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New atypical manifestations and prognostic factors of *Vibrio vulnificus* infection:

A 10-year retrospective study

Ruizhao Cai^{12#}, Miao Zhen^{12#}, Zhiguang Guan³, Menghe Li⁴, Qiangyun Liao⁵, Xing Liu⁶, Ying Wu⁷, Wen Lai⁸, Bin Shu^{12*}, Shaohai Qi^{12*}

1. Department of Burns, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong province, China.
2. Guangdong Engineering & Technology Research Center for Precise Diagnosis and Treatment of Burns and Wounds
3. Department of Burn and Plastic Surgery, The People's Hospital of Taishan, Taishan, Guangdong province, China.
4. Department of Burn and Plastic Surgery, GuangDong Yangjiang People's Hospital, Guangdong province, China.
5. Department of Burn and Plastic Surgery, Binhaiwan Central Hospital of Dongguan, Dongguan, Guangdong province, China.
6. Department of Burn and Plastic Surgery, Zhongshan City People's Hospital, Zhongshan, Guangdong province, China.
7. The Clinical Laboratory of Foshan Hospital of Traditional Chinese Medicine, Foshan, Guangdong province, China.
8. Department of Burns and Wound Repair Surgery, Guangdong Provincial People's

Hospital (Guangdong Academy of Medical Sciences), Guangzhou, Guangdong province, China.

, Contributed to this work equally.

* , Corresponding to:

Prof. Bin Shu,

Email: shubin29@sina.com

And

Prof. Shaohai Qi

Postal address: Department of Burns, 18 / F, Building 5, The First Affiliated Hospital of Sun

Yat-sen University No.58, Zhong Shan Er Lu, Guangzhou 510080, P. R. China

Tel: +86 13711537231

Email: qishh@mail.sysu.edu.cn

ORCID: 0000-0002-6945-4137

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Summary

Vibrio vulnificus (*V. vulnificus*) infection is rare but potentially fatal. This study explored the new atypical manifestations and prognostic factors of *V. vulnificus*-infected patients throughout hospitalization. we retrospectively reviewed 33 patients diagnosed as having *V. vulnificus* infection in Guangdong Province, China between 2010 and 2020. Medical records were analyzed. Multiple logistic regression and receiver operating characteristic (ROC) curve analyses were performed. New atypical manifestations were found, including cholangitis, urinary tract infection, and suppurative otitis media. Eleven of thirty-three (33.3%) *V. vulnificus*-infected patients died eventually. By univariate analysis, patients with cardio-cerebro-vascular diseases, lower platelet counts, higher levels of C-reactive protein and procalcitonin (PCT) had a statistically higher mortality. However, multivariate analysis showed that only PCT ($P = 0.036$) reached statistical significance. Also, the area under the ROC value estimate for PCT was 0.8816 (95% CI, 0.759-1.000; $P = 0.0009$). More than half of patients with *V. vulnificus* infection would die when $PCT > 20$ ng/ml, while no patient dies when $PCT \leq 20$ ng/ml. This study found new atypical manifestations of *V. vulnificus* infection. Also, PCT is an effective and independent predictor of mortality of *V. vulnificus* infection, and is suitable for clinicians to make early risk stratification and best therapeutic strategies.

Introduction

Vibrio vulnificus (*V. vulnificus*) is a halophilic opportunistic pathogen found

worldwide, primarily in warm tropical and subtropical waters (1,2). Infections with this pathogen are sporadic, but potentially life-threatening with a case-fatality rate ranging from 10% to 54% (3,4). *V. vulnificus* infections may progress rapidly and are generally acquired by consuming contaminated raw or undercooked seafood or exposing open wounds to contaminated seawater or shellfish (5). Susceptible people are those with chronic liver cirrhosis, diabetes mellitus, hemochromatosis, or immunocompromised medical conditions (5). Typically, these infections can result in three major manifestations: gastrointestinal diseases, primary septicemia, and primary wound infection (2,6). Other atypical presentations are pneumonia, osteomyelitis, eye contagions, meningitis, and endocarditis (7-10).

