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**Fungal isolates in peritoneal fluid culture does not impact peri-operative
outcomes of peptic ulcer perforation**

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Abstract

Background

Fungal isolates from peritoneal fluid sampling in patients with Perforated peptic ulcer (PPU) is not uncommon and its management unclear. This study aims to evaluate if the presence of fungus in peritoneal fluid cultures is a predictor of morbidity and mortality following laparotomy for PPU.

Method

This is a single-centre retrospective study including adult patients with perforated gastric and duodenal ulcers over a 10-year period (January 2004 to January 2014). Evaluation of predictors contributing to fungal growth was conducted using multiple logistic regression analysis. Operative factors and 30-day mortality and morbidity outcomes were compared against fungal growth using a multivariate generalized linear mixed model analysis.

Results

The median age was 58 (IQR 44-70) years with 110 (20.3%) females. In addition to hypertension and hyperlipidemia, diabetes mellitus (13.5%), ischaemic heart disease (2.6%) and heart failure (2.4%) were common. Fungus was cultured from peritoneal fluid in 209 (38.6%) patients. Median American Society of Anesthesiology (ASA) score was 2 (IQR 2-3) and median Mannheim peritonitis index (MPI) score was 15 (IQR 10-20). Free air was detected in 323 (59.6%) patients and 52 (9.6%) patients had gastrectomy. Median length of stay was 7 (IQR 6-11) days. All cause complications was seen in 53 (9.8%) patients,

1 of which 37 patients (6.8%) developed intra-abdominal collection, 20 patients
2 (3.7%) had anastomotic leakage and 12 patients (2.2%) required repeat
3 operation. 30-day mortality was seen in 47 (8.7%) patients. Multivariate
4 analysis showed age (median age 64, IQR 53 – 74) as a significant predictor of
5 fungal growth ($p < 0.001$) but fungal growth not a predictor of adverse peri-
6 operative outcomes.

7

8 Conclusion

9 Fungal isolates in peritoneal fluid cultures are more likely to occur in older
10 PPU patients. Presence of fungal isolates does not impact peri-operative
11 outcomes.

12

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Introduction

Peptic ulcer disease has high prevalence. Bleeding and perforation are two commonly encountered complications. Perforated peptic ulcer (PPU) is a surgical emergency and omental patch repair is the most common procedure performed. Despite advances in critical care, understanding of sepsis and surgical techniques, peri-operative morbidity (20-50%) and mortality (1.3-20%) remain high (1-6), with sepsis the predominant contributor. At outset, PPU is a sterile phenomenon with gastric acid causing chemical peritonitis. With time, secondary infection with sepsis ensues (7). This is supported by evidence reporting worse outcomes in patients with delayed presentation (5,7). Hence, it is likely that the peritoneal fluid bacteriology evolves with time. PPU is classified as 'contaminated' or 'dirty infected' surgical procedure and empiric antibiotics are routinely prescribed with therapeutic intent. In PPU patients, peritoneal fluid culture is sampled and it is predominant source of microbial isolation. Evidence based standard clinical practice recommends changing empirical antibiotics when the culture reports are available. It remains the duty of acute care surgeons to follow local antibiogram and antibiotic stewardship program recommendations (8, 9).

While the management guidelines for bacterial isolate is clear, fungal isolates from the peritoneal fluid poses a clinical dilemma with regard to anti-fungal therapy. Fungal isolates could represent oral commensal flora and it is unclear if antifungal therapy should be administered in patients with fungal isolates. Furthermore, not all patients are subjected to fungal testing of the peritoneal

