0.929	0.866–0.997	**0.040**				Iron≤21				1		
Sodium	0.978	0.949–1.007	0.134				21<Iron≤40.4				12.408	1.177–130.756	**0.036**
Potassium	1.253	1.062–1.477	**0.007**				40.4<Iron≤73.5				13.265	1.382–127.288	**0.025**
Chlorine	1.006	0.983–1.029	0.619				Iron>73.5				59.187	7.393–473.823	**0.000**
Calcium	0.872	0.761–1.001	**0.051**				L-ALC at admission	2.251	1.246–4.066	0.007	2.171	1.201–3.925	0.010
Anion gap	1.039	1.015–1.063	**0.001**										
Bicarbonate	0.948	0.921–0.976	**0.002**										
Phosphosate	1.188	1.112–1.270	**0.000**										
ALT	1.000	0.999–1.000	0.714										
AST	1.000	0.999–1.000	0.358										
ALP	1.001	1.000–1.001	**0.000**	1.001	1.001–1.002	**0.000**							
Magnesium	1.959	1.325–2.896	**0.001**										

(*Continued on next page*)

Table 3. *Continued*

	MIMIC-III database							LCPH					
	Univariable CR			Multivariable CR				Univariable CR			Multivariable CR		
Variable	HR	95% CI	p-value	HR	95% CI	p-value	Variable	HR	95% CI	p-value	HR	95% CI	p-value
Iron	1.007	1.004–1.009	**0.000**										
Glucose	1.001	1.000–1.002	**0.034**										
Amylase	1	0.999–1.000	0.835										
Lipase	1	0.999–1.000	0.963										
Free calcium	0.429	0.126–1.456	0.175										
IBC	0.997	0.995–1.000	**0.024**										
Ferritin	1.000	0.999–1.000	0.096	1.000	0.999–1.000	**0.018**							
PT	1.019	1.004–1.034	**0.011**										
APTT	1.018	1.012–1.025	**0.000**	1.014	1.004–1.025	**0.008**							
INR-PT	1.113	1.027–1.207	**0.009**										
BUN	1.014	1.010–1.019	**0.000**	1.014	1.004–1.023	**0.004**							
Creatinine	1.119	1.044–1.199	**0.001**	0.814	0.690–0.961	**0.015**							
Lactate	1.138	1.092–1.185	**0.000**										
Iron $\leq$ 21				1									
21<Iron $\leq$ 40.4				0.855	0.432–1.696	0.655							
40.4<Iron $\leq$ 73.5				1.177	0.618–2.241	0.619							
Iron>73.5				2.790	1.895–4.107	**0.000**							

BMI, body mass index; SOFA, Sequential Organ Failure Assessment; ALD, alcohol liver disease; ASHN, acute &subacute hepatic necrosis; RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; PT, prothrombin time; IBC, Iron-binding capacity; APTT, activated partial thromboplastin time; INR-PT, international normalized ratio-PT; BUN, blood urea nitrogen; Cr, creatinine; L-ALC, Low Absolute Lymphocytes count. Data are n/N (%) or mean $\pm$ SE. Bold font means $p < 0.05$.

