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Use of plasma DNA to predict mortality and need for intensive care in patients with abdominal pain

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ABSTRACT

Background: We investigated the value of plasma deoxyribonucleic acid concentrations in patients presenting with acute abdominal pain to predict need for intensive care or mortality.
Methods: Plasma deoxyribonucleic acid taken from patients with acute abdominal pain was analyzed for the *β-globin* gene using the quantitative polymerase chain reaction. The primary outcome measure was the combined 28-day mortality or admission to the intensive care unit.
Results: Of 287 consecutive patients with acute abdominal pain recruited, 12 patients were admitted to the intensive care unit and/or died. Median plasma DNA concentrations were higher in patients with cancer and major organ inflammation. Mean plasma DNA concentrations were three-fold higher in patients with systemic inflammatory response syndrome, five-fold higher in patients who died within 28 days, and eight-fold higher in patients admitted to the intensive care unit. The area under the receiver operator curve for plasma DNA concentrations and intensive care unit admission/mortality was 0.804. At a cut-off of 1100 GE/ml, the sensitivity was 67% (95%CI 35–90) and specificity was 89% (95%CI 84–92). At a cut-off of 175 GE/ml, the sensitivity was 100% (95%CI 73–100) and specificity was 30% (95%CI 25–36). Plasma DNA concentration predicted need for intensive care unit admission or death (adjusted odds ratio 1.4; $P<0.0001$).
Conclusions: Plasma DNA may have a role in patients with acute abdominal pain as a marker for inflammation and cancer, and a predictor of intensive care unit admission/mortality.

1. Introduction

Acute abdominal pain is a common presenting complaint encountered in emergency departments throughout the world [1–3] and is associated with potentially life-threatening pathology [4]. The challenge for frontline clinicians is primarily to safely and efficiently differentiate benign from life-threatening causes, and to make appropriate decisions regarding admission to hospital, to intensive care or for emergency surgery.

Strategies to improve the early diagnostic accuracy and risk-stratification of patients presenting with acute abdominal pain have included clinical scoring methods [5,6], blood and urinary markers [7,8], ultrasound and CT imaging [5,9–14] and minimally-invasive surgery [15]. The complexity of presentation of acute abdominal pain has not produced a simple score that is clinically useful. There is a need to identify clinical predictors or other markers of potential life-threatening acute abdominal pain.

Circulating nucleic acids are becoming increasingly important as diagnostic and prognostic tools in prenatal diagnosis [16–21], cancer [22–26], graft rejection [27], trauma [28,29], stroke [30–32] and acute coronary syndrome [33,34]. Although the mechanisms by which nucleic acids are released into the circulation are unknown, it is likely that cell death is one major factor [35,36]. The good correlation between the circulating DNA level and the severity of trauma or the presence of organ failure suggests that DNA is rapidly released into the circulation when there is a massive tissue destruction [29].

We hypothesized that the concentration of plasma DNA in patients suffering from acute abdominal pain would be significantly higher in patients with life-threatening causes of abdominal pain involving inflammation and tissue destruction than in patients suffering from less serious diseases. We determined whether plasma DNA concentrations taken from patients presenting with acute abdominal pain predict mortality and need for intensive care.

2. Materials and methods

Approval was obtained from the Institutional Review Board of the Chinese University of Hong Kong to conduct a prospective study investigating the role of

Abbreviations: GE, genome-equivalent; SIRS, severe inflammatory response syndrome.

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Table 1
Characteristics of the 287 patients presenting to hospital with acute abdominal pain[a]