Besides early diagnosis of the disease, identifying the risk factors of mortality in patients with *V. vulnificus* infection as early as possible seems to be important (11). Previous studies demonstrated that leukopenia, higher counts of band forms of leukocytes, decreased platelet counts, and severe hypoalbuminemia on admission were associated with mortality in patients with *V. vulnificus* infection (12). However, no study reported the relationship between outcomes and laboratory results throughout hospitalization. Also, procalcitonin (PCT) has been proved to be an excellent biomarker of bacterial infections and guidance for antibiotic therapy during the last 20 years (13-15). No data have been reported regarding the new prognostic factor like PCT for patients with *V. vulnificus* infection.

Therefore, we conducted a 10-year retrospective study to describe the clinical features of *V. vulnificus* infection and to identify new prognostic factor throughout

hospitalization.

Materials and methods

Our study enrolled patients with *V. vulnificus* infection diagnosed by the mass spectrometry detection (VITEK MS, France) from January 2010 to July 2020.

Medical records of patients were collected from the First Affiliated Hospital of Sun Yat-sen University and 6 other hospitals in Guangdong Province, China, including the People's Hospital of Taishan, Guangdong Yangjiang People's Hospital, Binhaiwan Central Hospital of Dongguan, Zhongshan City People's Hospital, Foshan Hospital of Traditional Chinese Medicine, and Guangdong Provincial People's Hospital..

Demographic data, underlying medical conditions, symptoms and signs, the worst laboratory results throughout hospitalization, microbiological findings, treatment and outcomes were retrieved and analyzed. This study was approved by the research Ethic Committee of each hospital (ID: IIT-2020-019). Permission to examine medical records of patients with *V. vulnificus* infection was granted from each institution, and identifying information was erased to ensure patient privacy.

Descriptive statistics are shown as means with standard deviations for continuous data and as percentages for categorical data. statistical analyses were performed using GraphPad Prism 8 software package. P values with values of less than 0.05 were considered as statistically significant.

Results

Patient characteristics and clinical manifestations

Thirty-three patients with *V. vulnificus* infection were identified during the study

period, including two outpatients. The male patients were 72.7% (24/33). The average age of patients was 57.6 ± 18.6 years. Twenty-six patients had various kinds of underlying diseases. Sixteen had cardio-cerebro-vascular diseases. Six had a history of gout. Three had diabetes mellitus. Nine had liver dysfunctions, including three Hepatitis B virus infection, two cirrhosis, two fatty liver, one hepatic cell carcinoma and one intrahepatic calculi. Moreover, eleven patients (33.3%) had a history related to raw or undercooked seafood or contaminated seawater exposure (Table 1).

In keeping with other studies, the main manifestations were primary septicemia (36.4%, 12/33), primary limb infection (48.5%, 16/33) and gastrointestinal diseases (3%, 1/33) (3,6,16). Notably, a diagnosis of *V. vulnificus* infection was not considered at the time of admission in the remaining four patients. Two patients presented with jaundice having intensely yellow sclerae and skin. One patient manifested a suppurative otitis media and another suffered from kidney failure with uremia. Infection with *V. vulnificus* was not confirmed until two strains were isolated from bile samples, another one from middle ear secretions and another from urine, which had not been reported before (Table 2) (6).

Treatment and outcomes

Antimicrobial drugs were administered to all patients. Twenty-six patients (78.8%) were treated with cephalosporins or tetracyclines with or without carbapenems or quinolones. The remaining seven patients were given carbapenems or quinolones alone. All patients were initially treated with empirically chosen antimicrobial drugs, but subsequently received specific antibiotic therapy as directed by the results of their

antibiotic susceptibility profiles. Debridements were performed in nineteen patients (57.6%). Subsequently, eight of them (42.1%) had to go through an amputation. Other supportive treatments, including fluid resuscitation and intensive care, were also performed whenever needed.

