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Bowel Ischaemia was Associated with Elevated Lactate and Pyruvate in Peritoneal fluid: A Prospective Observational Pilot Study

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Rezumat

Ischemia intestinală a fost asociată cu valori crescute ale lactatului și piruvatului în lichidul peritoneal: un studiu pilot observațional prospectiv

Scop: Diagnosticul ischemiei intestinale (II) reprezintă o provocare, având în vedere caracterul nespecific al manifestărilor clinice, biochimice şi imagistice. Scopul acestui studiu a fost identificarea unor biomarkeri în lichidul peritoneal care ar putea fi utilizați pentru îmbunătățirea diagnosticului II.
Metode: Acest studiu este de tip observațional, prospectiv, monocentric şi a inclus pacienți adulți la care s-a practicat laparotomie pentru suspiciune de ischemie intestinală. Au fost analizate probe de ser preoperator şi de lichid peritoneal intraoperator. Pacienții cu perforație intestinală au fost excluşi.
Rezultate: Au fost identificați 69 de pacienți; 5 au fost excluşi din cauza perforației intestinale, iar 6 din cauza datelor incomplete. Dintre pacienții incluşi, 34 au prezentat ischemie intestinală intraoperator şi au fost alocați grupului cu ischemie, iar 24 nu au prezentat semne intraoperatorii de ischemie şi au constituit grupul de control. Grupul cu ischemie a prezentat valori mediane semnificativ mai mari ale lactatului (3,9 vs. 1,2 mmol/L; p = 0,002) şi piruvatului (190 vs. 114 μmol/L; p = 0,003) în lichidul peritoneal. De asemenea, au fost înregistrate valori medii semnificativ crescute ale leucocitelor serice (16,23 × 10^9/L vs. 9,77 × 10^9/L; p = 0,001), neutrofilelor (13,97 × 10^9/L vs. 7,03 × 10^9/L; p < 0,001) şi proteinei C reactive (95,56 vs. 53,42 mg/L; p = 0,039). Aria de sub curbă (AUC) a fost cea mai mare pentru lactatul din lichidul peritoneal (0,770), urmată de piruvat (0,751); analiza combinată a biomarkerilor din lichidul peritoneal şi ser a generat o AUC de 0,901.
Concluzie: Ischemia intestinală a fost asociată cu creşteri semnificative ale lactatului şi piruvatului în lichidul peritoneal. Introducerea unei analize combinate care să integreze aceşti biomarkeri din lichidul peritoneal şi din ser ar putea îmbunătăți diagnosticul ischemiei intestinale în practica chirurgicală.

Cuvinte cheie: ischemie intestinală, lichid peritoneal, biomarker, lactat, piruvat, laparotomie de urgență

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Abstract

Purpose: Diagnosing bowel ischaemia (BI) can be challenging with non-specific clinical, biochemical and radiological findings. We aimed to identify biomarkers in peritoneal fluid that could be utilised to enhance the diagnosis of BI.

Methods: A prospective single-centre observational study was conducted with adult patients undergoing laparotomy for suspected BI. Samples of preoperative serum and intraoperative peritoneal fluid were analysed. Patients with bowel perforation were excluded.

Results: Sixty-nine patients were identified; 5 were excluded for bowel perforation and 6 for incomplete data. Thirty-four patients had BI intraoperatively and were allocated to the ischaemia group; 24 did not have features of BI intraoperatively and were allocated to the control group. The ischaemia group had significantly higher median peritoneal fluid lactate (3.9 vs 1.2 mmol/L; p = 0.002) and pyruvate (190 vs 114 μmol/L; p = 0.003); as well as significantly higher mean serum white cell count (16.23 x 10^9/L vs 9.77 x 10^9/L; p = 0.001), neutrophils (13.97 x 10^9/L vs 7.03 x 10^9/L; p < 0.001) and C-reactive protein (95.56 vs 53.42 mg/L; p = 0.039). The area under the curve (AUC) was the greatest for peritoneal fluid lactate (0.770), followed by pyruvate (0.751); the composite AUC for these peritoneal fluid and serum biomarkers was 0.901.

Conclusion: BI was associated with elevated peritoneal fluid lactate and pyruvate. Introducing a composite analysis of these peritoneal fluid and serum biomarkers could improve the diagnosis of BI in surgical practice.

