**KIDNEY RESEARCH**
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# Recent advances in novel diagnostic testing for peritoneal dialysis-related peritonitis

**Winston Wing-Shing Fung, Philip Kam-Tao Li**

*Department of Medicine and Therapeutics, Carol and Richard Yu Peritoneal Dialysis Research Centre, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, SAR, China*

Peritoneal dialysis-related peritonitis remains a significant complication and an important cause of technique failure. Based on current International Society for Peritoneal Dialysis guidelines, diagnosis of peritonitis is made when two of the three following criteria are met: 1) clinical features consistent with peritonitis; 2) dialysis effluent white blood cell count of >100 cells/μL; 3) positive effluent culture. However, early and accurate diagnosis can still be faulty, and emphasis has been placed on improving the timeliness and accuracy of diagnosis to facilitate early effective treatment. There have been advances in the novel diagnostic tests such as point-of-care molecular tests, genetics sequencing, mass spectrometry, and machine learning algorithm with immune fingerprinting. This article will discuss the latest evidence and updates of these tests in the management of peritoneal dialysis-related peritonitis.

**Keywords:** Diagnostic tests, Novel method, Peritoneal dialysis, Peritonitis

## Introduction

Peritoneal dialysis (PD)-related peritonitis (peritonitis) remains a significant complication and an important cause of technique failure and even mortality [1–3]. It is imperative to identify the organism causing peritonitis and to administer appropriate antibiotics early to minimize the risk of treatment failure [3–5]. Peritonitis has been identified as a core outcome of critical importance in the Standard Outcome in Nephrology Peritoneal Dialysis initiative [6].

Based on the International Society for Peritoneal Dialysis (ISPD) guidelines, diagnosis of peritonitis is made when two of the three following criteria are met: 1) clinical

features consistent with peritonitis (e.g., abdominal pain, cloudy dialysis effluent); 2) dialysis effluent white blood cell (WBC) count of >100 cells/μL or >0.1 × 10⁹ cells/L (after a dwell time of at least 2 hours), with >50% polymorphonuclear leukocytes (PMNs); and 3) positive effluent culture [7].

However, early and accurate diagnosis can still be a challenge because symptoms can be vague and the investigation results may not be readily available (e.g., during out-of-hours). Furthermore, the PD effluent culture may not always identify the causative organisms and is "culture-negative," ultimately affecting the guidance of choosing an effective antibiotic overall [8]. Because of the risk and the clinical impact of the delay in initiating antibiotics

for peritonitis, the ISPD currently recommends that empirical treatment be started as soon as peritonitis is suspected [7].

There have been some improvements in the timeliness and accuracy of diagnosing peritonitis over the years. In this review, three aspects of these novel diagnostic tests will be reviewed: confirmation of the diagnosis, identification of the causative pathogens, and risk stratification of treatment response. Overall, this article will discuss the latest evidence and updates for these important unmet needs in the management of PD-related peritonitis.

## Advances in confirmatory test and the advent of point-of-care testing

An ideal confirmatory test should have good diagnostic accuracy with high sensitivity, specificity, and predictive value. The test should be easy to perform, have a quick turnover time to minimize unnecessary delay, and be low cost and noninvasive. Thus, research has emphasized developing tests with improved accuracy and quick turnover time such as point-of-care testing. The PD effluent is traditionally sent for total cell counts with differentials after a minimum dwell time of 2 hours as the first investigation for suspected peritonitis. As mentioned, a WBC count of >100 cells/μL with >50% PMNs in the PD effluent is considered as a confirmation for peritonitis [7]. However, the total cell counts and differential counts in the PD effluent can vary greatly based on the dialysate fluid dwell time and the mode of dialysis [7,9]. Furthermore, the result may take some time to return and would cause a certain degree of delay. Thus, efforts have been made to investigate other means to detect signs of inflammation/infections other than the raised white cell counts in the peritoneal dialysate.

