



A pilot study of a novel molecular host response assay to diagnose infection in patients after high-risk gastro-intestinal surgery

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ABSTRACT

Purpose: SeptiCytE LAB measures the expression of four host-response RNAs in peripheral blood to distinguish sepsis from sterile inflammation. This study evaluates whether sequential monitoring of this assay has diagnostic utility in patients after esophageal surgery.

Materials and methods: Patients who developed a complication within 30 days following esophageal surgery and a random sample of 100 patients having an uncomplicated course. SeptiCytE LAB scores (ranging 0–10 reflecting increasing likelihood of infection) were compared to post-hoc physician adjudication of infection likelihood.

Results: Among 370 esophagectomy patients, 120 (32%) subjects developed a complication requiring ICU (re)admission, 63 (53%) of whom could be analyzed. Immediate postoperative SeptiCytE LAB scores were highly variable, yet similar for patients having a complicated and uncomplicated postoperative course (median score of 2.4 (IQR 1.6–3.3) versus 2.2 (IQR 1.3–3), respectively). In a direct comparison of patients developing a confirmed infectious ($n = 34$) and non-infectious complication ($n = 12$), addition of SeptiCytE LAB to CRP improved diagnostic discrimination of infectious complications (AUC 0.88 (95%CI 0.77–0.99)) compared to CRP alone (AUC 0.76 (95%CI 0.61–0.91); $p = .04$).

Conclusions: Sequential measurement of SeptiCytE LAB may have diagnostic value in the monitoring of surgical patients at high risk of postoperative infection, but its clinical performance in this setting needs to be validated.

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1. Introduction

Major gastro-intestinal surgery, such as esophageal, gastric, and pancreatic interventions, are associated with a high risk of developing postoperative complications [1,2]. Indeed, complication risks can be as high as 33.5%, and affected patients suffer from a significantly increased length of stay and excess mortality [2,3]. Most of the complications are of infectious origin (principally pneumonia and anastomotic leakage), which can readily lead to the development of sepsis in postoperative patients [1–3].

Timely recognition and treatment of and treatment of sepsis may improve outcome [4]. However, sepsis diagnosis is complicated by the

almost universal presence of Systemic Inflammatory Response Syndrome (SIRS) in patients who just had gastrointestinal surgery [5]. Currently, sequential measurements of C-reactive protein (CRP) and procalcitonin (PCT) are most commonly used to monitor onset of postoperative infectious complications [6]. Although these biomarkers have adequate negative predictive values (ranging from 91% to 100%), their positive predictive values remain poor [7–11]. A variety of alternative biomarkers have thus been proposed for the diagnosis of sepsis, however their diagnostic accuracy is variable across settings and their clinical utility not always evident [12,13].

Whole-blood transcriptomics-based technologies (i.e., measurement of RNA transcripts that are generated during gene expression in leukocytes) can detect rapid change when the host is exposed to infectious stress, and may therefore possibly yield an earlier diagnostic signal of sepsis than traditional protein biomarkers [14–17]. SeptiCytE™ LAB is the first RNA-based host response signature cleared by the US Food and

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Drug Administration for sepsis diagnosis [18]. It measures the expression of four genes (carcinoembryonic antigen-related cell adhesion molecule 4 (CEACAM4), lysosomal-associated membrane protein 1 (LAMP1), phospholipase A2 group VII (PLA2G7), and placenta-specific 8-gene protein (PLAC8)) in peripheral blood. In several evaluations SeptiCytE LAB discriminated better between critically ill patients with (overt) sepsis and a non-infectious SIRS than PCT [17,19,20]. Although the test performed less favorably in a recent cohort of difficult-to-diagnose cases of (nosocomial) sepsis after prolonged prior hospitalization [21].

We hypothesized that sequential measurement of SeptiCytE LAB could have superior diagnostic performance over established biomarkers in postoperative patients at high risk for infectious complications, as the test characterizes the host response to infection at a relatively early—and thus possibly more specific—stage [22]. To explore this idea further, we performed a pilot study in consecutive esophagectomy patients in order to 1) determine a normal range for SeptiCytE LAB measured directly following esophageal surgery, 2) evaluate temporal changes in SeptiCytE LAB scores as complications ensue in the postoperative setting, and 3) compare the ability of SeptiCytE LAB to discriminate infectious from non-infectious complications in postoperative patients to that of a more commonly used biomarker, CRP.