Keywords: bowel ischaemia, peritoneal fluid, biomarker, lactate, pyruvate, emergency laparotomy

Introduction

Bowel ischaemia (BI) is a surgical emergency requiring prompt diagnosis and treatment to prevent infarction, perforation, peritonitis and death (1). BI has an incidence of around 6 per 100,000 population and a mortality rate as high as 59% (2). Despite the considerable morbidity and mortality, diagnosing BI is often challenging due to non-specific clinical, biochemical and radiological findings (3,4). BI complications may be preventable with early diagnosis and treatment if the ischaemic insult is limited to mucosa. If treatment is delayed, mucosal ischaemia progresses to transmural infarction, leading to perforation, peritonitis and death (5).

A high degree of suspicion for BI is required in patients who present with acute abdominal pain that is out of proportion. There may be an embolic association such as atrial fibrillation but this is not ubiquitous (3). Despite extensive research, no clinically reliable biomarker for BI has been found due to poor sensitivity and specificity (5-8). Computed tomography (CT) is the preferred imaging modality for assessing BI, however the radiological features are inconsistent especially in the early phase (7,9,10).

Kobayashi et al found serosanguineous ascites in patients with strangulated obstruction (11). They utilised this finding to guide patient selection for laparotomy. They also found raised red cell count, haematocrit and lactate in peritoneal fluid in those with strangulated obstruction. We aimed to identify peritoneal fluid biomarkers that could be utilised either independently or in conjunction with serum biomarkers to enhance the diagnosis of BI.

Methods

This was a prospective single-centre observational pilot study conducted between January 2017 and December 2020 at a tertiary hospital in Canberra, Australia. The study inclusion criteria were adult patients over 18 years of age who were undergoing emergency laparotomy for suspected BI. The causes of BI encompassed vascular pathologies (i.e. arterial embolism; arterial or venous thrombosis; non-occlusive mesenteric ischaemia) as well as mechanical strangulations (e.g. adhesion; internal hernia; volvulus). Strangulated external hernias without an intra-abdominal component were excluded as they generally can be more reliably assessed for strangulation. Patients were also

excluded if there was preoperative or intraoperative evidence of bowel perforation or if they declined participation in the study.

Preoperatively, all patients received prompt clinical assessment from the surgical team, a blood test (full blood count and biochemistry) and an abdominal CT scan. Although arterial and portal venous phases were preferred for CT imaging, but they were not essential if a single-phase CT raised concerns for BI. The decision to proceed to an emergency laparotomy was made based on combined clinical, biochemical and radiological findings. An arterial blood gas was analysed at the start of the operation. A midline laparotomy was performed. A peritoneal fluid sample was collected immediately upon entering the peritoneal cavity and analysed for red cell and white cell counts, electrolytes, lactate, pyruvate, glucose and glycerol. The bowel was then examined for features of ischaemia, namely a dull appearance with loss of sheen, absent vasa recta pulsation and absent peristalsis (12,13). Patients with intraoperative findings of either reversible ischaemia or irreversible infarction were allocated to the ischaemia group. Patients who did not have BI intraoperatively were allocated to the control group.

Statistical Analysis

Statistical analysis was performed with SPSS Statistics (version 28.0.1.0). The distributions of the serum and peritoneal fluid biomarkers were analysed using the Kolmogorov-Smirnov test. Mean and standard deviation (SD) were derived for continuous variables with normal distribution. Median was used for continuous variables with skewed distribution. Student's t-test was used to assess the differences between the ischaemia and control groups; p-value < 0.05 was deemed as statistically significant. A receiver operating characteristic (ROC) curve was created to assess the diagnostic value of each statistically significant peritoneal fluid and serum biomarker. A calculated sample size of 51 patients was required (33 intervention and 18 control) ($\alpha = 0.05$, $\beta = 0.2$) (11).

Ethics Approval

This study was approved by the Institutional Health Human Research Ethics Committee (ETH 4.15.066).

Results

Sixty-nine patients were identified in the study period. Of these, 11 were excluded (5 due to bowel perforation and 6 due to incomplete data), leaving 58 for further analysis. Thirty-four patients had BI intraoperatively and were allocated to the ischaemia group; the other 24 did not have BI intraoperatively and were allocated to the control group. The patient characteristics are shown in *Table 1*. Although both groups had a similar mean age (69.88 ± 13.79 vs 69.92 ± 16.29 years); the ischaemia group had significantly higher median American Society of Anaesthesiologists score (4 vs 3; p < 0.001), greater rate of bowel resection (76.5% vs 8.3%; p < 0.001) and longer median postoperative intensive care unit (ICU) admission (3 vs 0 days; p = 0.02). The ischaemia group also had a higher inpatient all-cause mortality rate (20.6% vs 4.2%), but this was not statistically significant.