Leukocyte esterase reagents strips are a point-of-care test commonly used in urinalysis to detect the presence of leukocytes in urine, which may signify infection. These strips essentially detect the presence of esterase, which is an enzyme released by PMNs [10]. These strips have been reported to be useful in diagnosing spontaneous bacterial peritonitis, empyema, and meningitis [11–13]. Studies have also been done to assess whether these strips can be used to detect the presence of leukocytes in peritoneal dialysate [14–16]. Recently, Rathore et al. [17] assessed the efficacy of the strips to diagnose peritonitis in a prospective study

in a single center. They followed a total of 166 patients, and 21 patients were diagnosed to have peritonitis during the study period. Among these 21 cases of peritonitis, 20 were reagent-strip positive. This was compared to the 145 cases without peritonitis, of which only seven cases tested positive. Overall, the authors reported that the strips have a sensitivity, specificity, positive predictive value, and negative predictive value of 95.2%, 95.2%, 74.1%, and 99.3%, respectively [17]. These values were comparable to the diagnostic performance for cases based on clinical signs or total cell count. The sensitivity, specificity, positive predictive value and negative predictive value for cases based on clinical signs were 76.2%, 97.2%, 80%, and 96.6%, respectively, and the values for the total cell counts were 90.5%, 98.6%, 95%, and 98.6%, respectively [17]. A positive predictive value of 74.1% of the test is noted, which is lower than the 95% using total cell counts. One should note that the sample sizes are small, and studies with better designs would be needed to further assess the suitability of these reagent strips.

Apart from leukocyte esterase, several proinflammatory cytokines are possible biomarkers for diagnosing peritonitis. In particular, several nuclear factor kappa B (NF-κB) downstream mediators, such as interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2), have been examined [18]. IL-6 is present in the PD effluent and is a key regulator of acute peritoneal inflammation in response to infection [19]. Indeed, our group investigated the intraperitoneal level of IL-6 and COX-2 and found that patients with peritonitis had higher levels of COX-2 (4.97 ± 6.25 ng/mL vs. 1.60 ± 1.53 ng/mL, p = 0.007) and IL-6 (26.6 ± 17.4 pg/mL vs. 15.1 ± 12.3 pg/mL, p = 0.04) in the dialysate than those without peritonitis. There was also a significant correlation between number of episodes of peritonitis and IL-6 and COX-2 levels after 1 year [20]. Another cytokine implicated is the matrix metalloproteinase (MMP)-8, which is produced by activated neutrophils during acute inflammation and facilitates recruitment and trafficking of inflammatory cells [21]. MMP-8 has been detected in the PD effluent of polymicrobial peritonitis [22]. However, a cutoff value to increase their predictive efficacy is yet to be determined, which impairs these cytokines to be a useful biomarker. Therefore, it remains to have more large-scale clinical studies to determine a cutoff value and to assess the predictive efficacy of these proinflammatory cytokines as a diagnostic test in peritonitis.

Recently, a novel point-of-care device has been developed for rapid diagnosis of peritonitis. The test essentially detects the elevated presence of the two proinflammatory markers, IL-6 and MMP-8, in the peritoneal dialysate using a lateral flow assay. As the peritoneal fluid gets absorbed and has traveled through the nitrocellulose membrane in the device, the antibodies and the binding reagents situated at the detection line bind to the target markers, producing a visually interpretable result. The advantages of such a device are that it is easy to use and it only takes about 5 minutes to diagnose or exclude peritonitis [23]. Goodlad et al. [24] assessed the performance of this device in a real-world clinical environment, using a cohort of 107 PD effluent samples collected in their center. Among the 107 samples, 49 cases tested positive and 58 cases were negative using the device. Of the 49 device-positive cases, the device correctly identified 41 cases of peritonitis with the remaining eight cases being false positives. However, six of these cases had systemic sepsis with other causes of intraabdominal infection. Among the 58 device-negative cases, the device correctly identified 57 cases with the remaining one being a false-negative, which was likely a partially treated peritonitis. Overall, they reported that the device has a high negative predictive value of 98.3% (95% confidence interval [95% CI], 89.1–99.8) and positive predictive values of 83.7% (95% CI, 72.8–90.8); and high sensitivity and specificity of 97.6% (95% CI, 87.4–99.9) and 87.7% (95% CI, 77.2–94.5), respectively.

Given the high negative predictive value of 98.3%, the authors suggested that the test could offer a rapid exclusion of peritonitis for clinically healthy patients, minimizing waiting time and facilitating early discharge as well as sparing the use of unnecessary antibiotics. With symptomatic patients, a negative result may prompt investigation for an alternative diagnosis. However, follow-up studies are needed to further examine the utility of this device through day-to-day clinical practice and under various clinical situations (e.g., those with recurrent peritonitis, those with different dialysates like icodextrin, and those with chronic inflammatory disease).