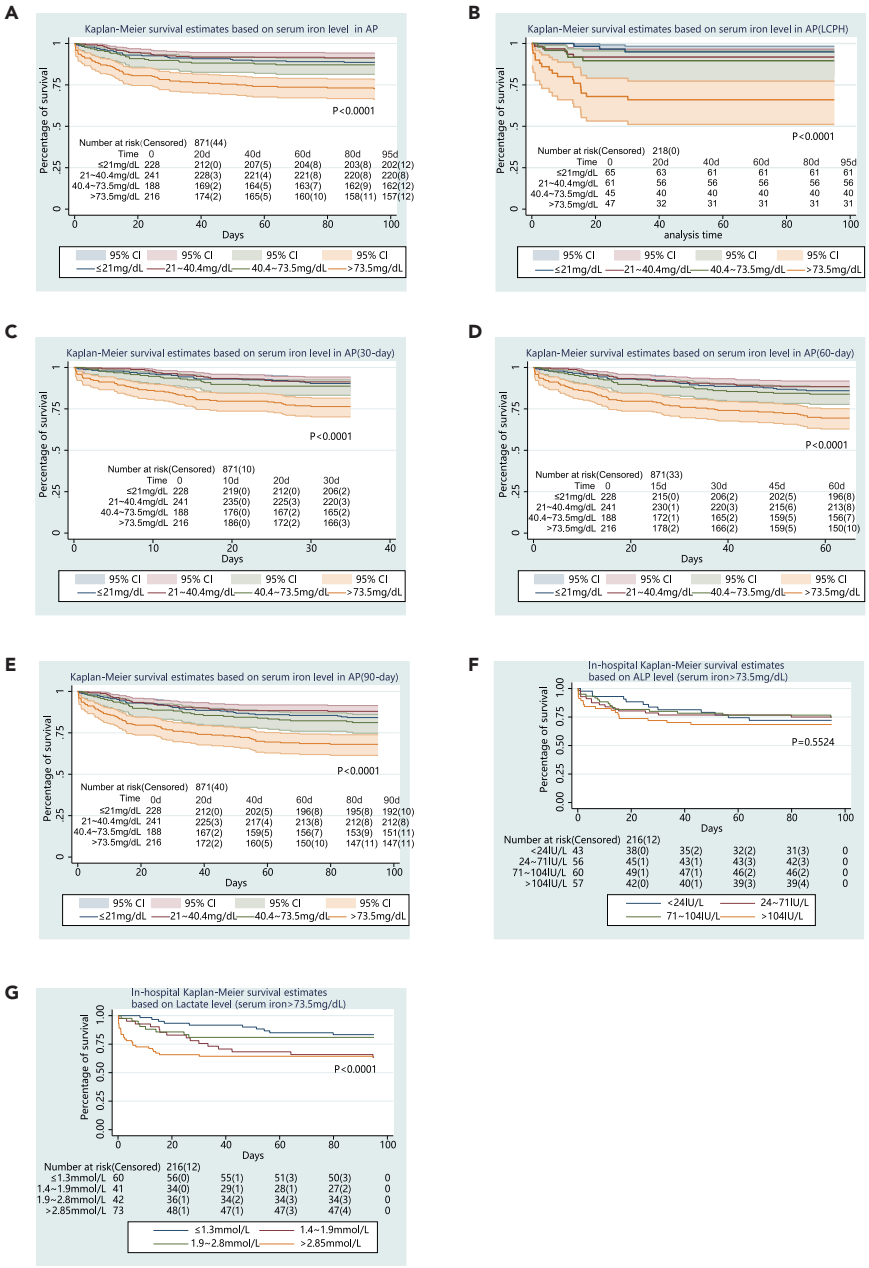

Figure 3. Kaplan–Meier survival analysis plot for all-time point overall survival for stratified serum iron levels and analyze the relation among ferroptosis factors in AP patients

(A–E) Kaplan–Meier survival analysis plot for overall survival for stratified serum iron levels in 30-day (C), 60-day (D), 90-day (E), and in-hospital (A, B).

(F and G) When serum iron concentration is above 73.5 mg/dL, the survival rate has no obviously difference among stratified ALP values (F), but it has strong positive association with stratified lactate values (G).

binding capacity, free calcium, and iron>73.5 mg/dL. The C-index analysis of the training cohort indicated that the nomogram provided high 30-day, 60-day, and 90-day survival C-indexes of 0.79 (95%CI: 0.75–0.83). The C-indexes exceed 0.7, and the calibration curve shows the good consistency between realistic model and ideal model (Figures 4C–4E). The DCA curves in Figure 6 display the large net benefits of the new model in predicting survival at 30-day, 60-day, and 90-day, and in-hospital compared with sofa score only (Figures 5A–5D). The NRI values at the 30-day, 60-day, 90-day, and in-hospital follow-ups were 0.938, 0.828, 0.803, and 1.028, respectively, in the combination model (sofa score, iron>73.5 mg/dL, ALP, lactate, and IBC). And they are 0.848, 0.773, 0.760, and 0.921, respectively, in sofa score only model.

The comparison among complications further confirmed the ferroptosis network interwoven by ALP, lactate, and serum iron in mortality in AP patients with critical illness.