Preoperative blood test analysis is shown in *Table 2*. The ischaemia group had significantly higher mean serum white cell count (16.23±9.23 x 10^9/L vs 9.77 ± 4.83 x 10^9/L; p = 0.003), neutrophils (13.97 ± 8.98 x 10^9/L vs 7.03 ± 3.22 x 10^9/L; p < 0.001) as well as C-reactive protein (95.56 ± 88.37 vs 53.42 ± 54.88 mg/L; p = 0.047) (*Fig. 1*). The ischaemia group also had significantly lower mean serum pH (7.32 ± 0.11 vs 7.39 ± 0.08; p = 0.017) and higher mean serum glucose (8.49 ± 3.60 vs 6.50 ± 2.09 mmol/L; p = 0.032) on arterial blood gas analysis (*Table 3*). There was no significant difference in the mean serum lactate between the groups (2.72 ± 2.87 vs 2.53 ± 3.74 mmol/L; p = 0.831).

Peritoneal fluid analysis showed that the ischaemia group had significantly higher median lactate (3.9 vs 1.2 mmol/L; p = 0.002), pyruvate (190 vs 114 μmol/L; p = 0.003) and lipase (18 vs 12 U/L; p = 0.046), as well as higher lactate-to-pyruvate (p = 0.005) and lactate-to-glucose (p = 0.01) ratios (*Table 4*; *Fig. 2*). The median peritoneal fluid red cell and white cell counts, bilirubin, glucose and glycerol were all higher in the ischaemia group, but their differences were not statistically significant.

Table 5 illustrates the area under the curve (AUC), diagnostic threshold, sensitivity and false positive rate that have been calculated for each significant peritoneal fluid and serum biomarker. Peritoneal fluid lactate had the highest AUC (0.770) followed by pyruvate (0.751). Combining

Table 1. Patient characteristics, operative and outcome data.

	Ischaemia (N = 34)	Control (N = 24)	p-value
Gender (male:female)	17:17	7:17	0.113
Age (years)	69.88 + 13.79	69.92 + 16.29	0.993
ASA score			0.009
1	0 (0%)	2 (8.3%)	
2	1 (2.9%)	6 (25.0%)	
3	13 (38.2%)	11 (45.8%)	
4	15 (44.1%)	4 (16.7%)	
5	5 (14.7%)	1 (4.2%)	
Indication for laparotomy (cases)			
Arterial or venous thromboembolism	3 (8.8%)	0 (0%)	
Non-occlusive mesenteric ischaemia	7 (20.6%)	4 (16.7%)	
Adhesive small bowel obstruction	8 (23.5%)	12 (50.0%)	
Closed loop small bowel obstruction	5 (14.7%)	4 (16.7%)	
Internal hernia	0 (0%)	2 (8.3%)	
Strangulated or obstructed hernia	4 (11.8%)	1 (4.2%)	
Intussusception	1 (2.9%)	0 (0%)	
Large bowel obstruction	2 (5.9%)	0 (0%)	
Volvulus	4 (11.8%)	1 (4.2%)	
Bowel resection (cases)	26 (76.5%)	2 (8.3%)	< 0.001
Postoperative ICU admission (days)	3 (0 - 62)	0 (0 - 11)	0.029
In-hospital mortality (cases)	7 (20.6%)	1 (4.2%)	0.074

The data has been presented as mean + SD for age; and as median (IQR) for postoperative ICU admission.
Abbreviations: ASA, American Society of Anaesthesiologists; ICU, intensive care unit; SD, standard deviation; IQR, interquartile range.

Table 2. Preoperative blood test.