Neutrophil gelatinase-associated lipocalin (NGAL), normally expressed by lymphocytes and renal tubular cells, is significantly increased in response to bacterial infection [25]. Studies also showed that severe acute peritonitis was associated with an increase of NGAL levels both in plasma and PD effluent [26–28]. Martino et al. [29] tried to validate the utility of using peritoneal NGAL levels for the diagnosis of peritonitis in their case-control study. They evaluated 182 patients during a study period of 19 months. Ninety-one patients had signs and symptoms of peritonitis and were allocated to the case group. The remaining 91 patients, who were scheduled for a routine visit, had no signs or symptoms of peritonitis and were designated as the control group. They then tested various biomarkers and compared them between the two groups. They found that the serum C-reactive protein (CRP) level, procalcitonin (PCT), peritoneal NGAL, and peritoneal WBC were all significantly different between the two groups, with the p-values of <0.001 for all markers. However, in the multivariate regression analysis model, only the WBC (odds ratio [OR], 24.84; p = 0.012) and peritoneal NGAL levels (OR, 136.6; p = 0.01) were independent predictors of peritonitis events [29].

The authors further tested the predictive efficacy of various biomarkers using the receiver operating characteristic (ROC) curve analysis. Both peritoneal WBC and the NGAL level have a very high area under the ROC curve (AUC) (0.973, p < 0.001 and 0.939, p < 0.001, respectively), suggesting a quite decent correlation for predicting peritonitis. Conversely, the AUCs of both CRP and PCT were only 0.719 (p = 0.048) and 0.754 (p = 0.042), respectively. To conclude, they suggested that the peritoneal NGAL may be a reliable diagnostic marker to predict peritonitis, especially when used as an adjunct to peritoneal white cell count. However, further study to test its utility in a real-life clinical setting would be needed. Furthermore, because peritoneal NGAL is influenced by local inflammation [30], studies to assess the relationship of peritoneal NGAL level in various inflammatory peritoneal states would be very helpful to distinguish local peritoneal inflammation during peritonitis from baseline local inflammation in the absence of acute peritonitis.

## Novel methods in pathogen identification

Currently, the standard method to identify the causative pathogens remains the sending of PD effluent to the microbiology laboratories for routine culture. Different culture methods include concentration, WBC lysis, and the BacT/Alert system (BioMérieux, Durham, NC, USA) [7,31]. However, these methods may take a few days before the respon-

sible organism is grown and identified [32]. They may take even longer and be difficult to grow if the bacterial numbers are small, unculturable, or fastidious or from patients who have recently received antibiotic therapy [33,34]. Thus, emphasis has been on other methods to rapidly identify the pathogens.

A well-established tool for rapid identification of bacterial species is 16S ribosomal RNA (rRNA) gene sequencing [35]. The 16S rRNA gene is highly conserved between different species of bacteria, yet it has hypervariable regions that provide a species-specific sequence useful for identification. The gene is also not prone to mutation [36]. Furthermore, the 16S rRNA gene sequence of almost all common bacterial pathogens has been studied and is readily available in the gene bank [37,38]. Overall, these findings make 16S rRNA an ideal target for sequencing as a unique fingerprint for pathogen identification.

Ahmadi et al. [39] evaluated this technique in the PD population. They assessed 45 patients with confirmed peritonitis and compared the pathogens identified using the molecular method to the conventional culture method. Among the 45 cases, the culture method identified 35 bacterial pathogens and two fungal organisms. Comparatively, bacterial DNA was detected in 38 cases and fungal DNA in two cases using the 16S rRNA gene sequencing technique, which showed similar pathogens identifications as the 37 samples from the culture method. This study suggested that the molecular method may be a useful emerging technique in concordance with the traditional culture method.

Recently, another study was done to further assess 16S rRNA sequencing following advances in next-generation sequencing [40]. The authors assessed 25 PD samples with peritonitis and similarly compared the molecular technique with culture-based assays. They defined the concordance as the co-occurrence of the same taxa in both culture and 16S amplicon sequencing assays at a level of less than 10% of the total population operational taxonomic unit for each sample. They showed that the majority of the 16S sequencing results gave an accurate representation of the main pathogens (at the genus level), achieving 80% concordance with bacterial strains identified in the culture-based assays. This was increased to 100% when the test was blinded for fungal and unculturable bacteria. They also reported that the technique was highly sensitive and could identify 33 different bacteria, compared to 13 bacterial species using culture-based techniques. In fact, they speculated that peritonitis may often be a polymicrobial disease [40].