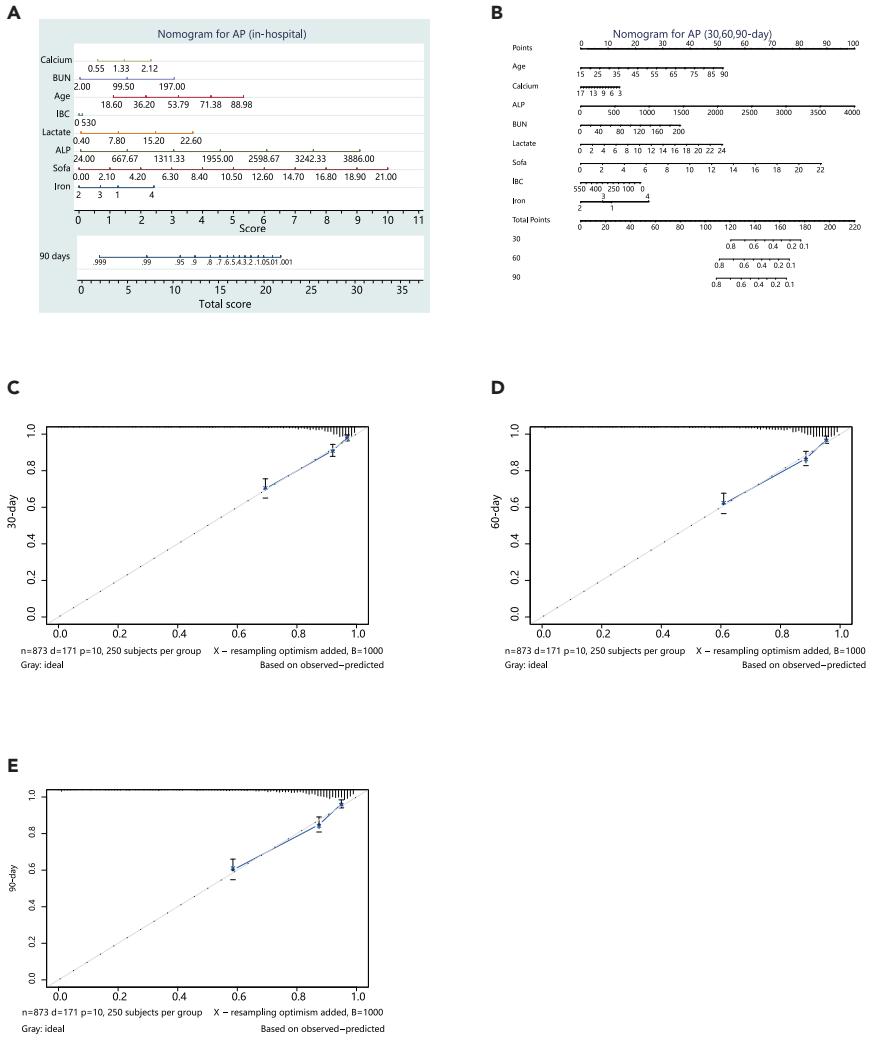

Figure 4. Nomogram predicting 30-, 60-, and 90-day, and in-hospital prognosis and calibration between the predicted probabilities and actual values in AP patients

(A and B) The point of each important ferroptosis factors was then summed up to obtain a total score that corresponds to a predicted probability of 30-, 60-, and 90-day (B), and in-hospital (A) survival at the bottom of the nomogram.

(C–E) Calibration plots showing the relationship between the predicted probabilities based on the nomogram and actual values of in 30 (C), 60 (D), and 90-day (E).

When we compared serum iron of AP patients in MIMIC-III database among different main comorbidities, serum iron was significantly increased in patients with chronic pancreatitis, ASHN, alcoholic liver disease (ALD), NAFLD, active hepatitis, and kidney disease (most of the p value is lower than 0.001) (Table S11); not in diabetes and all kinds of tumors. This again confirms the widespread existence of ferroptosis, which is basically consistent with the disease spectrum found in the current study. But iron binding capacity only obviously decreased in chronic pancreatitis ($p = 0.0218$) and kidney disease ($p = 0.026$) (Table S11).

Regardless of univariate analysis or multivariate analysis, ALP is closely related to the mortality of AP patients, and in the nomogram model, ALP accounts for a very high proportion of prognosis, almost equal to the sofa score (Figure 4A). After comparing in various complications, it was found that there was no statistically significant difference in the amount of ALP among these major complications, which further confirmed that the specific elevation of ALP was unique to AP.

NAFLD, in which ferroptosis plays an important role in its pathological process, has similar mortality risk factors to AP.

First, we compared the baseline data of the AP and NAFLD, and found that PO2, not SO2, was dramatically decreased in NAFLD ($p < 0.001$) (Table S2). This may be explained by that NAFLD patients are often accompanied by respiratory and circulatory diseases, so the basic value of SO2 is low. Even if in critical illness, SO2 will not drop too much.

As we thought, when we compared comorbidities (Table S12), we found that coronary heart disease and hypertension were significantly associated with NAFLD mortality, which further explained the differentiation on value of PO2.