2. Materials and methods

2.1. Study design

We performed a case-control analysis that was nested in the MARS (Molecular Diagnosis and Risk Stratification of Sepsis) cohort, which enrolled subjects in two Dutch university hospitals from 2011 to 2013. From this cohort we selected consecutive patients who had undergone elective esophageal resection. Ethical approval for the study was provided by the Medical Ethics Committees of both participating hospitals, and an opt-out procedure to obtain consent from patients was in place (protocol number 10–056). Blood samples for RNA analysis were collected within 24 h of surgery and whenever a complication occurred in the ICU during the first 30 postoperative days. This complication could be either a (suspected) infection, acute kidney injury (AKI), acute respiratory distress syndrome (ARDS), acute myocardial infarction (AMI), or readmission to the ICU for any (other) reason. For the present study, we selected cases having ≥ 1 postoperative complication and for whom (at least) a single paired RNA sample was available. In addition, we randomly selected 100 control subjects having an uncomplicated postoperative course after esophagectomy in order to establish (a range of) normal SeptiCytE LAB scores following major surgery.

Samples were collected in 2.5 mL PAXgene blood RNA tubes and processed in accordance with predefined acceptance criteria as set by the manufacturer of the assay (Immunexpress, Seattle, WA) [18]. Tests were performed in 96-well microtiter amplification plates on an Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (Thermo Fisher Scientific, Carlsbad, CA), yielding a threshold cycle number (C_t -value) per individual gene. A score was then calculated as $(C_{tPLA2G7} + C_{tCEACAM4}) - (C_{tPLAC8} + C_{tLAMP1})$. This ‘SeptiScore’ ranges from 0 to 10, and may be categorized into 4 probability bands according to the manufacturer's specification [18]. SeptiScores ≤ 3.0 (band 1) indicate that sepsis is unlikely, whereas scores 3.1–4.4 (band 2), 4.5–5.9 (band 3), and > 6 (band 4) represent increasing sepsis likelihoods.

2.2. Reference test for infection

Suspected infectious events were recorded prospectively upon each occasion that antimicrobial therapy was initiated by the clinician. All patients treated for a suspected infectious event in the ICU also met SIRS criteria and had a Sequential Organ Failure Assessment (SOFA) score ≥ 2 , thus fulfilling current Sepsis-3 definitions [23,24]. The likelihood of infection was subsequently classified as none, possible, probable, or definite based on daily discussions with the attending team as well as

a post-hoc review of all available clinical, microbiological, and radiological data collected during ICU stay by trained physicians according to predefined definitions [25]. This reference diagnosis was established without knowledge of SeptiCytE LAB results, yet observers were not blinded to CRP. However, CRP in and of itself could not lead to a diagnosis of infection in the absence of other clinical and inflammatory symptoms.

For use as a reference test in the current study, all observed complications were reclassified as infection ruled-out (patients with a post-hoc likelihood rated none, or patients who were never suspected of an infection), infection undetermined (patients with possible infection), or infection confirmed (patients with a post-hoc likelihood rated probable or definite). In case of multiple concurrent events, SeptiCytE LAB test results were related to the complication that occurred nearest in time to the moment the sample was taken.

2.3. Statistical analysis

Immediate postoperative SeptiScores were analyzed to determine a normal range following major surgery, both in patients who would later develop a complication as well as in the 100 control subjects. Subsequently, in esophagectomy patients having a complicated postoperative course only, we performed within-patient pairwise comparisons of scores measured in samples obtained directly after surgery and at complication onset. To assess the diagnostic potential of sequential SeptiCytE LAB measurement, we focused this analysis on differences between subjects having non-infectious, undetermined and confirmed infectious complications. In addition, we compared SeptiScores to CRP concentrations measured in plasma obtained at the same time point. To this end, we standardized differences between the post-operative moment and the moment of complication onset by calculating Z-scores. We compared discriminative ability of SeptiCytE LAB and CRP using receiver operating characteristic (ROC) curves. For this latter analysis we excluded subjects having an undetermined infectious state according to physician adjudication.