However, as both authors noted, this molecular technique mainly identifies the pathogens at the genus level and may not be able to distinguish pathogens with high genetic similarity [39,40]. While high sensitivity may be an advantage over the traditional culture method, the lack of specificity remains a limitation, making the test difficult when trying to distinguish nondominant fastidious contaminant species from the dominant bacterial species. Thus, further tests with larger sample size, more taxonomically informative bacterial sequences (more 16S rRNA regions and/or other genes), and the ability to accurately assess antibiotic sensitivity loci are needed to assess the suitability for this identification technique. Regardless, 16S rRNA gene sequencing is an emerging rapid and direct method to identify the pathogen and may be useful as an adjunct to the standard culture method, especially in those who have recently received antibiotics and in culture-negative cases.

Another similar method is the detection of bacterial DNA using quantitative polymerase chain reaction (PCR). Johnson et al. [41] previously compared this technique to conventional culture-based method in 14 cases of peritonitis and found a correlation with the conventional method with a reported sensitivity of 67% and specificity of 79%. Another group further assessed the suitability of this method coupled with the electrospray ionization mass spectrometry (PCR/ESI-MS) [42]. This assay essentially uses tailored PCR primers capable of generating amplicons from over 95% of the species associated with human infection, including samples with high concentrations of background human DNA [43–45]. At the same time, an automated desalting and DNA debulking platform and an ESI-MS platform are used to prepare amplicons for mass spectrometry [43,46] and to discriminate amplicon sequence variants from different species present in a single sample [47,48]. Similar to a previous study, Chang et al. [42] compared this novel technique with the conventional culture-based method. Of the 21 samples of PD effluent from patient with peritonitis, PCR/ESI-MS identified microorganisms in 18 samples. When comparing the samples that are positive for both PCR/ESI-MS and culture, there is 100% concordance between the two techniques at the genus levels and an 87.5%

concordance when compared at the species level. They suggested that PCR/ESI-MS is a potential tool [42].

We tried to validate the suitability of PCR/ESI-MS for rapid bacterial identification in our cohort with a much bigger sample size (73 cases). Compared to the bacterial culture, the assay only identified 34.3% of the causative organisms correctly and failed to identify any organism in 52.1% of cases [49]. The method also identified a different organism in 8.2% of cases [49]. The reason for the poor performance is not entirely clear and requires further study in another cohort. Given the conflicting results and unless this discrepancy is clarified, PCR/ESI-MS should not be used for identifying the causative organisms from PD effluent in peritonitis, and other diagnostic methods should be explored instead.

Matrix-assisted laser desorption/ionization mass spectrometry with time-of-flight detector (MALDI-TOF MS) is another novel method that may be used in the identification of pathogens in peritonitis. It involves the generation of mass spectra from the cellular samples, which are then matched to the known bacterial database for reference [50,51]. It has already been recognized as a fast and reliable method in pathogen identification [52,53]. It is also a cost- and time-effective alternative to the 16S rRNA gene sequencing [54].

Lin et al. [55] assessed the use of MALDI-TOF MS in the PD population as they compared this technique to the conventional culture-based method. They assessed whether there is any difference in the time taken to identify the pathogen and whether the difference can be translated into any meaningful clinical outcome. Among the 155 episodes of peritonitis, MALDI-TOF MS was able to identify the causative organism much earlier than the conventional culture-based method by a difference of 64 hours (mean ± standard deviation [SD], 71 ± 37 hours vs. 135 ± 55 hours; p < 0.001) [55]. Comparing the clinical outcome showed no difference in response of peritonitis between the two groups. However, the authors showed that there was a significantly shorter length of hospital stay in the MALDI-TOF MS group than the culture-based group (overall mean ± SD, 8.2 ± 4.5 days vs. 5.2 ± 4.8 days; p < 0.001) [55]. Their results suggested the usefulness of this novel technique in pathogen identification, allowing an earlier pathogen identification and timely pathogen-directed antibiotic therapy.