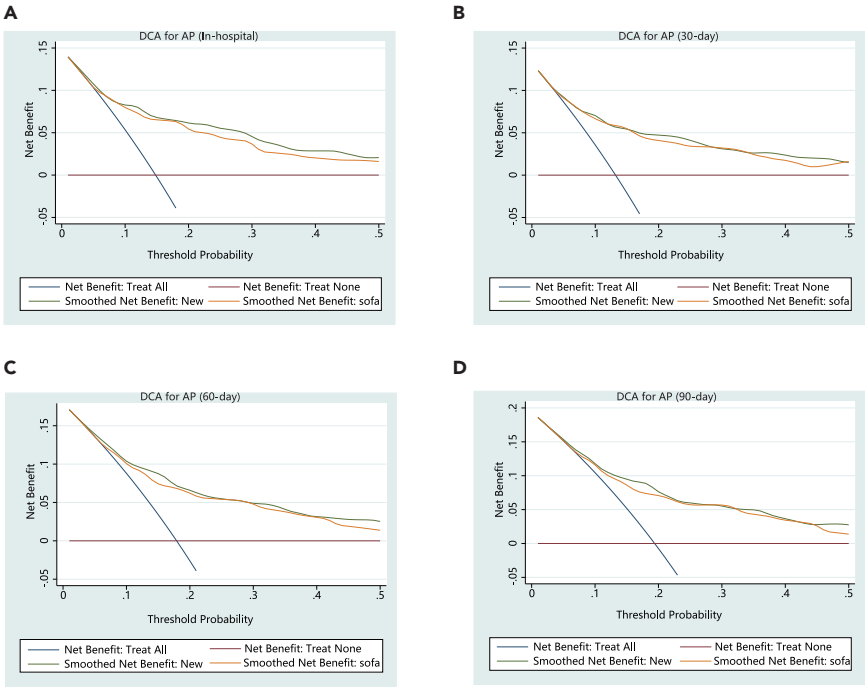

Figure 5. Decision-curve analysis about the benefit to interference ferroptosis factors compared with sofa score only in AP patients

(A–D) The abscissa is the threshold probability, and the ordinate is the net benefit rate.(A–D) The horizontal one indicates that all samples are negative and all are not treated, with a net benefit of 0. The oblique one indicates that all samples are positive. The net benefit is a backslash with a negative slope. 30 (B), 60 (C), 90-day (D), and in-hospital (A). The orange line represents sofa score only; the green line represents the combination of ferroptosis factors (New: ALP, lactate, BUN, iron binding capacity, free calcium, age, SOFA score, iron).

As for laboratory test, AP and NAFLD are also almost completely overlapped, except for a little bit of difference. Compared with serum iron, it can be found that the basic value of NAFLD is significantly higher than that of AP. Considering the long-term existence of ferroptosis in NAFLD patients, this difference can be explained. According to the comparison of the means value of serum iron of the two survival groups in AP and NAFLD, we set the NAFLD last group value to 104 mg/dL, and divided it into four groups evenly too. After univariate and multivariate analysis, we found that when serum iron exceeded 104 mg/dL, it was significantly associated with patient mortality (Figures 6A, 6B, and S2A–S2D; Tables S5, S6, S9, and S10). We also compared the ROC plot (Figures 6C and S3A–S3C) and KM curve (Figures 6D and S3D–S3F) between the two diseases and found that AP and NAFLD were highly similar after grouped by serum iron level. Further we found that Lactate, not ALP, still owns tight association with mortality stratified by serum iron as same as AP (Figures 6E and 6F). In the nomogram model, the correlations of various risk factors were also highly consistent (Figures 6G, 6H, and S4A–S4C). The C-index analysis of the training cohort indicated that the nomogram provided high 30-day, 60-day, and 90-day survival C-indexes of 0.786 (95%CI: 0.764–0.808).

ALP and lactate are the bridge between ferroptosis and main critical illness of AP and NAFLD, combination of them with serum iron could be an effective clinical intervene indicator to improve the prognosis of patients.

Usually, abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder, bones, Kidney tumors, and infections.[17] But as an important component of lysosome, ALP maybe also plays an important role in programmed cell death including ferroptosis. Through comparison, we found that the ALP values in AP and NAFLD patients were basically close at different time points in the survival group, but in the non-survival group, the mean ALP value of the AP group was significantly higher than that of the NAFLD group. That indirectly supports our conjecture: in AP, both iron and ALP are abundant at the same time in the blood suddenly, further aggravated ferroptosis and increased the mortality of patients. Moreover, we used an ROC curve to compare the predictive ability of ALP and or sofa score in all stages of mortality. When we combined all the related factors of AP death, including ALP, screened by multivariate regression, it improved the prediction ability remarkably compared with sofa score only in all stage mortality. The same comparison results were also obtained in NAFLD patients at 60-day, 90-day, and in-hospital mortality.

As an important substance in mitochondrial energy shuttling and metabolism,[18] lactate was also significantly elevated in AP and NAFLD patients. Univariate analysis found that there was a significant difference in lactate between the survival group and the non-survival group, whether in AP or NAFLD. The K-M curve adding with the lactate can also find that the survival of the patient is greatly reduced, which verifies the predictive potential of combination of lactate and serum iron, two related factors ignored by ferroptosis. As an independent factor or combined with ALP and serum iron, lactate has a high value in predicting and intervening in the prognosis of patients with AP and NAFLD critical illness. The ROC curve, K-M curve, nomogram, and DCA curve are displayed in Figures 4, 5, and 6.

