Establishment of a Clinically Correlated Human Pericardial Fluid Bank: Evaluation of Intrapericardial Diagnostic Potential

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Summary: The development of a clinically correlated human pericardial fluid bank and database is described. A unique feature of this registry is the availability of a large number of pericardial fluid samples for testing with respect to multiple factors and for correlation with angiographic findings and clinical syndromes expressed by the patients. The collection of data at the present time comprises frozen pericardial fluid samples obtained from patients who have undergone cardiac surgery; and historical, clinical, and laboratory data obtained from the patient records. Nearly 400 samples have been stored and analyzed thus far, with sample entry continuing. This registry is designed to evaluate the local factors that play a role in mediating or reflecting myocardial or coronary responses. Pathophysiologic processes of particular interest include restenosis, plaque ruptures, and angiogenesis. Study of the pericardial fluid bank should lead to enhanced understanding of molecular mechanisms, as well as to the explanation for the reasons underlying interpatient variability in these processes. It is further anticipated that this information might provide a foundation for the diagnostic use of pericardial fluid to individualize therapies targeting angiogenesis or plaque physiology.

Key words: angiogenesis, cytokines, database, growth factors, pericardial fluid, plaque stability, registry

Introduction

Explanations for differences in coronary disease outcomes among patients may be found in specific local cellular and

molecular events, which in turn might be reflected as well as modulated by the intrapericardial fluid which bathes the coronary vessels and myocardium. Although classically defined as an ultrafiltrate of plasma,¹ pericardial fluid actually varies significantly from plasma with respect to its composition and concentration of a range of factors.^{2,3} This is so because it is locally conditioned by vascular tissues as well as myocytes and mesothelium. Cytokines and growth factors present in the pericardial fluid microenvironment may thus relate to cellular events such as the activation of macrophages and endothelial cells in the coronary vasculature. Such activation, in turn, may affect expression of factors or enzymes resulting in angiogenesis or plaque instability.³

We have hypothesized that the pericardial fluid composition may be of potential significance for (1) understanding molecular mechanisms of these responses, (2) understanding the reasons underlying interpatient variability in these processes, and (3) providing diagnostic information to permit individualization of therapies targeting angiogenesis or plaque physiology. These hypotheses may be evaluated by analyzing specific components of pericardial fluid in relation to angiographic findings and clinical syndromes.

In initial studies, key factors thought to be important in angiogenesis and vessel wall growth control have been assessed. These include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, soluble vascular cell adhesion molecule (sVCAM), transforming growth factors (TGFs), monocyte chemotactic protein-1 (MCP-1), and macrophage colony-stimulating factor (M-CSF).

A pericardial fluid bank is a novel resource and is expected to provide new information concerning individual angiogenic capacity, plaque instability, and the local effects of the constellation of growth factors present in this environment.

Methods

The registry consists of samples and data of patients from participating centers who undergo cardiac procedures requiring pericardiotomy for either coronary bypass surgery or valve replacement. Patients are enrolled at the time of surgery and pericardial fluid is collected. As much fluid as possible is

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collected from each patient immediately following sternotomy and pericardiectomy. Following collection of pericardial fluid and an anticoagulated blood sample, which is centrifuged immediately, samples are labeled and the pericardial fluid and plasma tubes are immediately frozen at -80°C for future analysis.

Information obtained from the patients' records is acquired with consent from the patients. Four categories of clinical characteristics included in the database obtained from chart review are demographics, cardiac risk factors, coronary syndrome, and medications. Included as demographic information are age, gender, blood group, surgical procedure, and number of bypass grafts placed where applicable. Coronary syndromes are divided into stable angina (SA), unstable angina (UA), myocardial infarction.⁴ Cardiac risk factors include hypertension, cholesterol profile, smoking status (if within the last 10 years), diabetes status, and family history. Medications recorded include nitrates, calcium-channel blockers, beta blockers, heparin, aspirin, angiotensin-converting enzyme (ACE) inhibitors, diuretics, digoxin, insulin, oral hypoglycemics, and lipid-lowering agents. Multiple comorbidities are also noted. In addition to chart review, coronary angiograms are independently reviewed by two consistent observers to determine an angiographic score of collateral vascularization ranging from 1–3 as described by Cortina *et al.*⁵

Analysis of fluid and plasma samples is performed by enzyme-linked immunosorbent assay (ELISA) or other appropriate assays to evaluate the samples for selected molecules. Growth factors and cytokines currently assayed for are VEGF, bFGF, angiogenin, sVCAM, TGF- β 1, TGF- β 2, MCP-1, and M-CSF. Other factors will be tested as suggested by experimental results.

The associations among the molecular entities, clinical factors, and collateral vessel status are examined statistically in several ways. Data undergo square root and logarithmic transformations as needed to approach normal distributions. Correlation coefficients, *t*-tests, and analysis of variance (ANOVA) with Tukey's Honestly Significant Difference test for multiple comparisons are used to examine specific relationships between clinical factors and molecular characteristics. Logistic regression will be used to determine whether a subset of cytokine measurements and clinical data can identify subjects who have the capacity to develop collaterals.

Some individuals have comparatively limited volumes of pericardial fluid, which can limit the number of tests that can be performed on any given sample. In such instances, analyses are conducted to optimize the distribution of data among the various entities being evaluated.

