Molecular analysis of pericardial fluid: a 7-year experience

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Received 2 January 2006; revised 28 March 2006; accepted 20 April 2006; online publish-ahead-of-print 22 May 2006

Aims Aetiological investigations of pericardial effusion are often negative. We evaluate the interest of systematic analysis of fluid in order to diminish the number of pericarditis classified as idiopathic.

Methods and results We performed a systematic analysis of pericardial fluid and biopsy specimens, using cultures and molecular analyses for the identification of bacteriological, fungal, and viral agents, as well as histopathological examination of 106 pericardial fluid samples. The aetiological diagnosis was determined clinically and by non-invasive procedures in 40 and nine patients, respectively. In the remaining 57 patients, 14 neoplasias and 17 infections were diagnosed. Molecular procedures identified seven viral (Enterovirus, Herpes simplex virus, and Epstein–Barr virus in four, two, and one of the cases, respectively), one fungal (Cryptococcus neoformans), and nine bacterial infections. Four of these bacteria were not diagnosed by culture because of prior antibiotics treatment (Mycobacterium tuberculosis in two cases, Streptococcus pneumoniae in one case, and Actinomyces neuii in one case). The aetiology remained undetermined in 26 patients.

Conclusion The systematic use of molecular techniques permitted a significant increase in aetiological diagnoses of punctured pericardial effusions when compared with cultures (39.5 vs. 13.9%, respectively; \( P \leq 0.01 \)). It is particularly beneficial for patients with a previous antibiotic regimen or suspicion of tuberculosis.

KEYWORDS Pericarditis; Cardiac tamponade; Molecular biology

Introduction Detection and treatment of pericarditis remain a challenging problem and the aetiology is unknown in 40–85% of the cases. We have previously developed a diagnostic strategy that recommends the systematic use of a combination of non-invasive tests for the diagnosis of benign pericardial effusions. This strategy lead to a reduction in the number of pericarditis classified as idiopathic when compared with a prescription based on selecting laboratory tests intuitively.2 Pericardiocentesis is a life-saving procedure in decompensate cardiac tamponade and is indicated for effusions of \( > 20 \) mm in diastole visualized by echocardiography.1–5 As it has been reported that the basic analysis of fluid rarely discloses the aetiology of effusion,6 physicians are often reluctant to tap the pericardium unless it is necessary for treatment. Pericardiocentesis can be guided by fluoroscopy, echocardiography, or a surgical approach.

In this study, we performed a systematic analysis of all pericardial fluid samples obtained during a 7-year period to improve the diagnostic yield of patients with large pericardial effusions. Such a systematic analysis of pericardial fluid and biopsy specimens, using cultures and molecular analyses for the identification of bacteriological, fungal, and viral agents, as well as histopathological examination, has not been previously reported.

Methods Patients All patients admitted to the Department of Cardiology or Cardio-Thoracic Surgery at the Timone Hospital, Marseilles, for a scheduled drainage of pericardial effusion from May 1998 to June 2005 were enrolled in a prospective study. The indications were various: decompensate cardiac tamponade, suspicion of neoplasia or tuberculosis, and recurrent or chronic effusion, which does not resolve despite few weeks of anti-inflammatory or colchicine treatment. Patients with post-pericardiotomy syndrome were excluded.

Diagnostic procedures Non-invasive tests such as estimation of antibodies against specific infectious agents, anti-nuclear antibodies, rheumatoid factor test, and serum thyroid-stimulating hormone were systematically prescribed as previously published.1 Surgically resected pericardial tissue or pericardial effusions were split into five aliquots: one for

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pathological examination, one for standard bacteriological culture, one for viral culture, one for PCR amplification, and one for identification of fastidious bacteria and mycobacteria.

Culture of pericardial fluids
Pericardial fluids were systematically inoculated onto Columbia sheep blood and chocolate agar (BioMerieux, Marcy-l’Etoile, France) under both anaerobic and 5% CO₂ atmosphere. Cultivation of strict and facultative intracellular bacteria was attempted in human embryonic lung fibroblast using the centrifugation-shell-vial technique.² Pericardial fluids were also inoculated onto different cell lines (MRC5 fibroblast cells, Hep2, Bgm, and Vero cells) in shell vials, incubated at 37°C, and then examined for cytopathic effect (CPE) on days +2 and +3 after inoculation.³ Cultures exhibiting a CPE were tested with an indirect fluorescence antibody assay using monoclonal antibodies to detect the presence of cytomegalovirus (CMV) (anti-CMV IEA + EA monoclonal antibodies, Argene Biosoft, Varilhes, France), enterovirus (mouse monoclonal anti-enterovirus, DakoCytomation SA, Trappes, France), Herpes simplex virus 1 and 2 (monoclonal anti-herpes simplex 1 + 2 FITC, Argene), and adenovirus (monoclonal anti-adenovirus group, Argene).