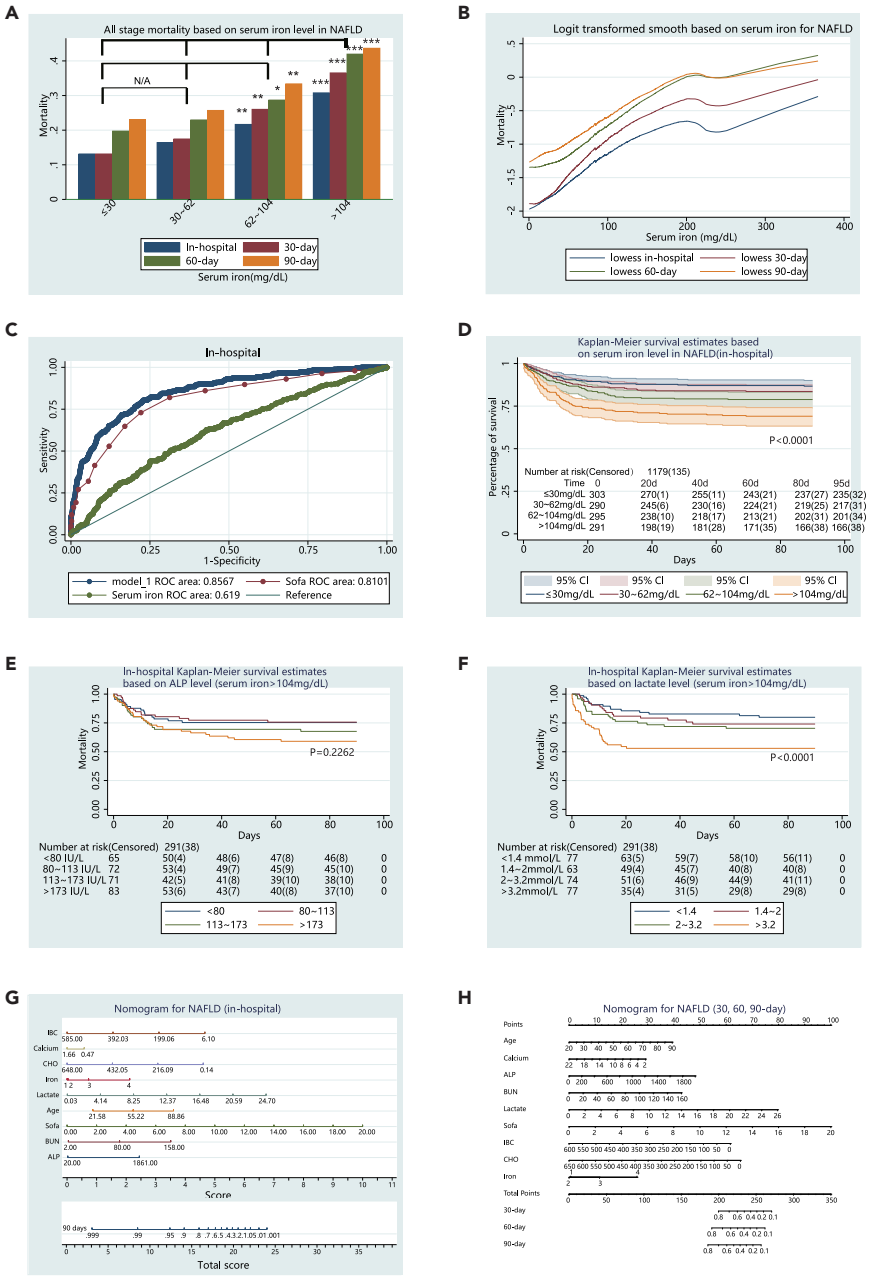

Figure 6. Based on same mortality and prognosis analysis, in NAFLD patients, ferroptosis factors presented the similar influencing pattern to AP patients

(A–H) Of 1188 NAFLD patients admitted to ICU were divided into 4 groups based on their concentration of serum iron (A); and its mortality changing trend in-hospital represented by lowess curves based on serum iron (B); ROC curves comparing serum iron and combination of ferroptosis factors with SOFA score for in-hospital (C) mortality; Kaplan–Meier survival analysis plot for overall survival for stratified serum iron levels in-hospital (D). When serum iron concentration is above 104 mg/dL, the survival rate has no obviously difference among stratified ALP values (E), but it has strong positive association with stratified lactate values (F). The point of each important ferroptosis factors was then summed up to obtain a total score that corresponds to a predicted probability of in-hospital (G), 30-, 60-, and 90-day (H) survival at the bottom of the nomogram. Model 1 means: combination of ALP, lactate, anion gap, creatinine, phosphatase, BUN, HGB, PT-INR, lymphocytes, magnesium, SpO_2, pH, iron binding capacity, free calcium. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$(The mortality of each of the two groups was compared. N/A indicates no statistical difference between the two groups).

