Perioperative Gene Expression Analysis for Prediction of Postoperative Sepsis

Carl Hinrichs,1† Katja Kotsch,1† Sandra Buchwald,2 Marit Habicher,2 Nicole Saak,2 Herwig Gerlach,3 Hans-Dieter Volk,1,4† and Didier Keh2†

BACKGROUND: Postoperative sepsis is one of the main causes of death after major abdominal surgery; however, the immunologic factors contributing to the development of sepsis are not completely understood. In this study, we evaluated gene expression in patients who developed postoperative sepsis and in patients with an uncomplicated postoperative course.

METHODS: We enrolled 220 patients in a retrospective matched-pair, case–control pilot study to investigate the perioperative expression of 23 inflammation-related genes regarding their properties for predicting postoperative sepsis. Twenty patients exhibiting symptoms of sepsis in the first 14 days after surgery (case group) were matched with 20 control patients with an uncomplicated postoperative course. Matching criteria were sex, age, main diagnosis, type of surgery, and concomitant diseases. Blood samples were drawn before surgery and on the first and second postoperative days. Relative gene expression was analyzed with real-time reverse-transcription PCR.

RESULTS: Significant differences ($P < 0.005$) in gene expression between the 2 groups were observed for $IL1B$ (interleukin 1, beta), $TNF$ [tumor necrosis factor (TNF superfamily, member 2)], $CD3D$ [CD3d molecule, delta (CD3-TCR complex)], and $PRF1$ [perforin 1 (pore forming protein)]. Logistic regression analysis and a subsequent ROC curve analysis revealed that the combination of $TNF$, $IL1B$, and $CD3D$ expression had a specificity and specificity of 90% and 85%, respectively, and predicted exclusion of postoperative sepsis with an estimated negative predictive value of 98.1%.

CONCLUSIONS: These data suggest that gene expression analysis may be an effective tool for differentiating patients at high and low risk for sepsis after abdominal surgery.

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Severe sepsis is one of the leading causes of death after major surgery (1). Despite intensive therapeutic efforts, mortality remains high. Soft-tissue trauma leads to an immunologic reaction consisting of hyperinflammation and immunodepression at the same time. Although increased plasma concentrations of proinflammatory cytokines can be found, also present is an impaired monocyte function, which is characterized by a reduced ability to produce tumor necrosis factor (TNF) after lipopolysaccharide stimulation and a decreased HLA-DR production that leads to a reduced antigen-presenting capacity (2, 3). Additionally, an impaired natural killer (NK) cell activity and a shift toward an immune reaction dominated by type 2 helper T cells is typical (4). Although antiinflammatory and immunosuppressive reactions are regarded as physiological and protective responses during stress, the same reactions may be harmful if aggravated (5). Major stress, such as that occurring after trauma, hemorrhage, burns, and cardiac surgery (6), is well recognized to substantially increase the susceptibility to infectious complications (7–9). Although these pathophysiological principles are known and acknowledged, it is still not possible to apply specific therapies. One major reason is the limited ability to adequately assess an individual’s host response. Moreover, immunologic dysregulation does not occur in every patient, and even if such risk factors as comorbidities and age are known, it is not yet possible to predict an individual’s susceptibility. Consequently, different attempts have been made to distinguish high- and low-risk patients by their individual immunologic reactions. Thus far, most
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studies have focused on clinical criteria (10) and measuring known circulating mediators or cell-bound receptors on peripheral blood cells (6, 11, 12); however, no reliable marker or method for risk prediction has been identified.

Real-time reverse-transcription PCR (RT-PCR) analysis allows measurement of mRNA production and thereby the functional status of immune cells. In recent years, RT-PCR analysis has proved its effectiveness in different diagnostic fields and has become readily available for clinical applications. In the present pilot case-control study, we examined the early perioperative expression of 23 selected inflammation-related genes in samples of whole blood from patients undergoing major surgery. We used the concept of postoperative immunodepression as a permissive condition and the results obtained from an analysis of gene expression to establish an assay to predict the risk of sepsis early in the postoperative period.

