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Admission Chemokine (C-C motif) Ligand 4 Levels Predict Survival in Pediatric Septic Shock

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Abstract

Objective—Stratification with an effective outcome biomarker could improve the design of interventional trials in pediatric septic shock. The objective of this study was to test the utility of chemokine (C-C motif) ligand 4 as an outcome biomarker for mortality in pediatric septic shock.

Design—A cross-sectional, observational study.

Setting—18 Pediatric Intensive Care Units in the United States of America.

Patients—156 pediatric patients with septic shock.

Interventions—Serum samples were obtained within 24 hours of admission to the Pediatric Intensive Care Unit. Serum levels of chemokine (C-C motif) ligand 4 were measured via ELISA and compared to mortality in a training set of 34 patients. These data were used to generate a cut-off value whose utility was evaluated through prospective application—without post-hoc modification—to a larger validation set of 122 patients.

Measurements and Main Results—Upon inspection of the training set data, a cut-off value of 140 pg/mL was chosen. When applied to the validation set, serum chemokine (C-C motif) ligand 4 levels greater than 140 pg/mL yielded a sensitivity of 92% and a specificity of 40% for mortality. A serum level of 140 pg/mL or less had a negative predictive value for mortality of 98%.

Conclusions—A serum level of chemokine (C-C motif) ligand 4 of 140 pg/mL or less, when obtained within 24 hours of admission, predicts a very high likelihood of survival in pediatric septic shock. Exclusion of patients with a chemokine (C-C motif) ligand 4 level of 140 or less from interventional clinical trials in pediatric septic shock could create a study population in which survival benefit from the study agent could be more readily demonstrated.

Keywords

Chemokine CCL4; Biological Markers; Sepsis; Shock; Septic; Pediatrics; Clinical Trials as Topic

INTRODUCTION

Pediatric septic shock is a major public health concern, afflicting over 42,000 children, and resulting in nearly \$2 billion in costs per year in the United States alone (1,2). Pediatric septic

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shock—the most severe manifestation of pediatric sepsis—kills over 4,000 children per year in the United States (1). Unfortunately, to date no specific therapies for pediatric septic shock have been successfully developed. Thus, treatment is limited to antibiotics and supportive care in a Pediatric Intensive Care Unit (PICU).

Development of specific therapies for septic shock in patients of any age is complicated by the fact that sepsis is a syndrome, rather than a specific disease entity. As such, there are heterogeneous underlying etiologies and pathophysiologies. Research trials for septic shock in pediatrics are further hampered by the lower incidence and mortality rates for sepsis in the pediatric population, compared to the adult population (1–3). The lower mortality rate in pediatric septic shock implies that even if a therapy had the same relative risk reduction in pediatric and adult septic shock, a larger sample size would be needed to demonstrate a statistically significant reduction in mortality in the pediatric population (4). The lower incidence of pediatric septic shock, however, often prohibits enrollment of such a large sample size.

One potential solution to this dilemma would be the development of biomarker-based stratification strategies allowing for the early and efficient identification of low or high risk patients (5). For example, excluding patients with a very low risk of mortality would create a study population with a higher mortality rate, and thus a smaller sample size would be needed to achieve the same statistical power for detection of any treatment effects (4).

In an ongoing translational research program involving microarray-based, genome-wide expression profiling in children with septic shock, we reported that chemokine (C-C motif) ligand 4 (CCL4; a.k.a. MIP-1 β) messenger ribonucleic acid (mRNA) levels, measured within 24 hours of admission to the PICU, were increased in non-survivors of pediatric septic shock, relative to the levels in survivors (6). Given that CCL4 protein is easily measured in serum, and a correlation between CCL4 protein level and outcome is biologically plausible, we felt that it could be a useful biomarker for the stratification strategies mentioned above. The current study was undertaken to test the hypothesis that the serum level of CCL4 protein, measured within the first 24 hours of admission to the PICU is a robust outcome biomarker in pediatric septic shock.

MATERIALS AND METHODS

Genomics of Pediatric Septic Shock Database

The Genomics of Pediatric Septic Shock (GPSS) Database was used to generate the data sets. The GPSS database has been previously described in detail (6–8). The study protocol was approved by the individual institutional review boards of each participating institution. Briefly, this noninterventional database supports a translational research program focused on genome-level expression profiles (microarray-based) of children with septic shock. Eighteen PICUs in the United States have contributed samples to the GPSS (see Acknowledgement). The database contains extensive clinical data, RNA samples, and concomitant serum samples. RNA and serum samples are collected within 24 hours of admission to the PICU and 48 hours after the initial sample collection. In the current analyses, we used only the serum samples drawn within 24 hours of admission to the PICU. The subjects enrolled in the GPSS database include children (≤ 10 yr of age) admitted to the PICUs of multiple institutions who have a diagnosis of septic shock. Pediatric-specific criteria are used to classify patients as having septic shock (9), and the only exclusion criteria are the inability to obtain informed consent or a white blood cell count of less than 1,000 cells/mm³. Standard clinical care is not under protocol for the enrolled subjects, and is left to the discretion of the attending physician. Patients are monitored for 28 days to determine survival. Accordingly, all mortality-related data in this report refer to this 28-day period.