Cytological and histopathological examinations
Cytospin preparations from pericardial effusion were air-dried and stained with May–Grünewald–Giemsa. Formalin-fixed and paraffin-embedded tissue samples were cut to a 5 μm thickness and stained with haematoxylin–eosin–saffron. Special stains were used for the detection of fungi and bacteria, which included periodic acid–Schiff, Giemsa, Brown–Hopps tissue Gram, Grocott –Gomori stained with May–Gru¨nwald–Giemsa. Formalin-fixed and paraffin-embedded tissue samples were cut to a 5 μm thickness and stained with haematoxylin–eosin–saffron. Special stains were used for the detection of fungi and bacteria, which included periodic acid–Schiff, Giemsa, Brown–Hopps tissue Gram, Grocott–Gomori methenamine silver, and Warthin–Starry stains.

Molecular procedures
DNA was extracted from pericardial fluids for the molecular detection of DNA viruses using the MagNaPure LC Total Nucleic Acid Isolation kit (Roche Diagnostics France SA, Meylan, France), according to the manufacturer’s instructions. The DNA was eluted into a 100 μL final volume. One microlitre of DNA was used to detect CMV, HSV-1, HSV-2, VZV, EBV, and HHV-6 using the Herpes Consensus Generic kit (Argene). Positive results were then identified using the Herpes Identification Hybridwell kit (Argene), according to the manufacturer’s instructions. Detection of parvovirus B19 DNA was performed as described.⁴ RNA was extracted from pericardial fluids for the molecular detection of enterovirus RNA with the MagNaPure LC RNA Isolation kit. The RNA was eluted into a 100 μL final volume. A 10 μL volume was used as the target in a one step real-time RT-PCR protocol performed on an ABI Prism 7000/7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

For bacterial and fungal DNA detection, DNA was extracted from 200 μL of pericardial fluid using the QIAamp Tissue kit (Qiagen, Hilden, Germany), as directed by the manufacturer, and amplified using the 536F (5′-CACGACCCGCGGTAAATC-3′) and rp2 (5′-AGGCTACCTTGTGACACT-3′) and Fchampuniv (5′-TCCGTAGG TGAACCTGCGG-3′) and Rchampuniv (5′-GCTGCTTCTTCACTGATG C-3′) primer pairs, as previously described.⁵ We used a PTC200 thermal cycler (MJ Research, Watertown, MA, USA) for PCR amplification. Positive PCR products were sequenced using the PCR primers, the dRhodamine terminator cycle ready sequencing kit (Applied Biosystems,), and an ABI Prism 3100 automated sequencer (Applied Biosystems). To decrease the likelihood of DNA contamination, DNA extraction, preparation of the master mixture, and amplification were carried out in different rooms. If positive PCR results were considered as non-reliable, additional PCR targeting a second gene was performed as previously reported.⁶ PCR was systematically performed, even though non-infectious aetiological diagnoses were determined. Negative controls were systematically included for each routine PCR test.

Results
We received pericardial fluid specimens from 106 patients over the course of the 7-year study. The mean ± SD age of the cohort was 54.5 ± 19 and ranged from 7 to 93 years. Apart from the analysis of infectious agents isolated from pericardial fluid, a diagnosis was determined for 63 patients (Figure 1). This included 14 cases of neoplasia diagnosed by histological examination. An aetiological diagnosis, which included hypothyroidism, collagen vascular diseases, Influenza virus, Q fever, and Bartonella quintana infection, was made using non-invasive procedures for nine patients, and 40 patients had a known cause at the time of admission, which included malignancy, collagen vascular diseases, hypothyroidism, post-radiation, and uraemia. Despite previous diagnoses, these 40 patients underwent a pericardiocentesis because of either haemodynamic reasons or suspicion of associated aetiology (mainly infectious causes). Molecular procedures were systematically performed on these samples, and the results were uniformly negative as expected. Superinfections, which are uncommon events, were then also excluded. These specimens were considered as true negative.