DISCUSSION

As the main reason of ICU admission, detailed process of AP is still in the mist mostly.[19] This cognitive deficit greatly hinders clinical staging and corresponding treatment. Recently, many studies have found that ferroptosis plays a critical role in lots of pathological process, especially among chronic diseases,[20] but the study on relation between ferroptosis and AP becomes popular within 2–3 years.[21]

Serum iron, as a popular lab indicator, is commonly examined in malnutrition and blood system disorders. Although ferroptosis involves changes in the concentration of intracellular iron ions, little attention has been paid to the relationship between changes in serum iron concentrations and ferroptosis. Changes in serum iron are more often associated with sepsis. It is mainly due to that ferroptosis is caused by the accumulation of Fe^{2+} within the cells, while Fe^{3+} is the main form outside the cells. Furthermore, the common serum iron test cannot distinguish Fe^{2+} and Fe^{3+}, and this is the obstacle for the expansion of ferroptosis study. Therefore, we use AP and NAFLD, the two most common sources of critical diseases in digestive system diseases, to gradually verify our conjecture.

In AP status, especially suffered with severe AP, it will destroy large number of parenchymal cells in a short period of time, which provides a mechanistic feasibility for the release and accumulation of intracellular iron into the periphery.

The important role of ferroptosis in the pathogenesis of NAFLD has been confirmed recently.[14] When NAFLD patients progresses from chronic disease to acute critical status, this dramatic change in pathophysiology can also cause a large amount of parenchymal cell damage, which provides the frame of reference and supporting evidence to explore the role of ferroptosis in AP too.

By studying the MIMIC-III database and tested by LCPH ICU data, after multivariate regression analysis, we found that regardless of AP or NAFLD, the mean serum iron in the non-survival group at all stages was significantly higher than that in the survival group. Moreover, in view of the long-term existence of ferroptosis, the mean value in NAFLD was significantly higher than that of AP patients correspondingly. Combined with the decrease in pH in the non-survival group of these two diseases, these environments are conducive to the conversion of Fe^{2+}, released into the blood after tissue destruction, into Fe^{3+}, which then binds to ferritin and re-enters cells, then inducing the chain reaction of ferroptosis. The decrease in iron binding capacity in non-survival groups also further verified our speculation.

Due to the correlation between serum iron and infection,[22] and taking into account the impact of infection on such diseases, we further focused on infection-related indicators, but strangely, the total number of WBCs and the proportion of neutrophils had no obvious correlation between the two groups. Surprisingly, the proportion of lymphocytes was closely related to the mortality in all stages. Because of data limitations, we cannot determine which type of lymphocyte has an increased proportion, but the correlation between ferroptosis and specific lymphocytes has also attracted attention.[23] But the above-mentioned result could not be verified in LCPH data for its small number of patients. Based on recent research,[15] the absolute count of lymphocytes within seven days of onset has provided us with hints. Further research based on the patient data of LCPH found that all patients, admission within seven days of onset, found that the rate of L-ALC (<0.8) patients in the non-survival group was significantly higher than that in the survival group, and there was a certain correlation with ferroptosis. We think immune disorder is the bridge and reason. This also confirms the role of some biomarkers in the early onset of AP in prognosis. However, research based on the MIMIC-III database cannot yield corresponding results. We mainly consider that the selected patients cannot guarantee that the collected laboratory data is collected in the early stage of AP onset, so there is a deviation in the results.

In addition, we have also studied other early indicators that have a significant impact on the prognosis of AP, such as hyperchloremia.[24] Although there are significant differences in blood chlorine concentration, the ratio of hyperchloremia has no significant statistical significance for the prognosis of two groups patients. We consider that the lower number of people included and the lower average blood chlorine value are the reasons, thus further verification is needed from more populations and more centers in the future.

Interestingly, there was some difference in the spectrum of comorbidities strongly associated with mortality of both diseases. In AP and NAFLD, ASHN, and kidney disease is strongly associated with patients' mortality, that is exactly matched with what we expected, and verified in LCPH data too. Acute & subacute hepatic necrosis can cause a large number of hepatocytes to die in a short period of time, and hepatocytes are rich in iron, which could provide solid substrate support for the subsequent ferroptosis. Kidney disease, that has been verified its close relation to ferroptosis,[25] could lower the ferroptosis threshold in AP, and explain the mechanism by which BUN and creatinine are significantly associated with these patients' mortality also.