CCL4 Measurements

CCL4 measurements were conducted using a Quantikine Human MIP-1 β ELISA kit as per the manufacturer's instructions (Catalog number DMB00, R&D Systems, Minneapolis, MN, U.S.A.).

Statistical Analysis

Receiver operating curves (ROCs) were generated using Stata/SE 10.0 for Windows (StataCorp LP, College Station, TX, U.S.A.).

Specificities, sensitivities, positive predictive values, negative predictive values, and their respective confidence intervals were calculated using the VassarStats website (<http://faculty.vassar.edu/lowry/VassarStats.html>, accessed on 10/15/2008). Mortality odds ratios and their confidence intervals were calculated using the online calculator developed by David J R Hutchon (<http://hutchon.net/confidOR.htm>, accessed on 11/12/2008). Continuous variables were compared by Student's t-test or Mann-Whitney Rank Sum Test as appropriate. Categorical variables were compared by χ^2 test. When indicated, significance was set at 0.05.

RESULTS

Patient Characteristics

The characteristics of both the training and validation data set patients are shown in Table 1. There were no significant differences between the two data sets in any of the parameters measured.

Training Data Set

CCL4 protein measurements were initially performed for a set of 34 previously reported patients (27 survivors and 7 non-survivors) (6). These data were used to generate a receiver operating curve (ROC) (Figure 1) which was used to evaluate the utility of CCL4 levels for the prediction of mortality and to generate cutoff values for subsequent validation studies. This ROC had an area under the curve (AUC) of 0.698 (95% confidence interval [CI] 0.513–0.844). Using these data, a CCL4 level greater than 140 pg/mL had 86% sensitivity (95% CI, 42–99%) and 44% specificity (95% CI, 26–64%) for predicting mortality. The positive and negative predictive values for mortality were 29% (95% CI, 12–52%) and 92% (95% CI, 62–100%), respectively, for this training patient cohort. The CCL4 concentration cutoff of 140 pg/mL was used—without *post hoc* modifications—for all further analyses.

Validation Data Set

In order to prospectively evaluate the ability of a CCL4 level of 140 pg/mL to discriminate between survivors and non-survivors, we measured the CCL4 level for all patients in the GPSS database with available serum samples. For this validation we excluded the patients who were used in the training data set, leaving 122 patients (109 survivors and 13 non-survivors). Figure 2 shows the ROC generated from these data, which had an AUC of 0.798 (95% CI, 0.713–0.863). Figure 3 represents the contingency table for these patients, with mortality and CCL4 level greater than 140 pg/mL as the contingency variables. A CCL4 concentration greater than 140 pg/mL had 92% sensitivity (95% CI, 62–100%) and 40% specificity (95% CI, 31–50%) for mortality. The positive predictive value for mortality was 16% (95% CI, 9–26%); however, the negative predictive value for mortality was 98% (95% CI, 87–100%).

Performance Across All Patients

In this analysis, the data from both the training and validation data sets were combined, and the performance of the pre-specified CCL4 cutoff was evaluated. A CCL4 concentration

greater than 140 pg/mL had 90% sensitivity (95% CI, 67–98%) and 41% specificity (95% CI, 33–50%) for mortality. The positive predictive value for mortality was 18% (95% CI, 12–28%), and the negative predictive value for mortality was 97% (95% CI, 87–99%).

Within this combined patient set, we then separated patients *post hoc* into two groups based upon their serum CCL4 concentration—one group with a concentration less than or equal to 140 pg/mL (n=58) and another group with a concentration greater than 140 pg/mL (n=98). As shown in Figure 4, the group with CCL4 less than or equal to 140 pg/mL had a significantly lower 28-day mortality rate (3% vs. 18%, $p=0.01$) and lower odds ratio for mortality (0.16 vs. 6.30) when compared with the group with CCL4 greater than 140 pg/mL.

DISCUSSION

CCL4 is a member of the CC chemokine family, and is produced by numerous cell types including monocytes, neutrophils, T and B lymphocytes, NK cells, and dendritic cells (10, 11). It plays a key role in chemotaxis as well as enhancement of the inflammatory response (10). CCL4 levels have been shown to increase during human endotoxemia and sepsis (12), and increased levels of CCL4 have been associated with mortality in patients with Sudan Ebola virus (13) and children with cerebral malaria (14). To our knowledge, no study has evaluated the relationship between CCL4 and mortality in pediatric or adult septic shock.

Our microarray data show that CCL4 mRNA levels are increased in non-survivors of pediatric septic shock, relative to survivors (6). The present study is founded on these original microarray data and was developed to test the hypothesis that the serum CCL4 protein level is a robust outcome biomarker when measured early in the course of pediatric septic shock.