Materials and Methods

Patients

The protocol was approved by the local ethics committee, and written informed consent was obtained from all patients before surgery. Adult patients with planned major abdominal or thoracic surgery were consecutively enrolled at 3 Berlin centers and monitored for the development of postoperative sepsis and severe sepsis until postoperative day 14. Sepsis and severe sepsis were defined according to the criteria of the American College of Chest Physicians and the Society of Critical Care Medicine (13). We planned to enroll at least 20 patients with postoperative sepsis or severe sepsis.

Blood Sampling, Data Collection, and Matching Procedure

Venous blood samples were collected at 3 times (before surgery and on the first and second postoperative days) with PAXgene™ Blood RNA Tubes [PreAnalytiX (BD/Qiagen)]. PAXgene tubes were frozen immediately at -20 °C and kept at -80 °C until batch processing. Patients’ demographic data, American Society of Anesthesiologists classification, main diagnosis, type of operation, concomitant diseases, laboratory values, microbiological data, vital signs (heart rate, temperature, urine output, blood pressure, and so forth), and clinical criteria of sepsis/severe sepsis were obtained from medical charts and collected into a database. At the end of the study, each patient with postoperative sepsis was matched with a patient without infection according to the following criteria: sex, age, main diagnosis, type of intervention, American Society of Anesthesiologists score, and concomitant diseases. The Sequential Organ Failure Assessment (SOFA) score and the Simplified Acute Physiology Score II (SAPS II) were calculated on the day of sepsis/severe sepsis diagnosis from routinely derived data.

RNA Preparation, cDNA Synthesis, and Real-Time RT-PCR

RNA was prepared with the PAXgene Blood RNA Kit (Qiagen) according to the manufacturer’s instructions. RNA preparation included a 30-min incubation with a bovine deoxyribonuclease to eliminate genomic DNA. RNA concentration and integrity were evaluated with the 2100 Bioanalyzer (Agilent Technologies). cDNA was then synthesized with the Quantitect Reverse Transcription Kit (Qiagen) according to the manufacturer’s instructions; a maximum of 1 μg RNA per sample was used in a total reaction volume of 20 μL. The Quantscript® reverse transcriptase included in the kit was used for cDNA synthesis. The reaction was performed for 28 min at 42 °C in a DNA thermal cycler (PerkinElmer); the reaction was then stopped with a 3-min enzyme-deactivation step at 95 °C. A control with no reverse transcriptase was performed to detect contamination with genomic DNA. The control was analyzed in the PCR step with an intron-specific primer combination. We discarded cDNA samples that had a positive signal in the control with no reverse transcriptase. For gene expression analysis, we selected 23 inflammation-related candidate genes. The first group of genes consisted of cytokine genes and genes related to cytokine signaling: TNFα [tumor necrosis factor (TNF superfamily, member 2)], IL1B [interleukin 1, beta], IL10 [interleukin 10], IL18 [interleukin 18 (interferon-gamma-inducing factor)], SOCS3 [suppressor of cytokine signaling 3], TGFβ1 (transforming growth factor, beta 1), and IL6 [interleukin 6 (interferon, beta 2)]. We also evaluated T cell- and NK cell-related genes, including CD3D [CD3d molecule, delta (CD3-TCR complex)], CD69 [CD69 molecule], PRF1 [perforin 1 (pore forming protein)], GNLY [granulysin], CCR3 [chemokine (C-C motif) receptor 3], KLRK1 (killer cell lectin-like receptor subfamily K, member 1), IDO1 (indoleamine 2,3-dioxygenase 1),...
and KLRD1 (CD94) (killer cell lectin-like receptor subfamily D, member 1). For the assessment of monocyte antigen-presenting capacity, we analyzed 2 genes associated with major histocompatibility complex class II (MHCII), HLA-DRA (major histocompatibility complex, class II, DR alpha) and CD74 (CD74 molecule, major histocompatibility complex, class II invariant chain). We also evaluated chemokine genes that have shown altered expression in septic patients: IL8 (interleukin 8), CXCL10 [chemokine (C-X-C motif) ligand 10], PF4 (platelet factor 4), CCL3 [chemokine (C-C motif) ligand 3], and CXCL1 [chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)]. In addition, we analyzed HMOX1 [heme oxygenase (decycling) 1], which encodes a molecule with cytoprotective facilities, and S100A8 (S100 calcium binding protein A8), which encodes a granulocyte-derived molecule associated with chronic and acute inflammation. cDNA was stored at 20 °C for a maximum of 4 weeks before RT-PCR analysis. Real-time PCR was performed with the QuantiTect Probe PCR Master Mix® (Qiagen) on the GeneAmp 5700 Sequence Detection System® (Applied Biosystems) with the instrument’s analysis software (GeneAmp 5700 SDS 1.3; Applied Biosystems). All primers and probes were designed with Primer Express software (Applied Biosystems) and validated by BLAST search, with the exception of the primers and probes for S100A8, CXCL1, IL18, and PF4, which were purchased commercially. The amplification primers or fluorogenic probes were designed to span exon borders to exclude cross-reactivity with genomic DNA. The PCR reaction was performed in a 13-μL final reaction volume containing 1 μL cDNA, 6.25 μL QuantiTect Master Mix, 0.5 μL fluorogenic hybridization probe, 3 μL primer mix, and 2.25 μL distilled water. After an initial step of 2 min at 50 °C to degrade any contaminating RNA sequences, we performed a denaturation and hot-start step with HotStarTaq™ DNA polymerase (Qiagen) at 95 °C for 10 min. A 2-step PCR thermal profile was used (40 cycles of 15 s of denaturation at 95 °C and 1 min of annealing/extension at 60 °C). In contrast to the 22 genes analyzed with fluoroscence labeled probes, HLA-DRA mRNA production was measured with SYBR Green® dye. The production of HPRT1 (hypoxanthine phosphoribosyltransferase 1) mRNA was used for data normalization (14) according to the expression $2^{-\Delta \Delta Cq}$, where Cq is the quantification cycle. The $2^{-\Delta Cq}$ values of the septic patients were normalized to the arithmetic mean of the mRNA production values for the reference group ($2^{-\Delta \Delta Cq}$ method) (15). The mean Cq values for the genes of interest and HPRT1 were calculated from duplicate assays. Samples were considered negative for gene expression when Cq values were $>40$ cycles. Samples were tested for contamination with genomic DNA and were excluded from the study if they tested positive. Untreated controls (i.e., Master Mix without added cDNA) were included on each plate, untreated control samples with a positive signal (Cq $<40$ cycles) were discarded. All tested samples were positive for the respective cDNA (Cq $<35$ cycles). Additional information on the RT-PCR is available in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol56/issue4.