Differences in categorical and continuous variables between groups were assessed using Chi-square, Wilcoxon signed rank or Mann-Whitney *U* tests, as appropriate. All analyses were performed in SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC) and R Studio (R Studio Team (2015), Boston, MA).

3. Results

During the study period, a total of 370 patients were admitted to the ICU after elective esophagectomy, of whom 120 (34%) developed a complication resulting in prolonged ICU stay or readmission to the ICU within 30 days. Among these, 74 (62%) subjects had immediate postoperative PAXgene blood samples available for analysis, and 63 (53%) also had a sample taken at complication onset (Fig. 1). Patients without a postoperative sample ($n = 46$) had a shorter ICU stay (9, IQR 2–16 versus 12, IQR 7–24, $p = .04$), less ICU readmissions (54% versus 97%, $p < .001$), and were treated for infection less frequently (70% versus 92%, $p < .05$) than patients with an available sample. However, in-hospital mortality was similar between the groups (13% versus 16%, $p = .79$).

Immediate postoperative PAXgene samples were additionally analyzed in a random subsample ($n = 100$) of the 250 remaining esophagectomy patients who had an uncomplicated postoperative course. These patients developed SIRS less frequently, required less vasopressors, and exhibited a trend towards lower APACHE scores and CRP levels compared to patients in the complicated cohort (Table 1).

Among the 63 patients with postoperative complications who were included in the pairwise analyses of SeptiCytE LAB, 34 (54%) subjects had a confirmed infection, 17 (27%) an undetermined infectious state, and 12 (19%) a non-infectious complication (i.e., 5 were empirically treated with antibiotics but classified as having no infection in retrospect, whereas 7 were never suspected of infection). Frequently