Eleven of the thirty-three patients (33.3%) with *V. vulnificus* infection died eventually. Patients with primary septicemia had a higher case-fatality rate than those with primary limb infections (66.7% [8/12] vs 18.8% [3/16]; $P = 0.019$). The presence of cardiovascular diseases was related to mortality ($P = 0.01$). No death occurred in patients with other manifestations, including those with atypical presentations. There was no significant difference in outcomes between those treated with antimicrobial drugs alone and those treated with surgery combined with antimicrobial drugs. Also, there was no difference in outcomes between those treated with cephalosporins or tetracyclines and those treated with other types of antimicrobial drugs.

Multivariate analysis of clinical factors related to mortality

By univariate analysis, patients who died had lower platelet counts and higher levels of C-reactive protein and PCT compared with patients who survived. Moreover, patients with cardio-cerebro-vascular diseases had a higher case-fatality rate ($P = 0.01$). However, when those significant variables obtained from univariate analysis in Table 1 were subjected to multivariate analysis, only one variable, PCT (OR, 0.317; 95% CI, 0.108-0.929; $P = 0.026$) throughout hospitalization reached statistical significance (Table 3). The receiver operating characteristic (ROC) curve analysis of PCT was performed. The area under the receiver operating characteristic

(AUROC) value estimate for PCT was 0.8816 (Std Error = 0.0626; 95% Confidence Interval, 0.7589-1.000). And this AUROC value was significantly greater than 0.5 ($P = 0.0009$), indicating that higher PCT was an independent risk factor for mortality of *V. vulnificus* infection (Figure 1). Using Youden's index, the optimal cutoff estimate for PCT for predicting mortality was 20 ng/ml. A marked increase in case-fatality rate was observed when the $PCT > 20$ ng/ml, whereas the $PCT \leq 20$ ng/ml, the case-fatality rate was zero ($P < 0.0001$) (Figure 2).

Discussion

In this study, several atypical manifestations of *V. vulnificus* infection were reported for the first time in the literatures. Moreover, this is the first retrospective study to focus on the role of PCT for prognosis of *V. vulnificus* infection. Our study showed that PCT throughout hospitalization was a practical predictor of mortality. We also found that PCT provided good discriminatory power for predicting mortality of *V. vulnificus*-infected patients and identified an optimal cutoff level of 20 ng/ml for this indicator in the prediction model.

V. vulnificus infection is a potentially fatal marine-related disease (2,17). Besides the three main manifestations mentioned above, it should be noted that patients may also have atypical presentations including pneumonia following the aspiration of seawater (7), corneal ulcer (8), spontaneous bacterial peritonitis (6), endometritis after intercourse in seawater (9), meningitis (10), septic arthritis (18), osteomyelitis, epiglottitis (19) and infections of the testes (20) and spleen (21). Now our retrospective findings indicate that urethritis, cholangitis, and suppurative otitis media

can be added to the list of “atypical” infections caused by *V. vulnificus*. This confirms once more that *V. vulnificus* is an opportunistic pathogen that may cause a variety of clinical manifestations. One third of the patients had a history of exposure to raw/undercooked seafood or contaminated seawater. Most patients had underlying diseases, which probably mean they had weaker immune systems. These suggested that physicians should consider *V. vulnificus* infection in patients with a history of marine environment exposure and poor immunity.

PCT is a quickly measurable biomarker, assumed to have high sensitivity and specificity for sepsis and infection (13,14). It has been proved to be an excellent diagnostic and prognostic tool in infectious processes. A PCT value of > 1.0 ng/ml is indicative of bacteremia (13,15). However, we found that PCT was often much higher than 1.0 ng/ml throughout hospitalization in daily treatment of *V. vulnificus* infection, which hadn't been reported previously. In the present study, the value of AUROC for PCT exceeded 0.8, demonstrating that the level of PCT would be useful in predicting the outcome for patients with *V. vulnificus* infection. More than half of patients with *V. vulnificus* infection would die when $\text{PCT} > 20$ ng/ml, while fewer patients die when $\text{PCT} \leq 20$ ng/ml. This may help clinicians make a risk stratification of *V. vulnificus*-infected patients and treat promptly and accurately.