**STATISTICAL ANALYSIS**

We used SAS 9.0 (SAS Institute) and the SAS macro LD_F2 for nonparametric longitudinal statistical analyses (16, 17). This macro assesses intergroup differences and changes over time. For the genes with statistically significant differences, we used the Wilcoxon paired test in post hoc analyses for postoperative group comparisons and for comparison of preoperative and postoperative values. The method of Bonferroni and Holmes was used for correcting P values, with a starting $P$ level of $<0.05/23$ for the LD_F2 macro (for 23 genes assessed), a $P$ level of $<0.05/10$ for intergroup comparison (5 differentially expressed genes according to the LD_F2 macro), and a $P$ level of $<0.05/80$ (20 genes showing significant differences, LD_F2) for comparison of preoperative and postoperative measurements.

An ROC curve analysis was done for all genes differentially expressed in the 2 study groups. Three genes (IL1B, TNF, and CD3D) that showed the largest median differences between the 2 groups were selected for a logistic regression analysis (SPSS 12.0; SPSS). To produce cutoff values for the combined gene expression assay, we then performed an ROC curve analysis based on the probability derived from the logistic regression. With the objective of optimizing clinical applicability, we included expression values of a single measurement (first day after surgery, because this was the first day with significant differences between the 2 groups) in the logistic regression.

The positive and negative predictive values were estimated from the sensitivity and specificity values for each gene, which were derived from the ROC curve analysis and the prevalence of sepsis in the study population (20 sepsis cases among a total of 220 study patients).

For correlation analysis, the Spearman Rho rank correlation test was used (SPSS 12.0; SPSS).

**Results**

**Patients**

We enrolled 220 eligible patients between September 2003 and May 2005. Four of these patients developed sepsis, and 16 developed severe sepsis. The median
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* Matching criteria were age, sex, American Society of Anesthesiologists (ASA) score, diagnosis, and type of intervention. The matched individuals in the 2 groups were highly similar for the characteristics shown.

a, case group; b, control group.