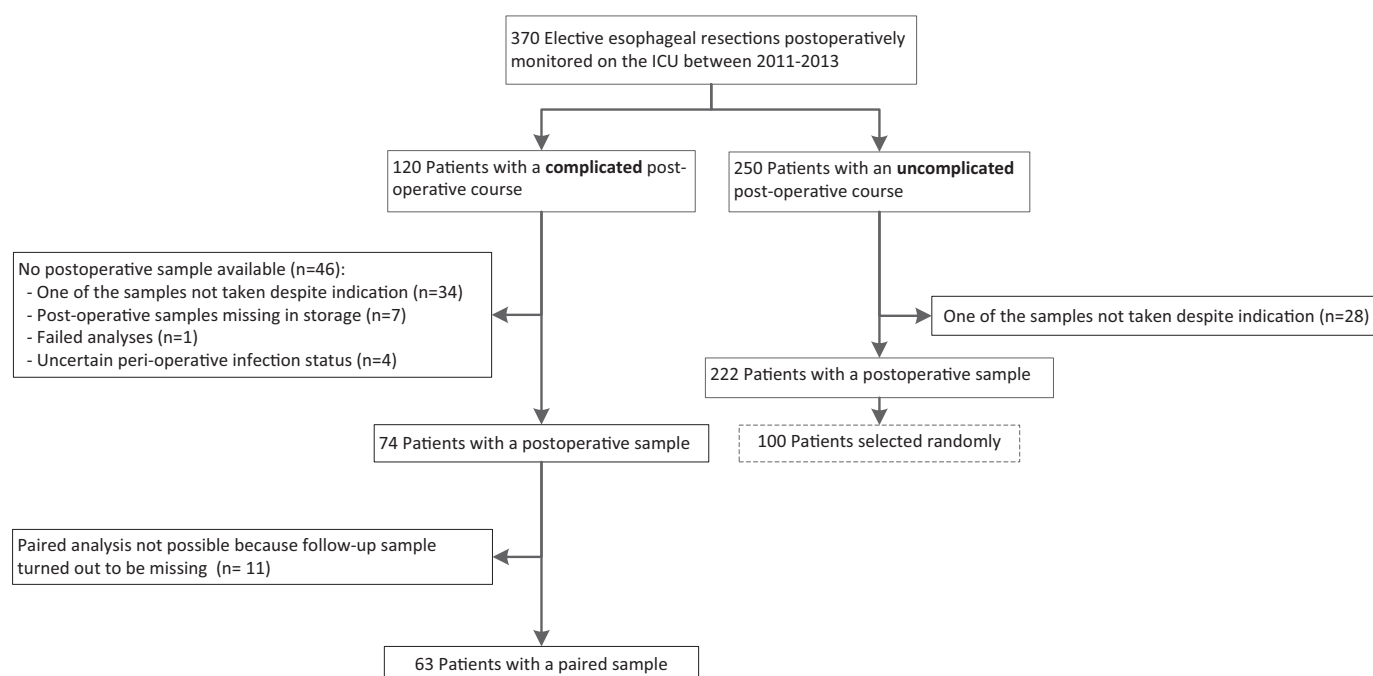


Fig. 1. Flowchart of patient inclusion. ICU; Intensive Care Unit.

observed infections included intrathoracic sources (34%; most commonly mediastinitis or pleural empyema due to anastomotic leakage) and pneumonia (24%) (Table 2). Of note, multiple complications could coexist.

3.1. SeptiScore distribution in the immediate postoperative setting

Among the total number of 174 analyzed patients, immediate postoperative SeptiScores were highly variable (range 0–10), with a median of 2.3 (IQR 1.4–3.1). Overall, 45 (26%) samples corresponded to probability band 2 or higher, which—in case sepsis were to be clinically suspected—would have incorrectly resulted in a “sepsis-likely” label according to the manufacturer's specification (Table S1). Median SeptiScores of patients having an uncomplicated postoperative course tended to be slightly lower than those of patients who would later

develop a complication (2.2 (IQR 1.3–3) versus 2.4 (IQR 1.6–3.3)), although this difference did not reach statistical significance ($p = .14$).

3.2. Temporal changes in SeptiScore

Fig. 2 shows SeptiScores as measured immediately after surgery and at the time of complication onset for the 63 patients in whom paired samples were available. Median lag time between surgery and the development of a complication was 5 (IQR 3–9) days. However, it should be noted that 8 (14%) of the repeat samples were already taken within 2 days of surgery. SeptiScores increased in all patients who developed a complicated disease course after surgery, but this rise was more pronounced in those developing infection versus another complication (median score differences 2.1 (IQR 0.4–3.6), 4 (IQR 2.5–5), and 4.7 (IQR 4.1–5.8) for subjects having a non-infectious, undetermined, and confirmed infectious event, respectively; $p < .0001$ (Table S2)).

Table 1

Characteristics of 174 esophagectomy patients stratified by their postoperative clinical course.

Variable	No complication (N = 100)	≥1 complication(s) (N = 74)	P-value
Age	64 (57–71)	65 (59–70)	0.85
Male gender	77 (77%)	48 (65%)	0.08
Charlson comorbidity index	2 (2–2)	2 (2–2)	0.25
Malignancy	90 (90%)	69 (93%)	0.45
APACHE IV Score	45 (38–55)	47 (41–58)	0.14
≥ 2 SIRS criteria (postop day 1)	26 (26%)	40 (54%)	<0.001
Vasopressor use (postop day 1)	50 (50%)	47 (64%)	0.24
CRP (postop day 1)	46 (3–92)	70 (6–108)	0.050
Time to complication onset (days)	NA	3 (2–6)	NA
ICU stay (days)	0.9 (0.8–1.0)	6.6 (3.7–17.2)	<0.001
Hospital stay (days)	11 (8–14)	28 (20–53)	<0.001
In-hospital mortality	0 (0%)	12 (16%)	<0.001
1-year mortality	3 (3%)	15 (20%)	<0.001

APACHE, Acute Physiology and Chronic Health Evaluation; COPD, Chronic Obstructive Pulmonary Disease; CRP, C-reactive protein; ICU, Intensive Care Unit; SIRS, Systemic Inflammatory Response Syndrome.

Continuous data presented as median and IQR and dichotomous data as n (%).

Table 2

Clinical events among 63 esophagectomy patients with ≥1 postoperative complication.