Some factors have already been identified as predicting mortality for *V. vulnificus* infection including advanced age, higher Acute Physiology and Chronic Health Evaluation (APACHE II), higher Mortality in Emergency Department (MEDS) scores, lower platelet counts on admission, and the presence of extensive skin lesions

(3,11,12). Previous studies showed that the APACHE II and MEDS scores were more representative and comprehensive than the other parameters, having good discriminative power for predicting in-hospital mortality in *V. vulnificus* patients with ROC curve analysis (11). However, the APACHE II and MEDS scoring system were not available in enough patients for analysis. And the level of PCT was found to be an effective alternative indicator for assessing the severity of *V. vulnificus* infection.

Though *V. vulnificus* isolates were susceptible to various kinds of antimicrobial drugs, some authors recommended doxycycline combined with third-generation cephalosporins for severe *V. vulnificus* infection (3,22,23). Others found that certain advanced fluoroquinolones may be an alternative choice (22,24). More than 70% of patients received cephalosporins or tetracyclines with or without carbapenems or quinolones in this study. There was no statistically significant difference in prognosis between patients treated with antibiotic alone and those treated with antimicrobial drugs plus surgery. Previous studies demonstrated that early surgical intervention after admission resulted in better prognosis (3,5). This may be partly due to selection bias, as patients with severe condition often delay the timing of surgery because of unstable vital signs. However, since *V. vulnificus* infection may progress rapidly and become fatal, we do believe that early surgery and antimicrobial drugs treatment are important.

There are some limitations in our study. First, our sample size is not large enough because of low incidence of infection. Moreover, the APACHE II and MEDS scoring system were not available in enough patients for statistical analysis. Also, the

effect of other newly identified infection biomarker such as presepsin was not assessed (15). Finally, some variables have wide CIs and this may cover up the statistical differences.

Conclusions

As more cases are reported, it is becoming evident that *V. vulnificus* may cause a variety of clinical manifestations, including cholangitis, urinary tract infection, and suppurative otitis media. Also, we demonstrated that PCT is an effective and independent predictor of mortality of *V. vulnificus* infection, and is suitable for clinicians to make early risk stratification and best therapeutic strategies.

Conflict of interest: None to declare

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Figure legends

Figure 1. Receiver operating characteristics curve for PCT for patients with *V. vulnificus* infection.

Figure 2. Frequency of PCT in relation to case- fatality for patients with *V. vulnificus* infection.

Table 1. Demographics, clinical features, and laboratory results of 33 patients with *V. vulnificus* infection.

Variable	All Patients (n=33)	Survivors (n=22)	Nonsurvivors (n=11)	<i>p</i>
Gender, male	24 (72.7)	14 (63.6)	10 (90.9)	0.212
Age (year)	57.6±18.6	55.6±21.4	61.7±11.0	0.282
Signs and symptoms				0.019
Septicemia	12 (36.4)	4 (18.2)	8 (72.7)	
Skin lesions	16 (48.5)	13 (59.1)	3 (27.3)	
Others	5 (15.2)	5 (22.7)	0 (0.0)	
Comorbid disease				
Cardio-cerebro-vascular disease	16 (48.5)	7 (31.8)	9 (81.8)	0.010
Gout	6 (18.2)	2 (9.1)	4 (36.4)	0.146
Diabetes mellitus	3 (9.1)	2 (9.1)	1 (9.1)	1.000
Laboratory findings				
WBC count ($\times 10^9/L$)	15.6±7.2	14.4±4.5	18.2±10.5	0.270
Hemoglobin level (g/L)	95.9±30.9	99.9±31.3	88.0±30.0	0.306
AST level (U/L)	253.6±431.6	133.2±319.9	483.4±533.3	0.066
ALT level (U/L)	130.1±174.1	74.8±77.8	246.1±255.9	0.066
CRP level (mg/L)	98.0±93.7	73.4±70.0	166.0±102.9	0.007
Platelet level ($\times 10^9/L$)	110.9±92.5	141.9±94.3	49.0±48.6	0.005