PPPDr, pylorus-preserving pancreatoduodenectomy; HP, hypertension; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; EsR, esophagus resection; CAD, coronary artery disease; PAOD, peripheral arterial obstructive disease; CA, cardiac arrhythmia.
time to the onset of sepsis/severe sepsis was 6 days (range, 1–14 days). In 14 patients, sepsis was diagnosed after the second postoperative day. In the case cohort, the mean SOFA score was 6.3 (95% CI, 4.4–8.2) and the mean SAPS II score was 36.9 (95% CI, 28.7–45.1) on the day of sepsis/severe sepsis diagnosis. To comply with recommendations of the peer reviewers, we retrospectively gathered preoperative SOFA scores and scores for the first and second postoperative days, when they were available. Mean SOFA scores for the sepsis and control groups were not significantly different preoperatively (n = 28) or on the first postoperative day (n = 21) (1 vs 0.86 and 2.8 vs 1.27, respectively). On the second day after surgery, the SOFA scores for the 2 groups were significantly different (4.1 vs 1.3, P < 0.05; n = 28). Ten patients had to be excluded from the control cohort before matching (n = 210) because of loss to follow-up, withdrawn consent, or an incomplete set of blood samples. Table 1 lists the characteristics of the matched pairs. The 2 groups were not significantly different with respect to either C-reactive protein concentration or leukocyte count at any measurement time. The site of infection was pulmonary in 11 cases, abdominal in 6 cases, and mediastinal, urogenital, and bloodstream in 1 case each. Bacterial sepsis was diagnosed in 10 cases, fungal in 4 cases, and both bacterial and fungal in 2 cases. In 2 cases, sepsis was diagnosed clinically without proof of microbes.

ALTERED POSTOPERATIVE INTERLEUKIN-1β (IL-1β) AND TNF mRNA PRODUCTION IN PATIENTS DEVELOPING SEPSIS

Peripheral blood monocytes from septic patients show a reduced release of proinflammatory cytokines, such as IL-1β, IL-6, and TNF (18). To ascertain whether decreased cytokine release occurs in sepsis patients in the early postoperative period before sepsis develops, we evaluated IL1B and TNF expression. Starting at comparable levels in the 2 groups, IL1B expression increased strongly in the control group, whereas expression in the septic group did not change significantly from preoperative values. Significant differences between the case and control groups were apparent on day 1 after surgery (Fig. 1A). TNF expression was reduced in the sepsis group, in contrast to the control group. This down-regulation was significant on the first postoperative day (Fig. 1B).

DECLINE IN EXPRESSION OF NK CELL- AND T CELL-DERIVED GENES

NK cells and T cells participate in the eradication of invading bacteria, not only by interacting with monocytes but also via direct mechanisms (19). Hence, a reduced activity or a reduction in the number of circulating NK cells and T cells could lead to an impaired defense against microorganisms. mRNA production by T cell–associated genes, including CD3D, CCR3, and CD69, and by NK cell–associated genes (KLRD1, KLRK1, and PRF1) shows similar time courses, with highly significant decreases in mRNA production ap-

Fig. 1. Altered IL1B and TNF mRNA production in patients developing sepsis.

(A), The postoperative increase in IL1B expression was impaired in the patients who developed sepsis. After surgery, the control patients showed increased expression that was significant on day 2 compared with the preoperative value. Although gene expression was comparable in the 2 groups before surgery, the median postoperative mRNA quantity was significantly decreased in the sepsis group compared with the controls. (B), Reduced TNF expression in the sepsis group over all 3 days. The median mRNA quantity was significantly decreased in the sepsis group compared with the preoperative value. Data are presented as the medians and 25th and 75th percentiles. Indicated are significant differences between different time points in a group (*) (P < 0.000 625) and significant differences between the groups (P < 0.005) at the indicated time point (#). Sepsis and control groups are indicated by gray and black lines, respectively.
parent between the preoperative and postoperative time points in both investigated groups (Table 2). This observation suggests a general effect of surgical intervention on the 2 groups, a finding consistent with published studies (4, 20). We found decreased mRNA production in the sepsis group for CD3D, PRF1, KLRD1, and KLRK1 but noted no such differences for the remaining genes. CD3D and PRF1 expression showed the most pronounced differences (Fig. 2, A and B), which were statistically significant. Interestingly, we noted a strong positive correlation between the postoperative expression of CD3D, which encodes a T cell surface antigen, and the postoperative expression of other T cell– or NK cell–related genes (r/H11005 0.471–0.884), which may reflect the influence of changes in lymphocyte cell counts on the quantity of mRNA in whole blood. The correlation between CD3D cDNA and leukocyte counts was not significant, however, whereas PRF1 expression was the only gene noted to exhibit a minor negative correlation with white blood cell counts preoperatively and on the first day after surgery [r = −0.36 (P = 0.021, preoperatively), and r = −0.34 (P = 0.031, on the first postoperative day)].