1 fluid. Fungal growth in peritoneal fluid cultures has been associated with poorer
2 surgical outcomes (10-16). Current mortality risk prediction models of PPU do
3 not include positive fungal isolates as part of its criteria (10). It is unknown
4 whether positive fungal cultures are merely an association or a predictor of
5 poorer outcomes. In 2003, a retrospective study by Shan YS et al including 145
6 PPU patients reported 43.4% incidence of positive fungal isolates and it being
7 an adverse risk factor for surgical site infection, length of hospital stay and
8 mortality (11). In a matched retrospective study from Taiwan, Li WS et al
9 reported that antifungal therapy did not improve outcomes including 30-day all-
10 cause mortality in PPU patients and antifungal therapy could be reserved for
11 patients who are critically ill and / or severely immunocompromised (12). In
12 2018, a retrospective study by Horn CB et al including 107 PPU patients
13 reported 25.2% administration of pre-operative antifungal therapy, 30.8%
14 patients having peritoneal fluid cultures tested of which 51.5% were tested
15 positive for fungus (13). The presence of fungal isolates did not impact length
16 of stay, intensive care unit stay, ventilator days, 30-day readmission rates or
17 intra-abdominal abscess formation. Routine pre-operative antifungal therapy
18 for all PPU patients is not evidence based and we do prescribe anti-fungal
19 therapy selectively. Due to conflicting reports, more evidence is needed. Our
20 study aims to evaluate if the presence of fungus in peritoneal fluid cultures is a
21 predictor of poor surgical outcomes. We hypothesise that fungus is a
22 contaminant of normal oral microflora, and a positive peritoneal fungal isolate
23 should not affect surgical outcomes of PPU.

24

25

1 **Methods**

2
3 A single-centre retrospective study is conducted including all adult patients with
4 perforated gastric and duodenal ulcers over a 10-year period (January 2004 to
5 January 2014). Electronic medical records were retrieved for ICD9
6 (International Statistical Classification of Disease) codes: 531.10, 532.10,
7 567.21, 567.29, 531.50, 532.50, 151.9 for primary or secondary diagnosis of
8 PPU, and ICD 10 codes (used in the hospital since 1st January 2012): gastric
9 ulcer (coded K25, subcategories 25.1, 25.2, 25.5 and 25.6) or duodenal ulcer
10 (coded K26, subcategories 26.1, 26.2, 26.5 and 26.6). Moribund patients,
11 patients who did not undergo surgery, patients in whom PPU could not be
12 confirmed intraoperatively and patients with perforation of other organs were
13 excluded from analysis.

14 Intra-operative peritoneal fluid sample is taken at the discretion of
15 operating surgeon. During office hours of weekdays, this is immediately
16 processed by microbiology laboratory. During weekends and after office
17 hours, this is processed at next immediate available opportunity. The
18 specimens obtained were processed for gram stain, bacterial cultures
19 (aerobic and anaerobic) and fungal smears. Isolates were identified based
20 on VITEK 2 system (bioMerieux). All isolates were sub-cultured on to
21 CHROMagar Candida (Becton Dickinson, USA). We did not record the
22 type of candida species separately however we have Candida albicans,
23 Candida tropicalis, Candida glabrata and Candida parapsilosis isolated.
24 Routine anti-fungal susceptibility testing is not done and is additionally
25 processed at the discretion of clinician. All patients who have yeast

1 isolated and are admitted to intensive care unit are managed by
2 antimicrobial stewardship programme and routinely intravenous
3 fluconazole 200-400mg/24 hour is prescribed depending on body weight
4 and creatinine clearance. Routine anti-fungal agent is not prescribed to
5 patients admitted to general ward. In patients who deviate from the
6 expected norms of recovery, an abdominal imaging was performed to
7 check for any intra-abdominal collections which in turn will be drained
8 and fluid tested for culture and sensitivity. Intravenous anidulafungin is
9 reserved to be prescribed strictly by infectious disease specialist.

11 Statistical analysis

12 Normal distribution of continuous variables was determined using the Shapiro-
13 Wilk normality test. Continuous variables were presented as mean $\pm$ standard
14 deviation or median with interquartile range (IQR) where applicable. Statistical
15 analysis was performed with the student's *t*-test or Mann Whitney U test for
16 continuous variables where appropriate and Pearson's Chi-squared test for
17 nominal variables to look for comparability in both groups. Evaluation of
18 predictors contributing to fungal growth was conducted using multiple logistic
19 regression analysis. Operative factors and 30-day mortality and morbidity
20 outcomes were compared against fungal growth using a multivariate
21 generalized linear mixed model analysis. Variables with a P value that is lesser
22 than or equals to 0.1 in the univariate analysis were included in the multiple or
23 multivariate model where applicable. All statistical analysis was performed
24 using SPSS version 23.0 (SPSS Inc, Chicago, III, USA). The level of statistical
25 significance was set at 0.05.