Results

To date, approximately 400 fluid samples have been obtained from patients undergoing surgical pericardiectomy. Volumes of fluid collected have ranged from 0.5 to 21.5 ml (4.1 ± 0.34 ml, mean $\pm$ standard error of the mean). Reviews of the chart-derived and angiographic data have been conducted

for these patients and assembled into a database as described above. The present demographics of this population are shown in Table I. Blood group percentages are illustrated in Table II.

To amplify the rate of recruitment of samples of pericardial fluid and the associated data, this study has been extended to include other centers.

Discussion

To date, comparatively little work has been devoted to exploring the potential diagnostic utility of pericardial fluid. Its use in this regard has been predominantly limited to the evaluation of intrapericardial malignancy or infectious disease. Only recently has pericardial fluid been considered as a fluid to sample in the assessment of myocardial or coronary vascular processes. It has been recognized in several small studies that this fluid contains a range of potent factors, with significant interpatient variations in composition and concentration.

Elevated bFGF levels have been found in a study of 12 patients with unstable angina.³ In another study of 17 patients, concentrations of bFGF in the pericardial fluid were found to be as high as 20-fold that of serum,² and the bFGF present was found to contribute significantly to a trophic effect on adult human cardiac myocytes.² Furthermore, remarkable levels of endothelin-1 have been identified in pericardial fluid,⁶ and patients with heart failure have manifested elevated intrapericardial levels of atrial natriuretic peptide (ANP) in an analysis of 20 individuals.⁷ It is noteworthy that the levels of several such factors are typically many times higher than the serum

TABLE I Demographics, coronary syndromes, and risk factors

Age	61.9 $\pm$ 11.8 years
Gender (%)	68.6 male; 31.4 female
CABG procedures (%)	90
Non-CABG procedures (%)	10
Stable angina (SA) (%)	19
Unstable angina (UA) (%)	59
Myocardial infarction (%)	26
Hypertension (%)	48
Diabetes mellitus (%)	29
Tobacco use (%)	37
Family history of CAD (%)	37
Hypercholesterolemia (%)	32

Abbreviations: CABG = coronary artery bypass grafting, CAD = coronary artery disease.

TABLE II Blood group percentages

A	46%	
B	9%	Rh – = 16%
AB	3%	Rh + = 84%
O	42%	

levels. Representative pericardial:serum ratios include those for ANP, 90:1;⁸ brain natriuretic peptide (BNP), 20:1;⁸ endothelin, 36:1;⁶ and bFGF as above. These examples illustrate the fact that these cytokines and growth factors present in the pericardial fluid may play a significant role in mediating local physiologic processes and support the effort to expand the scope of studies on pericardial fluids, as will be facilitated by this pericardial fluid repository.

Conclusions

Pericardial fluid analysis may provide new prognostic information with respect to angiogenesis, plaque stability, or other cardiac diseases. It may also provide therapeutic direction with respect to specific needs in the areas of growth factor and other coronary management approaches. This fluid bank and database were designed to allow analysis of relationships among different clinical syndromes and different growth factors and cytokines.

The existing sample collection and database, although substantially larger than any similar collection currently available, will be rendered more powerful by continued expansion. In hopes to broaden this database in the future for further studies, all hospitals/research centers are invited to participate in this registry and to contact the investigators using the "Address for reprints."

References

1. Gibson AT, Segal MB: A study of the composition of pericardial fluid, with special reference to the probable mechanisms of fluid formation. *J Physiol* 1978;277:367-377
2. Corda S, Mebazaa A, Gandolfini MP, Fitting C, Marotte F, Peynet J, Charlemagne D, Cavaillon JM, Payen D, Rappaport L, Samuel JL: Trophic effect of human pericardial fluid on adult cardiac myocytes. *Circ Res* 1997;81:679-687
3. Fujita M, Ikemoto M, Kishishita M, Otani H, Nohara R, Tanaka T, Tamaki S, Yamazato A, Sasayama S: Elevated basic fibroblast growth factor in pericardial fluid of patients with unstable angina. *Circulation* 1996 Aug 15;94(4):610-613
4. Braunwald E: Unstable angina: A classification. *Circulation* 1989; 80(2):410-414
5. Cortina A, Ambrose JA, Prieto-Granada J, Moris C, Simarro E, Holt J, Fuster V: Trophic effect of human pericardial fluid on adult cardiac myocytes. Differential role of fibroblast growth factor-2 and factors related to ventricular hypertrophy. *J Am Coll Cardiol* 1985;5:619-624
6. Horkay F, Szokodi I, Selmeci L, Merkely B, Kekesi V, Vecsey T, Vuolteenaho O, Ruskoaho H, Juhasz-Nagy A, Toth M: Presence of immunoreactive endothelin-1 and atrial natriuretic peptide in human pericardial fluid. *Life Sci* 1998;62(3):267-274
7. Amano J, Suzuki A, Sunamori M, Shichiri M, Marumo F: Atrial natriuretic peptide in the pericardial fluid of patients with heart disease. *Clin Sci* 1993;85:165-168
8. Klemm R, Laine M, Weckstrom M, Vuolteenaho O, Ruskoaho H, Huttunen P, Leppaluoto J: High concentrations of atrial natriuretic peptide and brain natriuretic peptide in rat pericardial fluid and their reduction by reserpine in vivo. *Naunyn Schmiedeberg Arch Pharmacol* 1995;Sept,352(3):331-336