The use of a cytokine panel to define the long-term risk stratification of heart failure/death in patients presenting with chest pain to the emergency department

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Abstract

Objective—To determine if a cytokine panel could be informative regarding subsequent heart failure (HF)/death.

Design and methods—In 216 subjects presenting with chest pain to an emergency department in 1996, EDTA plasma (−70 °C) was thawed for IL-6, MCP-1, IL-10, VEGF, EGF measurement.

Results—Subjects with any three cytokines elevated were at higher risk for HF/death compared to those with two cytokines elevated.

Discussion—A cytokine panel might be useful for risk stratification for HF/death.

Keywords

Acute coronary syndrome; Cytokine panel; Heart failure; Risk stratification; ED

Introduction

Inflammation, which is often assessed by measuring C-reactive protein (CRP) in patients presenting with acute coronary syndrome (ACS), has been shown to be an important predictor for subsequent heart failure (HF) and death [1]. However, more specific and earlier mediators of the inflammatory process (e.g., IL-6, MCP-1) may provide better or additional independent information [2]. In addition to IL-6 and MCP-1 there are other cytokines that may be measured that reflect or are indices of inflammation, cardiac fibrosis and cardiomyocyte function that have not been evaluated in this context (e.g., IL-10, VEGF, EGF) [3–6]. For the present study the objective was to test the hypothesis that a combination

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of these biomarkers (i.e., IL-6, MCP-1, IL-10, VEGF, and EGF as a panel test) could be used to identify those at high risk for future HF/death.

Methods

The study population and its characteristics have been previously reported [2,7]. Briefly, after ethics approval in 1996, consecutive unique patients presenting to the emergency department with symptoms suggestive of ACS were recruited for a cardiac marker study. For the present study, only the EDTA plasma specimen collected closest to 9 h post onset of chest pain (median time (interquartile range)=9 h (9–9); $n=216$ subjects) was used, consistent with our previous analyses with respect to HF/death outcomes [2].

Biomarker measurements

With renewed research ethics board approval, the EDTA plasma specimens were thawed for the first time in 2006 and a cytokine array was measured using the evidence investigator™ (Randox Ltd) biochip platform [2]. For the biochip analysis we prospectively decided to only include the following five biomarkers: IL-6, MCP-1, IL-10, VEGF and EGF. These were selected because there are data to support their roles in ACS and subsequent HF/death outcomes, and their long-term stability in storage has been demonstrated. Specifically, there is indirect evidence of the stability of these cytokines after 10 years of storage in that the reference ranges for MCP-1, VEGF, and EGF published in 2006 for this method were derived from samples collected as early as 1994 [8]. IL-6 and IL-10 also appear to be stable during long-term storage [9]. The observed interassay ($n=20$ assays) imprecision (CV) in our laboratory with the cytokine biochip, determined by measuring three levels of quality control material, ranged from 7% to 16% for the biomarkers. After measurement by the cytokine array, the same EDTA plasma specimens were assayed for NT-proBNP with the Elecsys® 1010 Roche analyzer (interassay CV<7%). This patient cohort previously had cTnI measured (AccuTnI, Beckman Coulter) in heparin specimens in 2003 [7], and both NT-proBNP and cTnI are stable during long-term storage [2,7]. As we had prior data suggesting that both NT-proBNP and cTnI add to the prognostic accuracy of risk prediction, we included these biomarkers in the hazard analysis models. [2].

Panel interpretation

The five biomarkers: IL-6, MCP-1, IL-10, VEGF and EGF may have different biological functions and perhaps different roles that may be synergistic and/or complementary to each other, which could enhance the risk for patients for subsequent HF/death.