Complications	Non-infectious (n = 12)	Undetermined (n = 17)	Confirmed infection (n = 34)
Total number of events	18	39	97
(Presumed) infectious source(s)			
Intrathoracic ^a	0 (0%)	6 (15%)	24 (25%)
Pulmonary	4 (22%) ^b	10 (26%)	11 (11%)
Abdominal	1 (6%) ^b	2 (5%)	7 (7%)
Other	0 (0%)	0 (0%)	2 (2%)
Other (non-infectious) complication(s)			
Readmission to ICU	10 (56%)	16 (41%)	33 (34%)
Acute respiratory distress syndrome	1 (6%)	3 (8%)	13 (13%)
Acute kidney injury	2 (11%)	1 (3%)	7 (7%)
Acute myocardial infarction	0 (0%)	1 (3%)	0 (0%)

ICU, Intensive Care Unit. Multiple complications could occur at the same time.

^a Intrathoracic infections include mainly cases of lung empyema and mediastinitis.

^b Initially suspected focus of infection among patients in whom infection likelihood was later classified as 'none'.

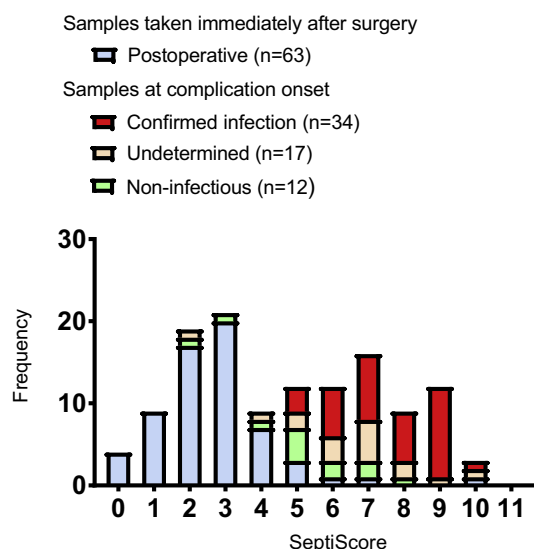


Fig. 2. SeptiScores among 63 esophagectomy patients with ≥ 1 postoperative complication. Frequencies represent the number of patients.

3.3. Discriminative ability of septicity LAB

Standardized differences (expressed as Z-scores) between samples collected immediately after surgery and at complication onset revealed a greater increase in patients with confirmed infections than in other patients for both SeptiCyte LAB and CRP (Fig. 3). However, among the 34 patients with confirmed infection, the observed standardized increase in SeptiScore was more pronounced than that in CRP, although this difference did not reach statistical significance (median Z-score 0.28 versus 0.08, $p = .08$).

In a direct comparison of patients developing a confirmed infectious ($n = 34$) and definite non-infectious complication ($n = 12$), ROC analysis yielded an AUC of 0.87 (95%CI 0.760–0.98) for SeptiCyte LAB, compared to an AUC of 0.76 (95%CI 0.61–0.91) for CRP ($p = .14$). Adding SeptiCyte LAB to CRP resulted in improved diagnostic discrimination (AUC 0.88 (95%CI 0.77–0.99), $p = .04$).

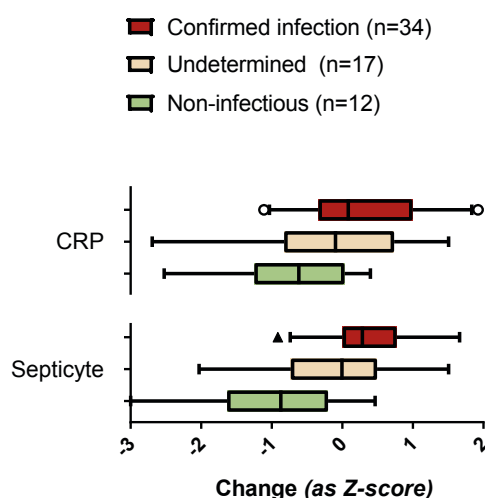


Fig. 3. Temporal changes in SeptiScores and CRP levels among 63 esophagectomy patients developing a postoperative complication. CRP, C-reactive protein. CRP concentration and SeptiScores were measured in the immediate postoperative sample and the sample taken at complication onset. Subsequently, observed differences between both time points were transformed into a standardized z-score ($z = (x - \mu) / \sigma$), having a mean value of 0 and standard deviation of 1. Boxes show median standardized differences with IQR, whiskers show 5–95th percentiles.