**EXPRESSION MARKERS WITH NO SIGNIFICANT DIFFERENCES BETWEEN GROUPS**

For most of the tested genes, we found no significant differences between the patients who developed sepsis and those who did not. These genes encoded pro- and antiinflammatory cytokines (IL10, IL18, TGFB1, IL6) and the gene encoding a suppressor of cytokine signaling (SOCS3), which is associated with intracellular TNF/IL-6 signaling. Interestingly, we also found no differences for HLA-DRA and CD74, which are associated with the MHCII. The genes encoding chemokines (IL8, CXCL10, PF4, CCL3, and CXCL1) were expressed similarly in the 2 groups.

**PREDICTIVE PROPERTIES OF CANDIDATE MARKERS**

Sepsis therapy should begin as early as possible (21). Discrimination of patients with and without risk of sepsis early in the postoperative course could help in initiating preemptive therapy, even before the occurrence of clinical sepsis symptoms. To evaluate the diagnostic capacity of the gene expression analysis, we performed an ROC curve analysis for the most promising genes in the study (Table 3). We included only the genes with statistically significant differences between the 2 groups in the analysis, as described above. This group consisted of the TNF, IL1B, CD3D, and PRF1 genes. To try to obtain a diagnosis as early as possible, we analyzed the relative expression of these genes on the first day after surgery. Cutoff values for the identification of case patients were derived from the ROC curve analysis. From the expression data for TNF, IL1B, CD3D, and PRF1 genes, we performed a logistic regression analysis and assigned a calculated probability value for each patient (Nagelkerke’s R², 0.660; Hosmer–Lemeshow significance, 0.116). A subsequent ROC curve analysis based on these values (Fig. 3) defined a cutoff that identified patients with contingent sepsis with a sensitivity of 85% and a specificity of 90%.

### Table 2. Overview of the results for 4 genes.a

<table>
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<th>First postoperative day</th>
<th>Second postoperative day</th>
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<tr>
<td>IL1B</td>
<td>Case 0.57 (0.11–2.57)</td>
<td>0.82 (0.15–1.52)b</td>
<td>0.83 (0.11–4.44)</td>
</tr>
<tr>
<td></td>
<td>Control 0.64 (0.27–2.66)</td>
<td>1.44 (0.52–4.14)b</td>
<td>1.93 (1.11–4.5)c</td>
</tr>
<tr>
<td>TNF</td>
<td>Case 0.33 (0.16–0.85)</td>
<td>0.14 (0.03–0.52)b</td>
<td>0.21 (0.04–0.46)</td>
</tr>
<tr>
<td></td>
<td>Control 0.53 (0.03–1.62)</td>
<td>0.39 (0.16–0.8)b</td>
<td>0.4 (0.18–0.92)</td>
</tr>
<tr>
<td>CD3D</td>
<td>Case 4.52 (1.68–10.89)</td>
<td>0.94 (0.18–2.44)b,c</td>
<td>0.84 (0.14–12.64)c</td>
</tr>
<tr>
<td></td>
<td>Control 5.01 (1.17–7.89)</td>
<td>2.22 (1.05–4.42)b,c</td>
<td>1.87 (0.59–4.5)c</td>
</tr>
<tr>
<td>PRF1</td>
<td>Case 5.69 (1.34–11.04)</td>
<td>1.06 (0.26–5.01)b,c</td>
<td>1.23 (0.33–11.47)c</td>
</tr>
<tr>
<td></td>
<td>Control 5.89 (1.12–13.5)</td>
<td>2.12 (0.55–9.48)b,c</td>
<td>2.36 (0.61–9.85)c</td>
</tr>
</tbody>
</table>