1 **Results**

2
3 A total of 673 patients were managed for PPU over the study duration. Seven
4 patients with missing data and 124 patients who did not have abdominal fluid
5 sent for culture were excluded. The remaining 542 patients were included in the
6 final analysis. The median age was 58 (IQR 44-70) years with 110 (20.3%)
7 females. Diabetes mellitus (13.5%), ischaemic heart disease (2.6%) and heart
8 failure (2.4%) were the most common comorbidities in addition to hypertension
9 and hyperlipidemia. Fungus was cultured from peritoneal fluid in 209 (38.6%)
10 patients.

11
12 Relating to operative factors and outcomes, the median American Society of
13 Anesthesiology (ASA) score was 2 (IQR 2-3) while median Mannheim
14 peritonitis index (MPI) score was 15 (IQR 10-20). Free air was demonstrated
15 on radiography in 323 (59.6%) patients and 52 (9.6%) patients required
16 gastrectomy. Median length of stay was 7 (IQR 6-11) days. All cause
17 complications was seen in 53 (9.8%) patients, of which 37 (6.8%) developed
18 intra-abdominal collections, 20 (3.7%) had anastomotic leakage and 12 (2.2%)
19 required repeat operation. After incident operation, 30-day mortality was seen
20 in 47 (8.7%) patients.

21
22 On univariate analysis, older age ($p < 0.01$) female ($p < 0.01$), use of steroids
23 ($p = 0.03$), chronic kidney disease ($p < 0.01$), low haemoglobin ($p < 0.01$),
24 elevated creatinine ($p < 0.01$), low albumin ($p = 0.09$) at admission, MPI score
25 ≥ 20 , ASA score ≥ 2 ($p < 0.01$) and higher physiology score ($p < 0.01$) predicted

1 presence of fungal isolates. On multivariate analysis, only age remained a
2 significant predictor for fungal growth (Table 1).

3

4 On univariate analysis, peritoneal fluid fungal isolates contributed to the need
5 for gastrectomy (versus omental patch repair) ($p < 0.01$), surgical intensive care
6 unit (SICU) admission ($p < 0.01$), ulcer size > 10 mm ($p < 0.01$), total length of
7 hospital stay > 9 days ($p < 0.01$), mortality ($p = 0.01$), intra-abdominal collection
8 ($p < 0.01$) and reoperation ($p = 0.04$). On multivariate analysis, none of the
9 outcomes demonstrated significance (Table 2).

10

1 **Discussion**

2
3 Our study has shown that old age is a predictor of fungal isolates from
4 peritoneal fluid and such isolates do not impact peri-operative outcomes.

5
6 Fungi are considered natural commensal organism of the gastrointestinal tract,
7 but in the setting of PPU, it remains unknown whether fungal growth in
8 peritoneal fluid cultures represents a contaminant or a true infection. Previous
9 studies have demonstrated that *Candida* species in the peritoneal cavity is
10 cleared without complications in most healthy patients and antifungal therapy
11 is not indicated as the mere presence of fungus does not affect outcome (14,
12 15). However, there is an increasing number of studies reporting significant
13 associations between fungal growth in peritoneal fluid and poorer clinical
14 outcomes (10, 16-18). There are also suggestions that only nosocomial fungal
15 peritonitis is associated with higher mortality but not community-acquired fungal
16 peritonitis (19). Regardless, the clinical significance of fungal growth in
17 peritoneal fluid remains unclear. The Surgical Infection Society and the
18 Infectious Disease Society of America recommends antifungal therapy in only
19 clinically severe cases and their recommendations are not based on strong
20 evidence (20, 21). Our study concludes that on multivariate analysis, presence
21 of fungal isolates does not impact morbidity or mortality in PPU patients
22 managed with emergency surgery at our institution.

23
24 In our study, 81.6% (524 out of 664) patients had their peritoneal fluid sampled,
25 and this is higher compared to 30.8% reported by Horn CB et al. (13). This is

1 significant as blood cultures may not have been done nor it may detect bacteria.