The diagnosis was obtained through the analysis of pericardial fluid only in 17 (39.5%) of the remaining 43 patients (Table 1). The aetiology remained undetermined in 26 patients. Molecular procedures identified seven viral (Enterovirus, Herpes simplex virus, and Epstein–Barr virus in four, two, and one of the cases, respectively) and nine bacterial infections. One Cryptococcus neoformans was diagnosed from the pericardial fluid by standard procedures (sheep blood and chocolate agar) and confirmed by PCR, as well as five bacteria (Citrobacter freundii, Escherichia coli, Streptococcus pneumoniae, and Staphylococcus aureus x 2). A case of S. pneumoniae and a case of Actinomyces neuii infection were identified by PCR only, but these two patients had received antibiotics prior to drainage. A clinical suspicion of tuberculosis was confirmed by the PCR analysis of pericardial fluid in two patients, although the cultures were negative. Each culture positive patient was also PCR positive. Aetiological diagnosis using molecular procedures was significantly higher than culture only. The number of cases with an aetiology determination increased from 13.9 (6/43) to 39.5% (17/43) (P < 0.01). None of the true negative sample was PCR positive. No additional diagnosis was obtained using the shell-vial culture for fastidious bacteria.

Discussion
Direct amplification from sterile sites provided an alternative approach for the detection of pathogens. In this study, the use of a systematic molecular approach allowed us to diagnose infections not detected by specific culture methods. Multiple control experiments were performed before interpreting the results. Contamination is a well-documented limitation of PCR-based molecular diagnosis approaches.¹¹ Strict conditions were applied to limit this risk as advised.¹² Negative and positive internal controls were included for each series; a total of 424 PCR samples were tested during this study. Furthermore, all the PCR results from the patients (54/106) who had a known cause of pericardial effusion at the time of admission or
in which an aetiology was determined by histological findings were uniformly negative. The highly specific results obtained allowed us to consider a positive PCR as a true positive when applied to patients with unknown diagnostic. In the absence of other aetiology and when the diagnosed micro-organism is a known agent of pericardial effusion, we believed that the aetiological diagnosis can be assessed by PCR. When the agent has not been described previously, the detection should be interpreted with caution. DNA is sufficiently stable that it can be amplified by PCR for long period after bacterial cultures become sterile. As described for the diagnosis of endocarditis based on PCR detection on cardiac valves, the presence of DNA does not necessarily indicate the persistence of viable bacteria or virus.

Molecular techniques enabled the diagnoses of seven viral infections. Except for the Epstein-Barr virus, a better sensitivity with the PCR method, compared with the culture method, may explain the difference. The use of 16s rRNA PCR confirmed the results of all patients who had positive bacterial cultures. In addition, the PCR approach determined the diagnosis in two patients who had received antibiotics prior to pericardiocentesis and in two patients with tuberculosis that were not diagnosed by culture. These findings are in agreement with the previous report that molecular detection was useful in the cases of infective endocarditis that were culture negative because of prior administration of antibiotics. Nearly, a three-fold increase in the number of diagnoses (17 vs. six), which represented 10.3% additional diagnoses overall, suggests that PCR (although costly) is useful for improving the frequency of determining the aetiology of pericarditis over culture methods alone. Molecular techniques have the biggest yield for additional diagnoses. The number of aetiological diagnoses among the 35 idiopathic pericarditis patients was substantially increased by 29.7% (11/37). The results of the culture as well as all molecular techniques and histology were obtained within a week. This was especially important because appropriate therapies were subsequently proposed for these patients (four patients in our study). When a viral aetiology is found, potential antibiotic treatment can also be promptly stopped. We have not been able to analyse the cost-effectiveness in our study. This is because the pertinent data (e.g. length of hospitalization, frequency of hospitalization within a year, and medication received) were not available uniformly for all study
subjects. Moreover, the second limitation of this study is that easy accessibility to molecular techniques may not be available everywhere yet.