PCT level (ng/mL)	62.2±77.4	31.2±55.9	121.0±80.8	0.002
Serum albumin level (g/L)	26.9±7.6	28±7.8	24.7±6.9	0.258
Serum creatinine level (μmol/L)	190.8±160.8	154.3±160.5	257.3±145.2	0.088
Bacteremia	15 (45.5)	8 (36.4)	7 (63.6)	0.163
Therapeutic modality				1.000
Surgical intervention plus antimicrobial drugs	19 (57.6)	13 (59.1)	6 (54.5)	
Antimicrobial drugs alone	14 (42.4)	9 (40.9)	5 (45.5)	
Surgical treatment				0.391
Amputation	8 (24.2)	4 (18.2)	4 (36.4)	
Without amputation	25 (75.8)	18 (81.8)	7 (63.6)	

WBC, white blood cell (normal range: 4-10 ×10⁹/L); AST, aspartate aminotransferase (normal range: 1-37 U/L); ALT, alanine transaminase (normal range: 1-40 U/L); CRP, C-reactive protein (normal range: 0-10 mg/L); PCT, procalcitonin (normal range: 0-0.05 ng/mL); Hemoglobin level (normal range: 120-160 g/L); Platelet level (normal range: 100-300 ×10⁹/L); Serum albumin level (normal range: 35-50 g/L); Serum creatinine level (normal range: 53-115 μmol/L).

Values are presented as mean ±SD or as n (%)

Table 2. Characteristics of four atypical cases of *Vibrio vulnificus* infection.

Case no.	Age (yrs.)/ Gender	Month of symptom onset	Underlying disease	Sites of sampling	Estimated route of infection	Main symptoms	Antibiotics	Outcome
Suppurative otitis media								
1	23/F	12	Not evident	Secretions from REC	External ear canal	Suppuration, Hearing loss	Cefazolin	Recovered
Cholangitis								
2	59/F	3	Intrahepatic calculus, Post-cholecystectomy	Bile	Gastrointestinal tract	High fever, Yellow sclerae and skin	Sulperazone	Recovered
3	61/M	7	Lung cancer, Pancreatic carcinoma	Bile	Gastrointestinal tract	Yellow sclerae and skin	Tienam	Recovered
Urethritis								
4	30/F	6	Uremia, Hypertension, ANCA associated vasculitis, Post-subtotal Thyroidectomy	Urine	Urethral canal	Hematuria	Latamoxef, moxifloxacin	Recovered

REC: Right ear canal. ANCA: Anti-neutrophil cytoplasmic antibody associated vasculitis

Table 3. Prognostic factors of mortality by multivariate analysis.

Variable	Relative Risk	
	(95% Confidence Interval)	<i>p</i>
CRP level	0.699 (0.091-5.380)	0.609
PCT level	0.317 (0.108-0.929)	0.026
Platelet level	1.713 (0.292-10.048)	0.574
Cardio-cerebro-vascular disease	0.568 (0.009-35.853)	0.611
Signs and symptoms	11.499 (0.418-316.061)	0.107

CRP, C-reactive protein; PCT, procalcitonin.

CRP (1= 0-20, 2= 21-100, 3 > 100; normal range: 0-10 mg/L); PCT (1= 0-30, 2=31-70, 3= 71-110, 4 > 110; normal range: 0-0.05 ng/mL); Platelet (1= 0-100, 2= 101-200, 3 > 200; normal range: 100-300 ×10⁹/L); Cardio-cerebro-vascular disease (yes or no); Signs and symptoms (1= septicemia, 2= skin lesions, 3= others)