Initial protein measurement performed in a smaller (training) sample set, from which the original microarray data were generated, confirmed the relationship between CCL4 mRNA expression, CCL4 serum protein levels, and mortality. These data were used to derive a cut-off value of 140 pg/mL, which was used in all subsequent analyses, *without post-hoc modifications*. When this cut-off value was applied to the larger (validation) sample set, the specificity and positive predictive value for mortality were not sufficiently robust to be useful; however, the sensitivity and negative predictive value for mortality were much stronger—both had values greater than 90%. The negative predictive value in particular was a very robust indicator—predicting survival with 98% certainty. Our group recently reported that interleukin-8 has a robust negative predictive value (95%) when used as an outcome marker in pediatric septic shock (15); however, the negative predictive value of CCL4 exceeds even that of interleukin-8, and we are unaware of any other biomarkers that have been shown to have a negative predictive value that is as robust. Thus far, our attempts at combining these two biomarkers have failed to yield additional utility. Further, clinical scoring systems such as the Pediatric Risk of Mortality III were designed for assessing risks of populations, and are not intended for mortality predictions of individual patients (16).

One could question the utility of a biomarker which predicts survival rather than mortality. We believe the main utility would lie in the design of future interventional clinical trials in pediatric septic shock. Through the exclusion of patients who otherwise meet entry criteria but have a very low mortality risk based on their CCL4 level, a study population with a higher mortality rate is established. As mentioned above, this higher mortality rate would allow for smaller sample sizes to achieve the same statistical power for the detection of treatment effects. Further, exclusion of patients who would survive with conventional treatment shields these patients from any potential risks of an experimental therapy that carries more than minimal risk. In a clinical setting, a test which predicts survival from sepsis with a high degree of confidence could be used to provide re-assurance to family members.

A limitation of this study is that it was performed on samples from a single database consisting of samples from children ≤ 10 years old. Thus, the results may not be generalizable to adults, adolescents, or populations that are significantly different from this database. This limitation is lessened by the fact that the GPSS database is comprised of samples from 18 separate PICUs within the United States. Also, the liberal inclusion criteria for the GPSS database make these results more generalizable across different patient populations. All children 10 years old or younger who meet the criteria for septic shock are eligible to enroll in GPSS, irrespective of their immunosuppression, chemotherapy, or transplantation status—the only exclusion criterion is a white blood cell count less than 1,000 cells/mm³, which precludes isolation of sufficient RNA for microarray analysis. Nonetheless, validation of our results in an independent set of pediatric patients with septic shock would be ideal.

CONCLUSIONS

We have demonstrated a relationship between serum levels of CCL4 protein and mortality in pediatric septic shock. Further, we have demonstrated that a serum CCL4 protein level of 140 pg/mL or less is a robust negative predictor for mortality in pediatric septic shock. This information can be used to design interventional clinical trials in pediatric septic shock which may require a smaller sample size and have a more favorable risk to benefit ratio.

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Figure 1.

Receiver operating curve (ROC) of chemokine (C-C motif) ligand 4 protein level as a predictor of 28-day mortality in the training set of 34 children with septic shock (7 non-survivors). Area under the curve = 0.698; 95% confidence interval = 0.513–0.844.

Figure 2.

Receiver operating curve (ROC) of chemokine (C-C motif) ligand 4 protein level as a predictor of 28-day mortality in the validation set of 122 children with septic shock (13 non-survivors). Area under the curve = 0.798; 95% confidence interval = 0.713–0.863.

Figure 3. Contingency table depicting sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of chemokine (C-C motif) ligand 4 (CCL4) cut-off of 140 pg/mL for 28-day mortality when prospectively applied to the validation data set.

Figure 4.

A chemokine (C-C motif) ligand 4 (CCL4) cutoff value of 140 pg/mL separates children with septic shock into two groups with significantly different risks for 28-day mortality ($p=0.01$, χ^2). Data generated from 156 patients (combining training and validation data sets).

Table 1

Characteristics Of Patients In Training And Validation Data Sets.

	Training Data Set	Validation Data Set
No. of patients	34	122
No. of deaths (%)	7 (21)	13 (11)
Median age, yr (IQR)	2.5 (1.0–3.9)	2.3 (0.8–5.4)
Median PRISM score (IQR)	18 (12–23)	15 (8–21)
Sex, no. male/female	22/12	73/49
Ethnic origin: n (%)		
White	21 (62)	88 (72)
African descent	8 (23)	23 (19)
Asian	3 (9)	2 (2)
Native American	0 (0)	1 (1)
Other (mixed racial)	2 (6)	8 (7)
Median CCL4, pg/mL (IQR)	160 (121–361)	194 (102–443)
No. of patients with CCL4>140 pg/mL (% mortality)	21 (24)	77 (16)

Definition of abbreviations: CI = confidence interval; IQR = interquartile range; PRISM = Pediatric Risk of Mortality; CCL4 = chemokine (C-C motif) ligand 4.

No differences are significant.