Characteristics	Value
Age (y)	51 [33–70]
Age >65 y	88 (31%)
Male sex — no. of patients (%)	133 (40%)
Previous history of chronic illness[b]	97 (34%)
Time from onset of abdominal pain to blood sample (h)	48 [48–120]
Onset <24 h	71 (25%)
Onset >24 h	216 (75%)
Admitted to hospital — no. of patients (%)	249 (87%)
Urgent operation — no. of patients (%)	65 (23%)
Diagnosis at discharge — no. of patients (%)	
Non-specific abdominal pain of low significance[c]	148 (52%)
Cancer	17 (6%)
Carcinoma of colon	8
Carcinoma of pancreas	4
Hepatocellular and renal cell carcinoma	2
Obstetric and Gynecological	15 (5%)
Acute pelvic inflammatory disease	9
Ovarian cyst	6
Organ inflammation	79 (27%)
Acute cholangitis/cholecystitis	28
Acute appendicitis	22
Acute diverticulitis	7
Acute peptic ulceration	7
Acute pancreatitis	7
Abscess/peritonitis/ischemic bowel/peritonitis	8
Organ obstruction	28 (10%)
Intestinal obstruction	20
Urolithiasis	8

[a] All continuous data are expressed as medians [interquartile range]. Numbers may not sum to 100 because of rounding, multiple factors (for example risk factors) or absent data. Categorical variables are presented as values (percentages).
[b] Chronic illness includes previously diagnosed hypertension (n=35), diabetes mellitus (n=30), ischemic heart disease (n=16), congestive heart failure (n=5), atrial fibrillation (n=6), hyperlipidemia (n=9), stroke (n=15), chronic obstructive airways disease (n=9), cancer (n=21) and psychiatric illness (n=2).
[c] Includes non-specific undiagnosed abdominal pain, gastroenteritis, simple hernias, hepatic cirrhosis, herpes zoster, and mesenteric adenitis.

plasma DNA analysis in patients presenting with acute abdominal pain to the Prince of Wales Hospital.

2.1. Subjects, sample collection and follow-up

Eligible patients aged ≥15 y presenting to the emergency department with acute abdominal pain were recruited consecutively into the study. Exclusion criteria included external blunt or penetrating trauma (due to an external force associated with a motor vehicle crash, fall or assault etc.), known non-surgical causes for abdominal pain (for example, diabetic ketoacidosis, urinary tract infection, gastro-esophageal reflux, or indigestion (dyspepsia)), and patients unable to give consent. Informed, written consent was obtained either from the patient or a relative in all cases. Forty healthy control subjects were recruited which were matched with the 287 patients for age (mean ± SD 46.4 y ± 16.4 vs. 50.8 y ± 20.0; unpaired t test P=0.20) and sex (male to female ratio 24:46 vs. 154:133; Fisher's exact test P=0.50). Control subjects included staff or their relatives with no history of recent acute illness within 3 months, chronic illness, smoking or medication.

2.2. Data collection, definitions and diagnostic imaging

Acute abdominal pain was defined as pain occurring within 7 days of onset and in an area extending below the lower ribs, above the inguinal line and between the mid-axillary lines. Data collected included age, sex, symptom onset time, history of previous abdominal pain, seizures, hypertension, diabetes mellitus, ischemic heart disease, atrial fibrillation, hyperlipidemia, smoking, antithrombotic and other medication.

In order to determine the exact nature and cause of the acute abdominal pain, patients received a standard clinical, laboratory and imaging workup. The patients were managed by emergency physicians according to standard practice and without any information about the plasma DNA results.

For patients requiring admission, their clinical information including the definitive diagnosis and pathological results of resected specimens were reviewed after discharge. All specimens were reviewed by a Specialist Pathologist who was blinded to the plasma DNA. Patients not requiring admission were contacted by telephone two week after the initial consultation to review whether they needed further consultation, admission or surgical intervention.

A final diagnosis was determined either by a specialist surgeon if the patient was admitted, or by an emergency medicine specialist if the patient was discharged directly from the emergency department. Clinicians were blinded to the plasma DNA level results at the time of diagnosis. Patient diagnostic groups for analysis were determined by the authors. Severe inflammatory response syndrome (SIRS) is said to occur if there are two or more of the following: temperature >38 °C or <36 °C; tachycardia >90 beats/min; respiratory rate >20 breaths/min or carbon dioxide and partial pressure (PCO_2) <4.3 kPa; white blood cell count >12×10^9/l or <4×10^9/l or >10% immature (band) forms [37]. Infection and sepsis are defined according to standard guidelines [37].