These emerging techniques offer an attractive alternative to the conventional methods, especially in terms of better sensitivity and quicker turnover time. However, some of the tests may be too sensitive and lack specificity at present. More studies with larger sample sizes are needed to assess their suitability. Thus, it is unlikely that these would replace the conventional culture method in the near future; these novel tests should be used as an adjunct, especially when the culture is repeatedly negative.

## Advances in predicting relapses/assess response

Several advances have also been made in developing tests that can assess the response of peritonitis and predict the risk of relapse. Our group has assessed both bacterial-derived DNA fragments and bacterial endotoxin, which are implicated as possible predictors [56,57]. For the bacterial-derived DNA fragments, we assessed 104 patients with peritonitis, in which a serial level of bacterial-derived DNA fragments was taken every 5 days during the antibiotic treatment. We showed that patients with relapsing or recurrent peritonitis episodes had significantly higher levels of bacterial DNA fragment in PD effluent than those without relapsing or recurrence, both 5 days before (31.9 ± 3.4 cycles vs. 34.3 ± 3.0 cycles, p = 0.002) and on the day of the completion of antibiotics (32.3 ± 2.6 cycles vs. 34.1 ± 1.7 cycles, p < 0.001) [56]. Furthermore, when bacterial DNA fragments detectable by 34 PCR cycles 5 days before the completion of antibiotics are used as the cutoff, the test has a sensitivity of 88.9% and specificity of 60.5% for the prediction of relapsing or recurrent peritonitis [56]. However, the sample size of our study is small and done in a single PD center. Thus, external validity needs to be substantiated before the result can be extrapolated to other centers. Further studies are also needed for a more accurate quantification of bacterial DNA fragment level in PD effluent so that a reliable diagnostic cutoff can be identified before it can be validated for use in clinical setting.

We have also assessed the suitability of dialysate bacterial endotoxin as a prognostic indicator for treatment failure in peritonitis [57]. Similar to the previous design [56], we studied 325 episodes of peritonitis and collected the PD effluent every 5 days for endotoxin levels and WBC count. Endotoxin was detected in the PD effluent of 23 episodes only. Nineteen episodes were caused by gram-negative organisms, and four episodes were of mixed bacterial

growth. For peritonitis caused by gram-negative bacteria, a detectable peritoneal endotoxin level on day 5 had a sensitivity of 66.7% and a specificity of 83.3% for predicting primary treatment failure [57]. In contrast, peritoneal leukocyte count of >1,000/mm$^3$ on day 5 had a highly superior sensitivity of 88.9% and a specificity of 89.1%. There was no significant difference in the endotoxin level between completed cured patients cure and those who relapsed (p = 0.5) [57]. Essentially, our study showed that a detectable peritoneal endotoxin 5 days after antibiotic therapy might predict primary treatment failure in peritonitis episodes caused by gram-negative organisms, although it was inferior to peritoneal WBC count. Again, it is likely that our sample size is too small, with few peritonitis episodes, before one could draw any meaningful analysis. Therefore, studies with a larger cohort and multicenter design would be needed before endotoxins can be validated as a prognostic indicator for clinical use.

Serial monitors of dialysate cell counts have also been suggested as a useful predictor of outcome. Our group previously showed that the dialysate WBC count taken on day 3 was an independent prognostic marker for treatment failure after adjusting for conventional risk factors (hazard ratio, 9.03; 95% CI, 4.40–18.6; p < 0.0001) [58]. Using WBC count of >1,090/mm$^3$ on day 3 as the cutoff, the sensitivity and specificity were 75% and 74%, respectively, for the prediction of treatment failure. At that time, external validation was needed to confirm these associations.

Recently, a group from Thailand developed a risk prediction tool for stratifying PD patients with peritonitis into different risks for treatment failure [59]. From their analysis, four predictors of treatment failure were identified that included diabetes, systolic blood pressure of <90 mmHg at presentation, a dialysate leukocyte count of >1,000/mm$^3$ on days 3 to 4, and a count of >100/mm$^3$ on day 5. The adjusted ORs for each predictor were 1.81 (95% CI, 1.09–3.01; p = 0.022), 4.36 (95% CI, 1.72–11.09; p = 0.002), 2.52 (95% CI, 1.50–4.23; p < 0.001); and 43.64 (95% CI, 25.69–74.16; p < 0.001), respectively [59].