Serum biomarker	Reference range	Ischaemia (N = 34)	Control (N = 24)	p-value
Haemoglobin (g/L)	135 - 180 g/L	130.18 + 28.67	126.08 + 21.64	0.559
Red cell count (x 1012/L)	4.3 - 6.5	5.04 + 3.90	4.11 + 0.73	0.253
White cell count (x 109/L)	4 - 11	16.23 + 9.23	9.77 + 4.83	0.003
Neutrophils (x 109/L)	1.8 - 7.5	13.97 + 8.98	7.03 + 3.22	< 0.001
Potassium (mmol/L)	3.5 - 5.2	4.15 + 0.61	3.87 + 0.43	0.061
Creatinine (µmol/L)	60 - 110	100.48 + 52.97	81.75 + 30.92	0.127
Glomerular filtration rate	> 90	66.52 + 24.51	69.79 + 20.13	0.594
C-reactive protein (mg/L)	< 6.0	95.56 + 88.37	53.42 + 54.88	0.047

The data has been presented as mean + SD; and independent sample t-test for significance.

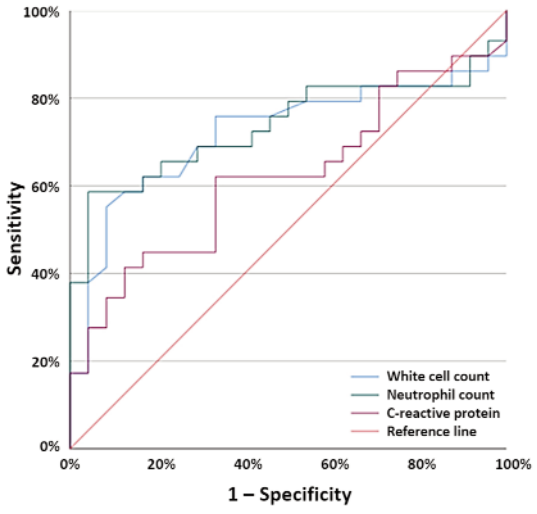

Figure 1. ROC curves for serum biomarkers.

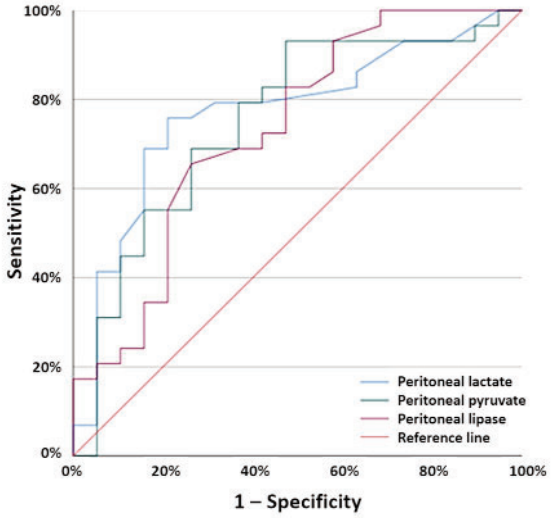

Figure 2. ROC curves for peritoneal fluid biomarkers.

Table 3. Preoperative arterial blood gas.

Arterial blood gas	Reference range	Ischaemia (N = 34)	Control (N = 24)	p-value
pH	7.34 - 7.44	7.32 + 0.11	7.39 + 0.08	0.017
Anion gap (mmol/L)	8 - 16	11.88 + 6.14	10.26 + 5.72	0.351
Potassium (mmol/L)	3.5 - 5.2	4.02 + 0.68	3.75 + 0.58	0.147
Lactate (mmol/L)	< 2.0	2.72 + 2.87	2.53 + 3.74	0.831
Glucose (mmol/L)	3.5 - 6.5	8.49 + 3.60	6.50 + 2.09	0.032

The data has been presented as mean + SD; and independent sample t-test for significance.

Table 4. Intraoperative peritoneal fluid.

Peritoneal fluid biomarker	Ischaemia	Control (N = 34)	p-value (N = 24)
Red cell count (x 10^6/L)	54,000 (603 - 1,239,000)	12,960 (790 - 675,000)	0.569
White cell count (x 10^6/L)	900 (0 - 153000)	355 (10 - 11700)	0.173
Potassium (mmol/L)	4.6 (1.6 - 9.7)	4.0 (1 - 13.5)	0.249
Bilirubin (µmol/L)	11 (2 - 40)	10 (2 - 73)	0.221
Lactate (mmol/L)	3.9 (0.5 - 27.9)	1.2 (0.3 - 16.7)	0.002
Pyruvate (µmol/L)	190 (35 - 578)	114 (19 - 657)	0.003
Glucose (mmol/L)	6.0 (0.3 - 17.5)	5.4 (0.3 - 15.8)	0.152
Glycerol (mmol/L)	0.29 (0.10 - 3.23)	0.23 (0.30 - 0.93)	0.932
Lipase (U/L)	18 (5 - 2781)	12 (4 - 49)	0.046
Lactate-to-pyruvate ratio	0.019 (0.007 - 0.11)	0.11 (0 - 0.04)	0.005
Lactate-to-glucose ratio	0.48 (0.10 - 44.33)	0.26 (0 - 4.63)	0.01