2.3. Sample collection, processing of plasma and DNA extraction

A 5 to 10 ml blood sample was withdrawn from the antecubital vein of each patient, collected into tubes containing EDTA, centrifuged at 1600 g for 10 min, and plasma was then transferred into plain polypropylene tubes for a further centrifugation at 16,000 g for 10 min, and stored at −80 °C pending further processing. DNA was extracted from 0.8 ml of plasma using the QIAamp Blood MiniKit (Qiagen, Hilden, Germany) following manufacturer's recommendations [38]. An elution volume of 50 µl was used.

2.4. Real-time quantitative PCR

Plasma DNA was measured using a real-time quantitative PCR assay targeting the *β-globin* gene as previously described [28]. Real-time PCR analysis was performed using an Applied Biosystems PRISM 7700 Sequence Detector (Applied Biosystems, CA). Standard curves were established by serial dilutions of known amounts of DNA extracted from human buffy coat. Five microliters of extracted plasma DNA was used for each amplification. Each real-time quantitative PCR assay was performed on an optical 96-well PCR reaction plate. In the present study, there were 287 patients and 40 healthy samples. Every sample was analyzed in duplicate. Serial dilutions of the standard curve included were included in each assay (batch). Ten assays (a batch) were carried out on 10 different plates. In order to ensure the consistency of all the assays, 2 different known amounts of plasma DNA samples were included in every assay. The precision of the real-time quantitative PCR assay has been reported previously (18), with a CV for the threshold cycle of 1.1%. Raw data were subsequently converted into units of copies of genomes per ml plasma. DNA levels are given to the nearest 25 genome-equivalents (GE)/ml. The detection limit of the real-time PCR was 1 GE per reaction (equivalent to 12.5 GE/ml plasma). Four negative controls were included in every assay. Negative controls are "no template controls" i.e. a complete reaction mix without plasma DNA samples. No DNA should be amplified and detected after the assay is completed, otherwise the assay is said to be contaminated.

Table 2
Univariate comparison of plasma DNA concentrations and outcome variables (n=287)[a]

Outcome variable	No		Yes		"x" fold increase	Mean difference	P value[b]
	N	DNA concentration	N	DNA concentration			
Hospital admission	38	225	249	950	4	725 (50 to 1400)	0.0343
Operation required within 28 days	206	700	81	1300	2	600 (100 to 1100)	0.0194
Readmission to hospital	226	575	61	1930	3	1350 (800 to 1875)	<0.0001
SIRS[c]	228	625	56	1825	3	1200 (625 to 1750)	<0.0001
Infection	239	725	48	1550	2	825 (200 to 1425)	0.0083
Sepsis	263	750	21	2300	3	1525 (675 to 2400)	0.0006
ICU admission alone	282	775	5	6000	8	5250 (3600 to 6875)	<0.0001
Mortality alone	278	725	9	3450	5	2650 (1375 to 3950)	<0.0001
ICU admission and/or mortality	275	700	12	4725	7	4025 (2975 to 5075)	<0.0001

[a] Values are rounded off to the nearest 25 GE/ml and shown as means and the mean difference (95% confidence intervals).
[b] Unpaired student t test is used for comparing means.
[c] SIRS, systemic inflammatory response syndrome.

2.5. Outcome

The primary outcome measure was the composite 28-day mortality or ICU admission. Secondary outcome measures were readmission and SIRS.

2.6. Statistical analysis

Descriptive statistics and data comparison tests – chi-square, Fisher's exact, Mann–Whitney and Kruskal–Wallis tests – were carried out using Statview® for Windows version 5.0 Statistical Analysis Software (Abacus Concepts, SAS Institute, Cary NC). Medcalc (Mariakerke, Belgium) was used for ROC analysis. The Bonferroni–Dunn test was used for multiple comparisons (i.e. when more than two groups were analysed), in order to reduce the probability that statistical significance arose from multiple testing rather real differences. The Kruskal–Wallis test was used because the number of cases in some groups (e.g. ICU and mortality) is small.