The group went on to develop a risk-scoring system that ranged from 0 to 11.5 using these four predictors with a specific assigned risk score: diabetes (1 score), systolic blood pressure of <90 mmHg at presentation (2.5 score), a dialysate leukocyte count of >1,000/mm$^3$ on days 3 to 4 (1.5 score), and a count of >100/mm$^3$ on day 5 (6.5 score). The

AUC for the prediction tools was 0.92 (95% CI, 0.89–0.94), and the p-value for the Hosmer-Lemeshow test was 0.93, indicating good agreement between observed outcome and predicted risk score [59]. For simplicity, the score was categorized into three risk group: low (<1.5), moderate (1.5–9), and high risk (>9) for treatment failure and the positive likelihood ratio for each risk group were 0.09 (95% CI, 0.05–0.15), 3.54 (95% CI, 3.03–4.12), and 25.16 (95% CI, 5.86–107.98), respectively. The observed treatment failure rate for each risk strata increased from 3.0% (95% CI, 1.6%–5.0%) in the low-risk group and 54.4% (95% CI, 48.8%–60.0%) in the moderate-risk group to 89.5% (95% CI, 66.9%–98.7%) in the high-risk group [59].

Overall, their risk-scoring system appears to accurately predict the risk of treatment failure and would be useful for risk stratification. Their result also validated our study and supported the use of serial dialysate cell count as a prognostic indicator of treatment failure. Large prospective external validation studies in different settings are needed to establish the transportability and generalizability of this prediction model.

## Future research and the emergence of artificial intelligence

Given there are major advances in the development of big data analysis and artificial intelligence (AI), emphasis should be placed on utilizing these innovations to facilitate the development of autonomous diagnostic tests that are both effective and efficient. An example would be the emerging concept of "immune fingerprinting" and the development of AI-assisted pattern recognition of these fingerprints through a machine learning algorithm. It has been noted that each type of pathogen may have a distinctive physiological and immunological pattern, and one of the objectives is to exploit these features to develop a rapid and effective diagnostic tool [60–62].

Zhang et al. [63] recently carried out a detailed analysis of dialysate in 83 patients with peritonitis. They identified a panel of local immune cells, inflammatory and regulatory cytokines and chemokines, and tissue damage-related factors that would constitute specific immune fingerprints for various pathogens. Using machine learning algorithms, they were able to identify and describe distinct patterns of immunological and inflammatory markers associated

with gram-positive and gram-negative organisms and with culture-negative peritonitis [63]. For instance, the combination of IL-15, IL-16, and soluble IL-6 receptor levels; total cell count; and MMP substrate turnover is associated with coagulase-negative staphylococcus infections, whereas a pattern of IL-1 beta, IL-15, MMP substrate, tumor necrosis factor-beta, and zymography is associated with enterococcal infection [63]. They concluded that these patterns were sufficiently immunologically distinct to allow a pathogen-specific diagnosis in PD patients, and that efficiency is enhanced by the machine learning algorithm [62,63]. The reported AUC based on the top five markers used specifically to distinguish each of the three pathogens of gram-negative, streptococcal, and coagulase-negative staphylococcal infections was all >0.9, suggesting these were highly predictive patterns [62,63]. Furthermore, they suggested that there are prognostic implications for some of the immune patterns because several immune markers are associated with technique failure [63]. This study demonstrated that immune fingerprinting is a viable and attractive adjunct in the diagnosis of peritonitis and might further promote the development of novel point-of-care diagnostic strategies in this field.

Taken together, the current ISPD diagnostic criteria for peritonitis remain reliable and indispensable. Nevertheless, these novel diagnostic and prognostic tests are promising and could certainly have impactful clinical applications in the future. Further studies are needed to assess the suitability of these emerging tests in real-world clinical settings.

## Conflicts of interest

Philip Kam-Tao Li received speaker honorarium from FibroGen, AstraZeneca, and Baxter. Otherwise, there is no conflict of interest to declare.

## Authors' contributions

Conceptualization: WWSF, PKTL
Project administration: PKTL
Writing-original draft: WWSF
Writing-review & editing: WWSF, PKTL
All authors read and approved the final manuscript.

## ORCID

Winston Wing-Shing Fung, https://orcid.org/0000-0001-7676-7628
Philip Kam-Tao Li, https://orcid.org/0000-0001-9879-8388

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