The data has been presented as median and IQR; and independent samples Mann-Whitney U test.

these significant biomarkers further increased the diagnostic value for BI to an AUC of 0.901 (*Fig. 3*).

Discussion

Early diagnosis and treatment of BI are crucial to reduce morbidity and mortality; however, an ideal biomarker facilitating early diagnosis of BI is still lacking. Several peritoneal fluid and serum biomarkers were evaluated in this pilot study. We found that BI patients had significantly higher serum white cell count, neutrophils, C-reactive protein, glucose as well as significantly lower serum pH. Serum lactate was not significantly raised in BI, thus reinforcing its shortcomings as a diagnostic biomarker. In comparison, peritoneal fluid lactate as well as pyruvate were significantly raised in BI.

Prolonged ischaemia activates a myriad of responses including increased production of reactive oxygen species and chemotaxis of polymorphonuclear leukocytes (14). This triggers an vicious production of proinflammatory mediators such as prostaglandins and leukotrienes, which promote further chemotaxis (14). This was supported in our study where leukocytosis and neutrophilia were observed in both serum and peritoneal fluid samples in BI. Paladino et al previously reported that leukocytosis was a predictor of mortality in BI (15). Nevertheless, leukocytosis and neutrophilia are not specific for BI; therefore, they should be considered with other diagnostic biomarkers. The diagnostic thresholds of white cell count and neutrophils for BI determined by this study were 9.90 x 10^9/L and 7.37 x 10^9/L, respectively.

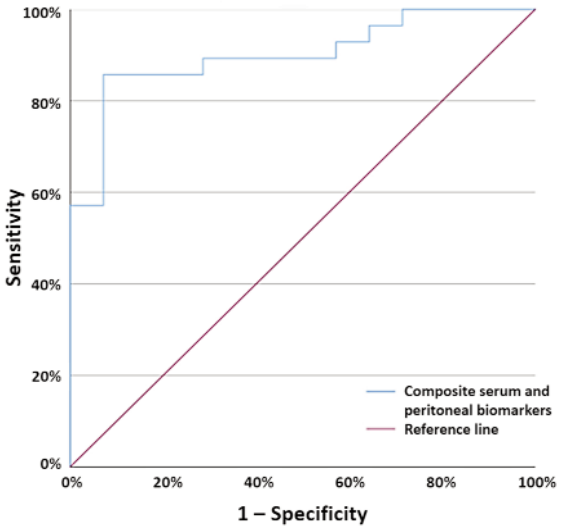

Figure 3. ROC curves for the combination of serum and peritoneal biomarkers.

Table 5. Potential candidate diagnostic biomarkers.

	AUC	SE	Diagnostic threshold	Sensitivity	False positive rate
Serum					
White cell count	0.720	0.073	9.90 x109/L	0.759	0.333
Neutrophil count	0.737	0.071	7.37 x109/L	0.724	0.417
C-reactive protein	0.621	0.077	18.9 mg/L	0.655	0.583
Arterial blood gas					
pH	0.697	0.075	7.4	0.706	0.579
Glucose	0.690	0.075	6.4 mmol/L	0.676	0.368
Peritoneal fluid					
Lactate	0.770	0.071	1.5 mmol/L	0.793	0.316
Pyruvate	0.751	0.075	140.5 µmol/L	0.690	0.263
Lipase	0.731	0.077	15.5 U/L	0.690	0.421

False positive rate calculated from "1 – specificity".
Abbreviations: AUC, area under the curve; SE, standard error.

Anaerobic metabolism under ischaemic conditions disrupts the energy-dependent sodium-potassium channels. This should lead to a rise in extracellular potassium. However, hyperkalaemia was not seen in BI in this study. Furthermore, there was no significant difference observed between serum and peritoneal fluid potassium levels in either group. One possible explanation is that the BI may have been confined to a segment that was too short to cause a detectable change in potassium levels in either serum or peritoneal fluid (16). Another explanation is that closed loop strangulation may have prevented a potassium shift into the systemic circulation to induce a measurable change. Renal clearance of electrolytes would have also contributed to upkeeping of potassium homeostasis and may have prevented hyperkalaemia (16).