Reference intervals have not been determined for either IL-6 or IL-10 with this array. However, for MCP-1, VEGF, and EGF there has been one publication with respect to reference intervals (e.g., males): MCP-1 156 ng/L, VEGF 142 ng/L (both at the 97.5th percentile), and for EGF 0.9 ng/L (2.5th percentile) [8]. However, to assess all the biomarkers in the present study equally, a decision was made to score them with respect to the median concentration in the cohort. Therefore, for this analysis, a decision was made to score each biomarker as positive if its concentration was on the side of the median associated with higher risk (i.e., above the observed median for IL-6, IL-10, VEGF and

MCP-1, and below the median for EGF). The panel of five biomarkers for each subject was then scored positive if any three biomarkers were positive. We set the panel cutoff at three positive biomarkers on the basis of prior observations of likelihood ratios (LR) for HF or death at 8 years [2]. We previously reported an interaction with IL-6 and MCP-1 for predicting HF/death (LR=22.7, $p=0.066$ as compared to IL-6 alone) [2]. Addition of VEGF, IL-10, EGF substantially increased the LR (i.e., the combination of five cytokines yielded a LR of 48.2 ($p<0.025$) compared to IL-6 and MCP-1). Panels of four biomarkers also yielded significant LRs ($p<0.025$), whereas panels of three biomarkers yielded varied significance as compared to panels of two biomarkers (e.g., IL-6, MCP-1, VEGF yielded a LR=30.4, which was significant as compared to IL-6 and VEGF ($p<0.05$) but not significant as compared to IL-6 and MCP-1 ($p>0.05$)). Therefore, for the present exploratory study we hypothesized that a minimum of three positive biomarkers in a panel would be necessary to improve risk stratification. To test this hypothesis, patients with three or more positive cytokines were compared to those with less, with HF/death as the combined endpoint.

Health outcomes and statistical analysis

Health outcome were obtained by linkages to the Registered Persons Data Base (RPDB) for mortality outcomes and the Canadian Institute for Health Information Discharge Abstract Database (CIHI-DAD) for hospital discharges associated with HF [2,7]. Both the RPDB and CIHI-DAD (i.e., administrative databases) have been reported to be highly accurate in obtaining these endpoints [2,7]. Based on the death date and earliest subsequent readmission for HF, indicators were created to reflect whether or not an event (death or HF readmission) occurred within 8 years post presentation (patients who died without previous HF readmission, follow-up were censored at the date of death). Kaplan–Meier curves were constructed to display time to an event (HF/death), with differences between groups assessed by the log rank test. The Cox proportional hazard model was used to compare time to an event for those with three positive biomarkers (panel positive) versus those with fewer (panel negative), after adjusting for age (continuous variable), sex, history of HF, NT-proBNP, and cTnI, the latter in accordance with previously published cutoffs: NT-proBNP >183 ng/L in the 9 h specimen and cTnI peak categories (<0.02 µg/L as reference) [2,7]. Between-group comparisons of central tendency were based on the Wilcoxon signed rank test and the chi-square test was used to test differences between proportions. Analyses were performed using SAS and GraphPad Prism.

Results

For the 216 subjects (61% male), the median (IQR) age was 66 years (53–76). Spearman correlation analysis of pairings between all five biomarkers revealed that IL-6 correlated with the other cytokines best, and EGF the least. However, none of the cytokine pairings was strongly correlated ($r<0.40$).

Kaplan–Meier analysis assessing individuals with a panel positive test (three or more positive biomarkers; $n=99$ subjects) demonstrated a greater probability for HF/death over the 8 years following the emergency department presentation compared to those with a negative panel ($n=117$) ($p<0.001$, Fig. 1). Of note, there were no differences in cTnI and NT-proBNP

concentrations between the subjects who had three or two biomarkers positive (median cTnI = 0.03 µg/L vs. 0.03 µg/L, $p = 0.091$; median NT-proBNP=189 ng/L vs. 179 ng/L, $p = 0.987$, respectively). The proportion of males versus females (44% vs. 49% with positive panel, respectively; $p=0.48$), those with history of MI (22% vs. 32%, $p=0.12$), and those with a diagnosis of MI during the presentation (20% vs. 19%, $p=0.80$) between the positive and negative panel groups were also not different. Cox proportional hazard models after adjusting for age, sex, history and the cardiac biomarkers yielded significant hazard ratios for the positive panel group versus the negative group at 6 months, 2 and 8 years (Table 1).