Systematic analysis of fluid decreases the number of diagnoses classified as idiopathic and has not been reported before. Maisch et al. 13,14 have also realized a comparable approach of a systematic analysis of the fluid including biochemistry, cytology, bacterial culture, and PCR for cardiotoxic virus and bacteria in 260 patients. The aim of these two published reports was to measure the efficacy and safety of intrapericardial treatment with cisplatin in these two published reports was to measure the efficacy and safety of intrapericardial treatment with cisplatin in the case of neoplastic pericardial effusion 13 and with triamcinolone in the case of autoreactive effusion.14 This systematic approach was used to select patients with neoplastic or autoimmune pericardial effusions, and the analysis of different aetiologies was not discussed. Despite a similar approach applied on a large cohort of patients, we unfortunately cannot compare our results with these series in order to appreciate the impact of rapid detection of pathogens by PCR.

Conclusions

Pericardial fluid analysis using molecular methods is complementary to a systematic strategy involving non-invasive procedures. We believe that the puncture of pericardial fluid should be proposed as a second line of investigation when serological tests are negative (including anti-nuclear antibodies and serum thyroid-stimulating hormone), except in the case of urgent drainage for tamponade. It can also be proposed when a neoplasm, tuberculosis, or an infection is suspected. It can be discussed in the case of idiopathic effusion, which does not resolve despite several weeks of anti-inflammatory treatment. When histopathological examination is not conclusive, systematic evaluation of pericardial fluid using microbiological cultures and molecular techniques may improve the frequency of identifying the specific aetiology in patients with pericardial effusion. The use of PCR is particularly beneficial for patients with a previous antibiotic regimen or suspicion of tuberculosis.

Acknowledgements

P.-Y.L. collected the data, followed the study, and wrote the first draft. P.-E.F. provided the results of the bacterial tests and contributed to the discussion. R.C. provided the results of the viral tests. G.H. and D.M. were responsible for the diagnosis and the treatment of the patients. D.R. designed the study, analysed the data, and wrote the manuscript.

Conflict of interest: none declared.

References

Clinical vignette

doi:10.1093/eurheartj/ehi790
Online publish-ahead-of-print 1 March 2006

Left ventricular pseudoaneurysm with a fistula to right ventricle

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A 68-year-old woman with a history of anterior myocardial infarction presented, as mechanical complication, an apical ventricular septal defect that was repaired surgically. Some years later, she consulted for difficulty in breathing, showing a holosystolic murmur suggestive of ventricular septal defect. A two-dimensional echocardiogram was performed and showed a paraventricular space free of echoes joining the above-mentioned ventricular cavity for a neck of union. The Doppler colour demonstrated abnormal flow between two chambers. An additional abnormal high-velocity flow connected the pseudoaneurysm to the right ventricle (Panel A). Coronary and left ventricular catheterization was performed and confirmed the echocardiography diagnosis (Panel B and movie I and II).

The left ventricular pseudoaneurysm is associated, in most cases, with acute myocardial infarction. However, we should not forget that surgery is the aetiology of this pathology. Few cases have been described following the repair of septal defect.

Panel A. Left side: Two-dimensional echocardiogram from plane of four apical chambers showing the break of the anteroapical wall of the left ventricle with pseudoaneurysm, the abovementioned location. Right side: Plane of two apical chambers showing the pseudoaneurysm in the apical position with a fistula to right ventricle. LV: left ventricle; PA: pseudoaneurysm.

Panel B. Left side: Two-dimensional echocardiogram from plane of four apical chambers showing the break of the anteroapical wall of the left ventricle with pseudoaneurysm, the abovementioned location. Right side: Plane of two apical chambers showing the pseudoaneurysm in the apical position with a fistula to right ventricle. LV: left ventricle; PA: pseudoaneurysm.

Panel A. Left side: Two-dimensional echocardiogram from plane of four apical chambers showing the break of the anteroapical wall of the left ventricle with pseudoaneurysm, the abovementioned location. Right side: Plane of two apical chambers showing the pseudoaneurysm in the apical position with a fistula to right ventricle. LV: left ventricle; PA: pseudoaneurysm. Arrow: fistula.

Panel B. Left side: Two-dimensional echocardiogram from plane of four apical chambers showing the break of the anteroapical wall of the left ventricle with pseudoaneurysm, the abovementioned location. Right side: Plane of two apical chambers showing the pseudoaneurysm in the apical position with a fistula to right ventricle. LV: left ventricle; PA: pseudoaneurysm. Arrow: fistula.