After clarifying the correlation between serum iron and mortality in critically illness patients with AP or NAFLD, we continued to analyze its impact on patients' prognosis. When we divided the serum iron of into four groups, and only when the serum iron was greater than 73.5 mg/dL (AP) or 104(NAFLD) had a clear impact on the prognosis of AP patients. The followed ROC curve and nomogram model further verified the statistically significant effect of serum iron on the prognosis of these, as a single factor. And more important, this cut-off value was verified its' predict ability in LCPH data. That shows the practical character of this model in another center.

Then, we continued to compare our model with AP related existing models and found the common predictive ability of BUN, age, creatinine, and Sofa score for mortality among all these models. However, so far, other models have different focuses and scope. Jiang[26] and Xu's[27] articles are also based on the MIMIC-III database, the former includes too few patients, while Xu uses TBIL as the grouping basis and paid more attention to changes in liver function. The patients selected by Shi[28] are mainly mild AP, which is significantly different from our model's prediction goals. Moreover, the above-mentioned models have not taken into account the effect of ferroptosis and have not comprehensively analyzed indicators such as serum iron, lactate, and ALP. Our model is a great supplement and extension to existing AP models.

The distribution of serum iron basal value in the two diseases also indirectly hinted the possibility of implication of ferroptosis also. Considering that ferroptosis is a long-standing pathological phenomenon in NAFLD, and AP appears suddenly, the basal value of serum iron in NAFLD is significantly higher than that in AP patients.

At this time, based on the cell organelle impairment theory, we found the correlation among ALP (lysosome), lactate (mitochondria), and these two diseases. As we all know, ALP, a marker of lysosome, is present in all tissues throughout the body, but is particularly concentrated in liver, bile duct, kidney, bone, intestinal mucosa, and placenta, and it is more linked with abnormal liver function,[29] biliary tract inflammation or obstruction.[30] Although some people have linked ALP with liver disease[31] and bone metabolism[32] as a related factor, none of them found that ferroptosis directly induced the increase of ALP, which in turn may produce the positive feedback, thus accelerating and aggravating ferroptosis. Regardless of multivariate or univariate analysis, ALP was closely related to the mortality of patients in various time periods. The nomogram model clarified the influence of ALP on the prognosis of AP patients, which is close to the ICU gold standard-SOFA score. The K-M curve divided by serum iron concentration showed a clear trend of change after ALP was added. The ROC curve, nomogram model, and KM curve also presented the degree of influence of ALP on the prognosis of NAFLD patients, similarly to that of AP patients.

Lactate, the metabolism marker of mitochondria, displayed the strong association with AP and NAFLD as well, but more remarkable in NAFLD. After statistical analysis, lactate showed its value on mortality, prognosis and intervening in AP and NAFLD, although not as special as ALP. But after K-M curve analysis, ALP is more of a parallel indicator of serum iron, reflecting the result of induced cell rupture and necrosis by ferroptosis. However, lactate and serum iron, as important intermediate substances within ferroptosis metabolism, should be linear indicators of the process of ferroptosis.

Above all, we proposed the potential association between elevated serum iron, ferroptosis and progress of AP and NAFLD under critical illness.

However, this study does have several intrinsic limitations. First, the samples enrolled in are too small, and it needs more patient records from other centers. Second, in a sense, these studies are more based on indirect data, although there are many related literatures to support, but still need to further excavate the underlying mechanism. Finally, we need better methods to identify and trace Fe^{2+}, ALP, and lactate in the cytoplasm, interstitial space, and blood, thus to discover the direct mechanism of ferroptosis in the progression of these two diseases.

Increased iron, lactate, and ALP is a simple but unlimited potentially useful biological marker characterizing severity in AP. This correlation suggests ferroptosis and follow-up cell programmed death may be a defining feature of this population. Furthermore, the above-mentioned indicators are easily acquired from common clinical data and could be used as an indicator in the screening and prediction at the epidemiological level. However, while our findings are limited by current shortage of understanding of AP in molecular scope, they require confirmation on a more common and solid medication status in future pervasive studies.

Limitations of the study

Due to the outdated MIMIC-III database, the lack of necessary imaging diagnostic criteria and diagnostic indicators corresponding to the severity of AP, and the difficulty in clarifying the interval between the onset time and admission time, the research results obtained from this database require further validation with more data and multi-center research results. Also, due to the small sample size of AP, we used the indirect internal validation by NAFLD. That affects the evaluation of results. Lastly, because the outdated mimic database accompanied without the time interval between admission and AP onset, we could not test the prediction value of serum iron level (sensitivity analysis).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107774.