4. Discussion

This pilot study explored whether temporal changes in SeptiCyte LAB could be used to help diagnose infectious complications after esophageal surgery. Although SeptiScores varied widely between individuals, median scores immediately after surgery were comparable between subjects who went on to have either an eventful or uncomplicated subsequent postoperative course. However, the increase of SeptiScore over time was greater in patients developing postoperative infections than in those with other complications. Furthermore, this appeared to be more pronounced than the simultaneous rise in CRP observed in these patients.

SeptiCyte LAB was originally developed to help diagnose infection in critically ill patients presenting to an ICU with SIRS. Previous studies evaluating its diagnostic performance in this setting have reported variable discriminative ability for SeptiCyte LAB, with AUC's ranging from 0.73 (0.68–0.79) to 0.99 (95%CI 0.96–1.00) in different cohorts [17,19–21,26,27]. In particular, specificity was lower in patients presenting with suspected pneumonia as well as in those who had already been subjected to a prolonged clinical course in hospital prior to ICU admission [21,26]. In the current study we observed favorable discrimination (AUC of 0.87 (95%CI 0.76–0.98), albeit after exclusion of patients having an uncertain infectious state.

Observed variations in diagnostic performance are most likely explained by differences in study size, clinical setting, and distribution of underlying infectious etiologies. The optimal intended use scenario for SeptiCyte LAB therefore requires further exploration. Our data suggest that adding SeptiCyte LAB to CRP may improve diagnostic discrimination in patients following major surgery [7–9]. However, any possible use of SeptiCyte LAB for routine screening of postoperative patients will require careful evaluation before it can be considered. As physicians base their probabilistic decision-making mainly on clinical information (rather than biomarkers alone), it is unlikely that SeptiCyte LAB will be able to significantly decrease antimicrobial drug use when the pre-test probability for infection is high. The test will also be much more expensive than routinely available alternative biomarkers, and a stepped diagnostic approach might thus be appropriate. Clearly, the clinical utility and cost-effectiveness of such strategies can only be evaluated in a randomized controlled diagnostic trial.

Our pilot study has several limitations related to its relatively small sample size, which limits statistical power, as well as the unavailability of paired PAXgene samples in almost half the target population. Also, not all study patients presented to the ICU having a true diagnostic dilemma regarding the presence or absence of infection, which precludes final conclusions regarding diagnostic accuracy. In addition, we did not collect SeptiCyte LAB follow-up samples in patients without a postoperative complication, hindering the comparison of temporal changes in SeptiScores between patients having infectious complications, non-infectious complications, and uneventful postoperative courses. Furthermore, although our pilot series aimed to explore the potential clinical utility of successive SeptiCyte LAB measurements in patients following major surgery, we evaluated score changes between two timepoints only. Thus, our findings cannot be directly extrapolated to a setting of sequential daily monitoring of this biomarker in postoperative patients. Also, for the prompt initiation of PAXgene specimen collection researchers were dependent on the clinical recognition of complications by attending physicians, which may have led to between-patient variability in timing of samples. Finally, even though the post-hoc likelihood of all suspected infections was carefully adjudicated by trained physician-observers according to standardized definitions, diagnostic misclassification cannot be ruled out [25]. However, patients with the greatest uncertainty regarding their reference diagnosis (i.e., those with an undetermined infectious status), were excluded from some comparative analyses, as has been done before in similar studies [17,21,28].

5. Conclusions

Repeated measurement of SeptiCytE LAB may have diagnostic value in the monitoring of surgical patients at high risk for postoperative infection. However, this has to be further evaluated in prospective studies that enroll both patients who are merely at risk for developing postoperative infections, as well as in those with suspected sepsis after major surgery.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcrc.2019.07.020>.

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