*a Data are presented as median 2−ACq values, as normalized to HPRT1 gene expression. Ranges are in parentheses.

b Significant differences between the 2 groups (P < 0.005).

c Significant differences between the pre- and postoperative time points (P < 0.000 625).
Discussion

Real-time RT-PCR analysis is a relatively new technique in medical diagnostics. It allows highly sensitive quantification of mRNA in samples. In recent years, it has been applied to routine diagnostics in different ways, such as in quantifying viral load in HIV or Epstein–Barr virus infections. In the present study, we assessed RT-PCR analysis as a diagnostic tool for monitoring the immune system in perioperative patients by quantifying the expression of 23 genes related to inflammation. Cytokine-encoding genes TNF and IL1B and the T cell– and NK cell–related genes CD3D and PRF1 were differentially expressed in a group of patients who developed postoperative sepsis at a median of 5 days before the clinical diagnosis of sepsis. From a logistic regression analysis of TNF, CD3D, and IL1B expression, we were able to predict sepsis with a specificity of 90% and a sensitivity of 85%. Control patients matched for relevant baseline parameters exhibited similar SOFA scores preoperatively and on the first day after surgery. This matching minimizes the influence of disease severity as a confounding factor on gene expression.

TNF and IL1B play a pivotal role in the host’s response to invading microorganisms. Although earlier concepts of the pathophysiology of sepsis regarded these cytokines mainly as mediators of shock and cell damage, newer findings have demonstrated that a certain level of production of these cytokines is necessary for a patient to survive sepsis. In this context, Riese et al. found an association between complications and a postoperatively reduced capacity of macrophages to secrete cytokines.

In a group of patients with an uncomplicated postoperative course, Hensler et al. found an increased monocytic release of IL-1β postoperatively upon lipopolysaccharide stimulation, whereas the TNF production remained unchanged in these cells. Although secretion of the latter cytokine by type 1 helper T cells was down-regulated, these results are generally consistent with the present gene expression data. The similar results obtained for septic patients underline the pathophysiological relationship between infectious and noninfectious inflammatory responses.

NK cells and cytotoxic T cells synergistically interact with macrophages in the clearance of bacterial infection. NK cell–produced interferon γ potentiates the antimicrobial activity of macrophages. An increased number of NK cells is associated with a longer survival of patients with severe sepsis, and a reduced number of NK cells after severe injury promotes the development of subsequent sepsis. Granulysin has a direct antimicrobial effect in vitro against fungi and gram-positive and gram-negative bacteria, and the lysis of mycobacteria by granulysin is intensified in the presence of perforin 1. In this context, the decreased expression of such genes as CD3D and PRF1 in T cells and NK cells in patients who developed sepsis in our study suggests a pivotal role of cell-mediated immunity in the risk for postoperative sepsis. A strong correlation between most of the expression data for NK cell/T cell–related genes evaluated in this study suggests an important influence of changes in leukocyte subpopulations on the measurements we have presented.
Because the study design did not include data for differential blood cell counts or the measurement of leukocyte subpopulations by fluorescence-activated cell sorting or similar methods, the pathophysiological interpretation of the results is somewhat limited. Differences in the amounts of mRNA measured can be caused by up- or down-regulation of transcription in the leukocytes or by differences in the leukocyte subpopulations between the case and control groups. Interestingly, we found no significant differences in the expression of MHCII-related genes. Decreased expression on monocytes of genes encoding HLA-DR antigens is a common finding in septic patients and constitutes a reliable marker of immunodepression (28, 29); however, the surface expression of HLA-DR antigens is subject to several posttranscriptional mechanisms (30), which may explain differences in the expression of these genes and the quantities of these antigens on cell surfaces. Nevertheless, Pachot et al. found decreased production of mRNAs for MHCII-associated genes in whole blood samples from patients in a state of septic shock, compared with the production in samples from healthy volunteers (31). Similarly, we found the postoperative production of HLA-DRA mRNA in both groups to be reduced compared with preoperative values, a finding that reflects the situation before and after an inflammatory stimulus. Given that the HLA-DRA gene is expressed in different kinds of circulating blood cells, the down-regulation seen on monocytic cell surfaces in immunocompromised patients may be blurred in samples of whole blood.