Multiple logistic regression analysis was used to determine the independent contribution of multiple variables to the outcome. Variables were initially entered into the model based on prior clinical reasoning rather than statistical significance (i.e., age, gender, plasma DNA, positive CT finding, positive US finding, chronic illness, time from symptoms onset to sampling, pulse rate >90 beats/min, temperature >38 °C, respiratory rate >20 breaths per minute, leukocyte count $>12\times10^9$/L, Alvarado score, amylase >100 and need for operation). Later statistically insignificant variables ($P>0.05$) were removed stepwise until only significant independent variables remained.

3. Results

Characteristics of the 287 adult patients who presented with acute abdominal pain possibly requiring surgical intervention during the period of recruitment are shown in Table 1. Differences in median plasma DNA concentrations between the five clinical groups — cancer (500 GE/ml), obstetric/gynecological (250 GE/ml), inflammatory (675 GE/ml), obstructive (325 GE/ml), and non-specific abdominal pain (225 GE/ml) are statistically significant ($P<0.0001$; Kruskal–Wallis test). Six patients had gastrointestinal hemorrhage associated with abdominal pain (2 patients had Mallory–Weiss Syndrome and 4 patients had melena). Compared with those patients with non-specific abdominal pain or benign conditions mean plasma DNA levels were significantly elevated in patients with cancer (500 vs. 250 GE/ml; $P<0.0167$; Bonferroni–Dunn test) and acute abdominal inflammation (650 vs. 250 GE/ml; $P<0.0167$; Bonferroni–Dunn test).

Mean plasma DNA concentrations and primary and secondary outcomes are shown in Table 2. Mean plasma DNA concentrations were four-fold higher in patients who were admitted to hospital, three-fold higher in patients with SIRS, five-fold higher in patients who died within 28 days, and eight-fold higher in patients admitted to the ICU. Plasma DNA concentrations correlated with SIRS score ($P<0.0001$; Kruskal–Wallis test; $P<0.005$ between SIRS scores 0 and 3, 1 and 2, and 1 and 3; Bonferroni–Dunn test).

Fig. 1 shows the significant increase in median plasma DNA concentrations between healthy control subjects, patients immediately discharged, patients admitted, patients requiring operation, and patients admitted to ICU and/or who died ($P<0.0001$; Kruskal–Wallis test).

Fig. 2 shows the receiver operator curve for plasma DNA concentrations and ICU admission/mortality (area under the curve is 0.804; 95% CI 0.753 to 0.848). At a cut-off of 1100 GE/ml, the sensitivity is 67% (95%CI 35–90) and specificity is 89% (95%CI 84–92). At a cut-off of 175 GE/ml, the sensitivity is 100% (95%CI 73–100) and specificity is 30% (95%CI 25–36). At a cut-off of 2150 GE/ml, the sensitivity is 58% (95%CI 28–85) and the specificity is 95% (95%CI 92–97).

Table 3 shows the adjusted odds ratios for predicting ICU admission or mortality. When all variables were entered into the model, only the presence of positive findings at CT and plasma DNA concentration predicted need for ICU admission or death.