Peritoneal fluid glucose was hypothesised to be low in BI due to anaerobic glycolysis of limited substrate, with raised pyruvate and lactate as the products (17). Although reduced peritoneal fluid glucose was not seen in this study, peritoneal fluid pyruvate and lactate were significantly raised in the BI patients. This pattern could have been driven by oxygen deprivation during ischaemia rather than glucose deficiency. The diagnostic thresholds of peritoneal fluid pyruvate and lactate determined were 1.5 mmol/L and 140.5 µmol/L, respectively. Glycerol is a degradation byproduct from cellular membrane phospholipids. The median peritoneal fluid glycerol in this study was not elevated in the BI patients. This suggested that glycerol lacks sensitivity as a biomarker, possibly due to cellular breakdown occurring as a late phenomenon in infarction and necrosis.

Kobayashi et al assessed the diagnostic value of peritoneal fluid in strangulated obstruction (11). They sampled peritoneal fluid preoperatively via diagnostic paracentesis, or intraoperatively during laparotomy, and reported serosanguineous fluid as a significant finding in strangulated obstruction. This study advanced on Kobayashi's finding by including all patients undergoing emergency laparotomy for suspected BI, regardless of its aetiology or the peritoneal fluid consistency, provided that the fluid was not contaminated from bowel perforation. This casted a broader net to identify peritoneal fluid biomarkers that are independent to the macroscopic appearance of the fluid. The objectivity of biomarker analysis removed inter-rater bias from visual assessment of peritoneal fluid, thus enhancing the reliability and generalisability of peritoneal fluid analysis.

The absence of reliable diagnostic biomarkers for BI was highlighted in current international guidelines and systematic reviews (7,18). Most biomarkers currently analysed in the clinical setting are serum-based and serve a corroborative rather than diagnostic role (7). Analysing peritoneal fluid biomarkers provide a novel approach to diagnosing BI. We propose patients whose assessment for BI is equivocal can be offered a paracentesis for peritoneal fluid analysis. The results can further guide the decision between emergency operation versus serial examination. With advances in modern endovascular techniques, peritoneal fluid analysis may also play a role in assessing bowel viability following early reperfusion, therefore potentially reducing unnecessary abdominal operations. Nevertheless, we can envision some barriers to implementing peritoneal fluid analysis. The volume and location of peritoneal fluid, and the expertise of clinician, could have a major influence on the safety and success of paracentesis. Kobayashi et al consistently found serosanguineous fluid intraoperatively in BI despite

preoperative paracentesis being unsuccessful in some cases. Improving training (standardising techniques) and facilities (incorporating ultrasound guidance) may help to overcome some of these barriers.

Study Limitations

We acknowledge several limitations in this study. The peritoneal fluid was sampled intraoperatively; therefore, the biomarker composition could have been influenced by prior resuscitation, anaesthesia and surgical trauma. Although the prospective study design would have likely matched some confounders between the groups, but the significant differences in American Society of Anaesthesiologists score and postoperative ICU admission could indicate further confounding variables that were not accounted for during the study. As this was a small-cohort single-centre pilot study, further larger scale studies are encouraged to validate these findings.

Conclusion

This pilot study identified peritoneal fluid lactate and pyruvate as significant novel biomarkers associated with BI. Performing a composite analysis with peritoneal fluid and serum biomarkers could enhance the diagnosis for BI and help to guide the decision for surgery.

Authors' Contributions

LongHai Jin – writing and editing; investigation. Rong Xian Chia – writing and editing; data curation; data analysis. Miho Mugino – investigation. Krishanth Naidu – investigation, editing. Eleni Baird-Gunning – investigation. Alice Richarson – data analysis. Soon-Ngee Lau – editing. Sivakumar Gananadha – conceptualisation; writing and editing; supervision.

Conflicts of Interest

All authors declare that they have no conflict of interest.

Consent

Consent for publication has been provided by all patients and authors of the study.

Informed consent has been provided by all patients and/or legal guardians for the study.

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Availability of Data and Materials

Data and materials can be provided upon request. Please contact LongHai Jin.

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