In the past, 2 different approaches have been taken for predicting the perioperative risk of postoperative sepsis. A number of investigators focused on the measurement of plasma cytokine concentrations, whereas others approached the problem by quantifying the expression of HLA-DR on monocytic surfaces via fluorescence-activated cell-sorting analysis. Whereas assays based on cytokine measurements do not allow identification of patients at risk with sufficient specificity and sensitivity, fluorescence-activated cell-sorting analysis of monocytic HLA-DR has proven effectiveness in different clinical settings, including in a multicenter approach (32–34). Nevertheless, this technique requires the preparation of blood samples directly after obtaining them, which limits its applicability in day-to-day clinical diagnostics. Blood withdrawal into PAXgene tubes requires rapid transport and cool storage (35), which are available in most hospitals in Western

**Table 3. High sensitivity and specificity of the combined gene expression assay.**

<table>
<thead>
<tr>
<th>Gene (cutoff)</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>PPV</th>
<th>NPV</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3D (1.04)</td>
<td>100%</td>
<td>60%</td>
<td>100%</td>
<td>95.5%</td>
<td>0.844 (0.723–0.965)</td>
</tr>
<tr>
<td>PRF1 (1.26)</td>
<td>85%</td>
<td>70%</td>
<td>35.4%</td>
<td>96.0%</td>
<td>0.814 (0.680–0.948)</td>
</tr>
<tr>
<td>IL1B (1.03)</td>
<td>80%</td>
<td>70%</td>
<td>29.2%</td>
<td>95.8%</td>
<td>0.805 (0.671–0.939)</td>
</tr>
<tr>
<td>TNF (0.15)</td>
<td>100%</td>
<td>60%</td>
<td>100%</td>
<td>95.5%</td>
<td>0.869 (0.758–0.979)</td>
</tr>
<tr>
<td>Combination assay (0.60)</td>
<td>90%</td>
<td>85%</td>
<td>47.2%</td>
<td>98.3%</td>
<td>0.918 (0.826–1.009)</td>
</tr>
</tbody>
</table>

* The gene expression assay for each gene was able to predict sepsis with an average sensitivity and specificity. Results for the combination assay (for 3 genes: CD3D, IL1B, and TNF) were defined as positive if the mRNA quantity fell below the cutoff for ≥2 genes. The combination assay was able to predict sepsis with high specificity and sensitivity. Sensitivity and specificity represent maximal accuracy according to ROC curve analysis. The negative predictive value (NPV) and the positive predictive value (PPV) are estimated from the sensitivity and specificity, as indicated in the table and a prevalence of 20 cases of sepsis in a study population of 220 patients. AUC, area under the ROC curve.

![Fig. 3. ROC curves for the assay for the combination of 3 genes (TNF, CD3D, IL1B).](Image)

A maximum specificity of 90% and a maximum sensitivity of 85% were achieved. See Table 3 for the area under the ROC curve, confidence intervals, and positive and negative predictive values.
industrialized countries. This technique therefore provides a useful supplement to the established methods. Despite the promising results, the data we have presented are subject to certain limitations. Because of the retrospective study design, all of the data and the derived cutoff values require a prospective validation. The study setting limits the application of the gene expression assay to perioperative patients. Furthermore, the number of patients is relatively small for logistic regression, indicating a risk of overfitting the data to the study population and possibility limiting the applicability of the assay to other patient populations. Therefore, a prospective multicenter approach with a larger study population would be preferable for validating the presented data.

Patients undergoing major surgery are at high risk for postoperative sepsis. Provided that the data presented here can be confirmed prospectively, the described RT-PCR assay offers the possibility of identifying patients at high risk for septic complications. It is based on a simple, easy-to-perform blood test, without having to consider such clinical symptoms as the criteria for systemic inflammatory response syndrome. This assay could open the way to the early application of preemptive therapies and thereby help in reducing postoperative mortality due to infectious complications.

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