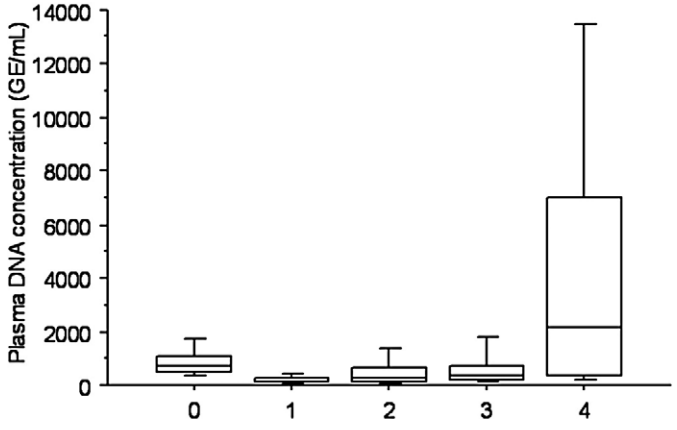

Fig. 1. Plasma DNA concentrations in patient groups and healthy controls. Plasma DNA concentrations as determined by real-time quantitative PCR targeting the *β-globin* gene (*y*-axis). Groups are shown on the *x*-axis and include healthy control subjects (0), patients immediately discharged (1), patients admitted (2), patients requiring operation (3), and patients admitted to ICU and/or who died (4). There were significant differences across the groups (Kruskall–Wallis test $P<0.001$) and between groups 0 to 3 and group 4 (Bonferonni–Dunn $P<0.055$). The lines inside the boxes denote medians whilst the boxes mark the interval between the 25th and 75th percentiles. The whiskers denote the interval between the 10th and 90th percentiles.

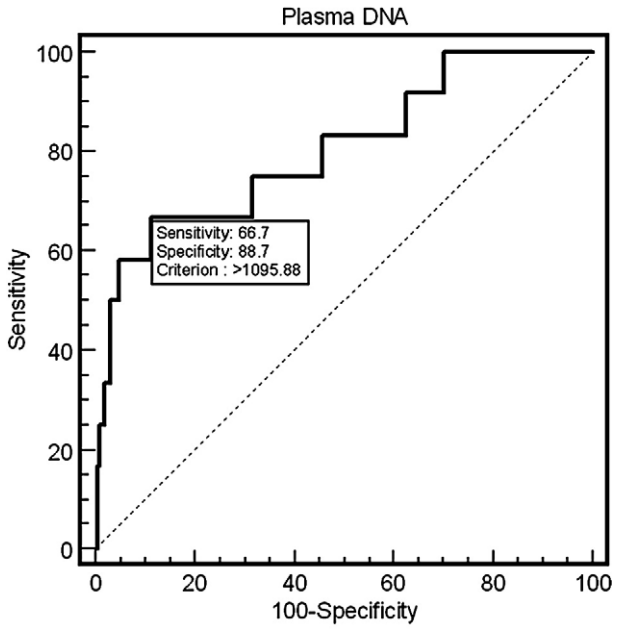

Fig. 2. Receiver operator curve for plasma DNA concentrations and ICU admission/mortality. This figure shows the receiver operator curve for plasma DNA concentrations and ICU admission/mortality (area under the curve is 0.804; 95% CI 0.753 to 0.848). At a cut-off of 1100 GE/ml, the sensitivity is 67% (95%CI 35–90) and specificity is 89% (95%CI 84–92). At a cut-off of 175 GE/ml, the sensitivity is 100% (95%CI 73–100) and specificity is 30% (95%CI 25–36). At a cut-off of 2150 GE/ml, the sensitivity is 58% (95%CI 28–85) and the specificity is 95% (95%CI 92–97).

Table 3
Adjusted odds ratios for predicting ICU admission or mortality ($n=287$)[a]

Factor	Adjusted odds ratio	95% CI	*P* value
Positive CT findings	4.5	1.1–17.8	0.0346
Reference — negative CT findings	–		
Plasma DNA per 1000 GE/ml[b]	1.4	1.2–1.6	<0.0001

[a] Initial independent variables entered into the model included age, gender, plasma DNA, positive CT finding, positive US finding, chronic illness, time from symptoms onset to sampling, pulse rate >90 beats/min, temperature >38 °C, respiratory rate >20 breaths per minute, leukocyte count $>12\times10^9$/L, Alvarado score, amylase >100 and need for operation. The dependent variable was need for ICU admission or death within 28 days. Insignificant independent variables were removed from the model stepwise until only those variables with *P* value <0.05 remained.

[b] The odds of ICU admission or death increased 40% for every 350 GE/ml increase in plasma DNA.

4. Discussion

This study shows that circulating plasma DNA concentrations, assessed by measuring *β-globin* gene concentrations using real-time PCR, are increased in patients presenting to an emergency department with acute abdominal pain requiring admission, are highest in patients with major inflammatory diseases and cancer, and correlate with increasing severity of systemic inflammation. Plasma DNA is clearly a non-specific rather than a specific diagnostic marker which may be useful for determining need for admission to hospital, need for intensive care, and for 28-day mortality risk.