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AUTHOR CONTRIBUTIONS

All authors reviewed and approved the final manuscript. F.L., X.Q., and Y.M. were responsible for the study concept; and Y.D., T.J., J.L., and F.L. for study design. Data extraction was undertaken by Y.D. M.L., T.J., and X.Q. were responsible for data analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
STATA/SE for Windows (version 15.1)	Stata Corporation	SCR_012763
R Project for Statistical Computing (version 3.6.1)	http://www.r-project.org/	SCR_001905
Other		
Linshui County People Hospital ICU database	Linshui County People Hospital	N/A
MIMIC-III database (version 1.4)	http://mimic.physionet.org/	SCR_017384

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Fuyao Liu (fuyaoliu@cqmu.edu.cn).

Materials availability

No reagent or material generated in this study.

Data and code availability

- The individual patient data reported in this study cannot be deposited in a public repository because these data are confidential medical records. To request access, contact Fuyao Liu, MD, PhD (fuyaoliu@cqmu.edu.cn) for de-identified summary data.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANTS DETAILS

Main data were acquired from MIMIC-III database (Free and public). Other data in this retrospective study was approved by the Institutional Review Board and Ethical Committee of Linshui Conuty People Hospital (LYLL2023100). Informed consent was waived by our Institutional Review Board because of the retrospective nature of our study. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. In Linshui Conuty People Hospital, all participants are Asian, other demographic information including age and gender are provided in Table 1.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Linshui County People Hospital.

METHOD DETAILS

Data sources

To test our hypothesis that alterations of serum iron might provide a more suitable signature of AP and NAFLD, we analyzed a publicly accessible critical care database named Medical Information Mart for Intensive Care III (MIMIC-III, version 1.4)[42] examining adults surviving in-hospital, 30, 60, and 90 -day following hospital admission for AP(ICD-9 code 5770) and NAFLD(ICD-9 code 5715, 5718, 5719, 5728, 5730, 5738, or 5739). And we also collect data of 218 patients aged ≥ 18 years with AP were admitted in Linshui Conuty People Hospital(LCPH) ICU from July 1, 2015 to July 31, 2021 as external validation. All the data were collected and collated by two independent researchers.

Participants

All patients with acute pancreatitis(MIMIC-III, LCPH) or NAFLD, the value of iron was available after admission to the ICU[15,24](The onset of AP was within 7 days, especially in LCPH), were included in the study. AP or NAFLD patients were defined as individuals meeting the following criteria: (1) ICD-9 diagnostic code containing the terms "Acute pancreatitis or Non-alcoholic fatty liver disease". Exclusion criteria were as follows: (1) <18 or ≥90 years; (2) usage of chalybeate (Figure 1).

Primary and others outcomes

The survival information of patients comes from MIMIC-III and Linshui Conuty People Hospital(LCPH) ICU. The primary outcome was defined as all-cause death in-hospital, whereas the others outcomes(for MIMIC-III data only) were defined as 30, 60, 90-day all-cause death after admission.

Sub-group assessment of ferroptosis and acute pancreatitis or NAFLD

We divided the patients into 4 groups evenly based on level of serum iron in AP and NAFLD. We then examined the relationship among serum iron, mortality, and prognosis in these groups combined with lactate, ALP, and others important ferroptosis markers.

QUANTIFICATION AND STATISTICAL ANALYSIS

Unit of iron and calcium is mg/dl. Variables with more than 80% missing values are deleted. The missing values are treated by using multiple imputation method. Kolmogorov-Smirnov test is used to evaluate the normality of continuous variables. Normally distributed variable was presented as mean $\pm$ standard deviation. Non-normally distributed continuous variable was presented as median [25th–75th percentile]. Pearson's χ 2 test was used for categorical variables, and the Mann–Whitney U test was used for non-normally distributed continuous variables. Student's t-test was used for variables with normal distribution. Cox regression model were used to evaluate the relationship between baseline value and the risk of mortality. Univariate and multivariate logistic regression analyses were used to identify independent risk factors for all time points mortality. Univariate and multivariate Cox regression analyses were used to assess the hazard ratio (HR) of serum iron with mortality. Survival curves were estimated using the Kaplan–Meier method and compared by the log-rank test. Receiver operating characteristic (ROC) curves were constructed, as well as the area under the curve (AUC). Harrell's concordance index (C-index) and the area under the receiver operating characteristic curve (AUC) were used to evaluate the predictive accuracy of the constructed nomogram. A calibration curve was used to evaluate the consistency between the predicted probabilities and the actual outcomes [15]. The clinical value of the prediction model was tested using decision-curve analysis (DCA). Statistical significance was set at $p < 0.05$ (two-sided). Data analysis was performed using STATA software (STATA/SE for Windows Version 15.1, Stata Corporation, College Station, TX, USA) and R software (version 3.6.1, CRAN).