Discussion

This study is different from others in that the panel of cytokines (IL-6, MCP-1, IL-10, VEGF, EGF) was measured relatively early after the onset of pain, reducing the possibility that the cytokine levels reflected a response to the extent of myocardial injury. This independence is important, since during ACS many physiological processes are likely at play, and one would surmise that several pathological events are ongoing, such as the activation and recruitment of leukocytes for the inflammatory process, the development of fibrosis, and alterations in myocyte function. For example, the EGF family including its cellular receptors (specifically Erb2 or HER2) appear to have a role in normal cardiac physiology. The loss of cardiac ErbB2 can cause a dilated cardiomyopathy which coincides with the data about EGF inhibitors and heart failure in patients with breast cancer [3]. Both IL-10 and VEGF have been reported to have a role in the development of ACS and in subsequent health outcomes [4,5]. *In vitro* data support the role of these proteins in influencing cardio-myocyte phenotype [6]. Furthermore, our previous data have demonstrated that both IL-6 and MCP-1 are useful for risk stratification for HF/death [2]. Thus, this study is the first to assess the combination of all five biomarkers as a panel test for predicting HF/death in an ACS population (i.e., a population at high risk of myocardial injury).

There are some important points and study limitations to consider when evaluating this panel test. First, the cytokine panel must be compared against other emerging biomarkers that have also been shown to be independent predictors of subsequent HF (e.g., growth differentiation factor-15) [10]. Furthermore, as we have previously shown an improvement when NT-proBNP was added to IL-6 and MCP-1 for risk stratification, studies of other models are warranted that include heart failure biomarkers with different combinations of the cytokines [2]. For example, the combination of IL-6, MCP-1 and NT-proBNP at 8 years yielded a LR of 43.6 compared to the LR of 48.2 with all five cytokines ($p>0.10$). From the present initial study, it is not certain the magnitude of effect the panel would have when added to NT-proBNP, however, one conceivable advantage of the cytokine panel is the possibility that additional therapeutic targets may be identified by measurement of the panel during the acute event, which is currently a major limitation for measuring NT-proBNP in the ACS setting. Second, at the time of this analysis we used a cTnI assay with conventional sensitivity; next generation, high-sensitivity cTnI assays might have different utility. A third limitation in our study is its retrospective design, using a population which received less treatment than would a present day cohort, and possibly had a higher risk for the target endpoints [2]. This point must be emphasized, as the extent on which current beneficial

therapy affects the test panel's utility must be thoroughly investigated. A fourth limitation is the concentration cutoffs and biomarkers used, in so far as the most appropriate concentration for risk stratification has not been determined for these cytokines and there may be more appropriate biomarkers that are less influenced by pre-analytical factors (e.g., VEGF). The independence (if true) of these data to the extent of injury suggests that assessing the magnitude and composition of the cytokine response acutely may provide important insights into the robustness of that process and with it, prognostically important information. However, confirmatory studies and different statistical analyses/techniques exploring the utility of this panel of biomarkers are required before one can consider its use clinically.

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Fig. 1. Kaplan–Meier survival curves for the positive versus negative panel groups.

Table 1

Hazard ratios for HF/death at 6 months, 2 years, 8 years comparing positive versus negative panel groups.

Model	Time since presentation	HR relative to negative panel	Lower 95% CI	Upper 95% CL	Chi-square <i>p</i> -value
1	6 months	3.755	1.585	8.896	0.003
	2 years	3.054	1.639	5.693	<0.001
	8 years	2.729	1.795	4.148	<0.001
2	6 months	3.171	1.327	7.579	0.009
	2 years	2.581	1.371	4.858	0.003
	8 years	2.736	1.788	4.185	<0.001

Model 1 adjust for age at presentation, sex, history of HF.

Model 2 adjust for age at presentation, sex, history of HF, NT-pro-BNP, and cTnl.