In this study we hypothesized that as plasma DNA is likely to be released from damaged, inflamed and necrotic tissues, and that in the appropriate clinical context e.g. acute abdominal pain, it might act as a marker for serious intra-abdominal pathology. We have demonstrated a role for plasma DNA analysis in identifying the presence of potentially life-threatening conditions for patients presenting with acute abdominal pain, for example patients with high SIRS scores, inflamed organs, and intra-abdominal cancer. The test is insensitive in detecting other conditions.

The relative lack of sensitivity of this test in picking up certain pathologic conditions may firstly be related to the relatively small amount of the affected tissues. Plasma DNA is a non-specific marker that may be released from any nucleated cell. Quantities of DNA released from other tissues into plasma may be greater than that released from an inflamed appendix. Some of the highest levels of plasma DNA are seen in patients with multiple trauma in which there is a large amount of damaged tissue [27]. In patients with acute stroke with catastrophic clinical effects, the amount of damaged neurological tissue may be quite small.

Elderly patients have elevated circulating plasma DNA levels which is most pronounced in those with chronic illness [35]. Similar to other studies in an emergency department setting, plasma DNA levels may give a general indication of the health of a patient presenting as a potential emergency. Moderate increases in plasma DNA may not necessarily indicate an acute event but may be associated with the chronic inflammatory and atherosclerotic processes which are often associated with aging. In our study chronic illness was associated with increased plasma DNA concentrations but when entered into the logistic regression model for ICU admission/mortality the adjusted odds ratio was not significant. Therefore in our study it is likely that differences in plasma DNA reflect acute rather than chronic processes. Higher levels of plasma DNA were noted in patients requiring an operation within 28 days although not necessarily at first admission, in patients requiring readmission after discharge, requiring admission to ICU and in those patients likely to die. Similar prognostic potential has been noted in patients with trauma, stroke and acute coronary syndrome [28–32]. It may initially be surprising that patients requiring readmission have higher levels than patients with SIRS. However the case mix of patients requiring readmission includes some patients with organ inflammation e.g., acute cholecystitis, and cancer who may have initially been treated conservatively, discharged and later readmitted either as emergencies or for elective procedures.

Others have shown that plasma DNA could predict mortality and sepsis in patients admitted to ICU [39,40], and we have also previously shown that early elevation of plasma DNA concentrations in patients with severe trauma predicts likely admission to ICU and mortality [28,29]. Also plasma DNA concentrations have been shown to be elevated in patients with cancer [23–26]. However few studies have looked at plasma DNA concentrations as potential screening or risk-stratification markers in patients with undifferentiated presentation such as the acute abdomen.

In the present format, the whole procedure of plasma DNA quantification, starting from blood collection, takes approximately 120 min. Technological advancement in the development of rapid DNA analysis now makes a bed-side test possible within 10 to 15 min of sampling [41].

The strengths of this study lie in its large sample size, and its consecutive and complete set of data. The heterogeneity of the diagnostic groups may be regarded as a strength or weakness depending on the reader's perspective. Undifferentiated abdominal pain is a common presentation which requires risk assessment and diagnosis. In this sense the study is very practical. The limitations of the study include the fact that it is a single centre study, the non-specific nature of *β-globin* DNA which may be derived from any nucleated cell in the body, the absence of other common markers of severity against which plasma DNA may be compared, and the relatively small number of cases with a positive primary outcome i.e. ICU admission and mortality. These may limit the generalizability of our results, and as such our study should be regarded as preliminary.

Further studies are required to validate the optimal plasma DNA cut-off levels for diagnosis and prognosis, and to confirm the generalizability of the results. A combination of inflammatory and tissue specific nucleic acid markers may aid more precise diagnosis and risk-stratification. In conclusion, this study shows that plasma DNA concentrations are elevated in patients presenting with acute abdominal pain, especially in those with cancer and organ inflammation, and predicts need for ICU admission and mortality.

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