2 In our experience of managing patients with pyogenic liver abscesses, we have

3 shown that blood culture is positive in only 41.6% of patients (22). Hence, if

4 body fluids are not subjected to microscopy routinely in patients with suspected

5 sepsis, microbial therapy may not truly be optimized. The incidence of fungal

6 isolates in peritoneal fluid in our study is consistent with other reports. (15, 19)

7 We have previously validated several PPU risk prediction models against our

8 cohort. (23) The factors affecting mortality (female gender, liver cirrhosis, SICU

9 stay, ASA score > 2) demonstrate statistically significant association with fungal

10 growth on univariate analysis but not on multivariate analysis. In our study,

11 patients with fungal isolates were generally 10 years older than those without

12 fungal isolated and only old age was associated with fungal isolates. This is

13 interesting and could be due to various reasons. Older patients may have a

14 delayed presentation to hospital, are more likely to be taking chronic

15 medication, have increased prevalence of diabetes mellitus or may have a

16 larger ulcer. All of these factors could increase the possibility of oral commensal

17 flora to egress through the PPU and gain access to the peritoneal cavity. There

18 was no detectable difference with regard to delayed presentation to hospital or

19 association with diabetes mellitus. However,

20 our study did show a tendency for elevated creatinine and larger size of ulcer

21 in patients with fungal isolates. Both large size and elevated creatinine are

22 associated with poor outcomes in patients with PPU (7). It has been shown that

23 patients with fungal isolates had a tendency towards increased SICU stay

24 ($p=0.05$) (18). Also, more patients with fungal isolates needed SICU admission

25 and the length of hospital stay was higher for patients with fungal isolates, but

1 this was not statistically significant on multivariate analysis. It is known that
2 immunocompromised patients are predisposed to developing fungal infections
3 and in the present study, steroid use was significant on univariate analysis but
4 not on multivariate analysis (24, 25). This could be due to small sample size as
5 only eight patients (1.5%) were on steroids.

6
7 The larger the size of ulcer, the more likely the consideration of resection
8 surgery due to fear of omental patch leak (5). Gastric resection surgeries are
9 associated with increased morbidity and mortality as compared to classic
10 omental patch repair (23, 26). In our current study, gastrectomy was more likely
11 required in patients with fungal isolates; however, this was not significant on
12 multivariate analysis. This is consistent with other studies (27-29). In the local
13 context, this could be accounted for by lower co-morbidity, fewer patients with
14 pre-operative shock, and less patients with resection surgery.

15
16 The strengths of our study are large database, a decade of experience and
17 liberal policy of sending peritoneal fluid for culture. In addition, multivariate
18 analysis was done to reduce confounding variables. The weakness of our study
19 is that this was conducted in a single center, there were seven (1.9%) missing
20 records and 124 patients (18.4%) did not have peritoneal fluid sampled. In our
21 opinion, the results are unlikely to be influenced by such weaknesses which is
22 inherent of any retrospective study. Secondly, we have not made a distinction
23 between community acquired versus nosocomial PPU unlike some authors
24 (12). Lastly, we have not reported the type of bacterial or fungal isolates and
25 their sensitivity pattern. While our study does provide good evidence that fungal

1 isolates do not impact outcomes; we are unable to answer other questions,
2 such as patient selection for antifungal therapy, duration of antifungal therapy
3 and type of antifungal agent. These answers can only rightfully be obtained by
4 prospective controlled studies. With PPU being an acute pathology with
5 emergency presentation; we foresee that conducting such studies will be
6 challenging, and hence retrospective studies like ours are essential to enhance
7 the understanding and evidence base on this topic. We also caution readers
8 with generalization of our results as patient demography and clinical profile is
9 varied not only across continents but also different institutions within same
10 nation.

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1 **Conclusion**

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3 In conclusion, fungal isolates are more likely to occur in older patients, and
4 outcomes of PPU patients are not determined by the presence of fungal
5 isolates.

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1 **Conflict of interest**

2 No competing financial interests exist.

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1 **Tables and figures**

2 Table 1: Demographic predictors for fungal growth

3 Table 2: Operative factors and surgical outcomes affected by presence of

4 fungus