Summary

**Aim:** To improve the understanding of factors that influence adenosine deaminase (ADA) activity in large pericardial effusions.

**Methods:** A prospective study was carried out at Tygerberg Academic Hospital, South Africa. Patients underwent echocardiographically guided pericardiocentesis. ADA activity, as well as biochemistry, haematology, cytology, and in some cases, histology, were determined. Human immunodeficiency virus (HIV) status was assessed in all patients.

**Results:** Two hundred and thirty-three patients presented to Tygerberg Hospital with large pericardial effusions requiring pericardiocentesis. Tuberculous pericarditis accounted for 162 effusions (69.5%). An ADA cut-off level of 40 U/l resulted in a test sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic efficiency of 84.0%, 80.0%, 91.0%, 66.0% and 83.0%, respectively. Pericardial exudates with an ADA activity ≥ 40 U/l were associated with increased total leukocyte and neutrophil counts. Patients with tuberculous pericarditis and ADA ≥ 40 U/l also had increased lymphocyte counts. Pericardial ADA activity < 30 U/l was associated with severe depletion of CD4 cell counts in HIV-positive patients. ADA levels were higher in cases with histological evidence of granulomatous inflammation than in cases with serofibrinous pericarditis.

**Conclusions:** An ADA cut-off level of 40 U/l results in best diagnostic test results. ADA production appears to be influenced by factors associated with the antituberculous immune response.

CARDIOVASCULAR JOURNAL OF SOUTH AFRICA Vol 16, No. 3, May/June 2005 143

Adenosine deaminase activity – more than a diagnostic tool in tuberculous pericarditis

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Tuberculosis (TB) is the most important cause of serious pericardial disease in sub-Saharan Africa, and this is likely to worsen with the increase in the human immunodeficiency virus (HIV) epidemic. It is often difficult to establish a definite diagnosis in tuberculous pericarditis, and a number of diagnostic tests are required to support a clinical diagnosis. In countries where TB is endemic, the increase in adenosine deaminase (ADA) activity observed in the pericardial fluid of patients with TB has been used to establish the diagnosis of tuberculous pericarditis.

ADA is a polymorphic enzyme that is involved in purine metabolism. It catalyses the deamination of adenosine and deoxyadenosine to produce inosine and deoxyinosine, respectively. It also plays a role in the differentiation of lymphoid cells and the maturation of monocytes to macrophages. The presence of ADA in pericardial and other body fluids reflects the activity of the cellular immune response in the respective compartments and in particular, the activation of T lymphocytes and macrophages.

**Aim**

In order to improve the understanding of factors that influence the ADA activity in large pericardial effusions, pericardial ADA results obtained from a prospective study of patients who required therapeutic pericardiocentesis were analysed and, where possible, correlated with pericardial leukocyte counts, peripheral CD4+ lymphocyte counts and histopathological features.

**Materials and methods**

A prospective study was carried out at Tygerberg Academic Hospital, South Africa. A total of 233 consecutive patients presenting with large pericardial effusions to the Echocardiography Unit between February 1995 and June 2001 were enrolled and followed up for a minimum of 12 months. The study was approved by the Ethics Committee of Stellenbosch University. At the time of enrollment, patients underwent echocardiographically guided pericardiocentesis followed by daily intermittent catheter drainage.

Pericardial fluid was sent for biochemistry, haematology and cytology. If an aetiological diagnosis could not be made within seven days, a pericardial biopsy was performed under...
general anaesthesia. The biopsy tissue was sent (in formalin) for histopathological evaluation. Each piece of tissue was sectioned and stained with haematoxylin and eosin (H&E), as well as with Ziehl-Neelsen (ZN) stain. Where applicable, patients were started on a six-month course of antituberculous therapy. HIV-positive individuals were treated with daily co-trimoxazole and referred to the Infectious Diseases Clinic for staging and management of their HIV.

ADA activity (U/l) was determined in all pericardial fluid specimens according to the method described by Giusti. This is a calorimetric method based on measurement by Berthelot’s reaction of the formation of ammonia, which is produced when ADA acts on excess adenosine. One unit of ADA is defined as the amount of enzyme required to release one μmole of ammonia per minute from adenosine at standard assay conditions. The enzyme is stable for at least 24 hours at 25°C, seven days at 4°C and three months at –20°C.

The final diagnosis was made according to pre-determined criteria. Tuberculous pericarditis was diagnosed if the patient met one or more of the following criteria: (i) identification of Mycobacterium tuberculosis in pericardial fluid or biopsy specimen by ZN stain and/or by TB culture, (ii) histological evidence of necrotising, granulomatous inflammation of the pericardium, (iii) positive ZN smear and/or TB culture and/or histological evidence of TB from one or more extracardiac sites, in the absence of any other cause for pericardial effusion, and/or (iv) clinical features of tuberculous pericarditis associated with a sustained positive response to anti-tuberculous therapy in the absence of any other cause for pericardial effusion.

Non-tuberculous pericardial effusions were defined as those with no histological evidence of TB, that were effusion/sputum ZN and TB culture negative, and where a definite alternative diagnosis was established. These included malignant effusions; effusions associated with connective tissue disease such as systemic lupus erythematosus (SLE), mixed connective tissue disease, systemic sclerosis and rheumatoid arthritis; uraemic pericarditis; septic pericarditis; and post-traumatic/post-surgical pericardial effusions.

Statistical analysis
Statistical analysis was done with the Kruskall-Wallis one-way ANOVA, the Bonferroni two-way ANOVA and Chi-square tests. A p-value < 0.05 was considered statistically significant. The correlation between two variables was plotted on a scatter plot and Spearman rank coefficients were used to express the relationship. The significance of the correlation was denoted by a p-value < 0.05. All statistical analyses were done using Statistica version 6.0.

The utility of determining ADA activity as a diagnostic tool for pericardial TB was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic efficiency expressed as a percentage. Sensitivity was defined as TP/(TP + FN) × 100; specificity was defined as TN/(TN + FP) × 100; PPV was defined as TP/(TP + FP) × 100; NPV was defined as TN/(TN + FN) × 100, and diagnostic efficiency was defined as (TP + TN)/(TP + FP + TN + FN) × 100, where TP = true positive, TN = true negative, FP = false positive and FN = false negative. These were compared by means of relative operating characteristic (ROC) curves and the cut-off value that maximised the true positive rate was selected.

Results
During the study period, 233 patients presented to Tygerberg Hospital with large pericardial effusions requiring pericardiocenteses. This included 101 (43.0%) females and 132 (67.0%) males. Tuberculous pericarditis accounted for 162 effusions (69.5%), malignant effusions for 22 (9.4%), effusions associated with connective tissue diseases for 12 (5.2%), septic pericarditis for five (2.1%), and ‘other’ effusions for 32 (13.7%). In total, 84 patients were HIV positive, including 81 patients who had pericardial TB (50.0% of TB patients). The mean (SD) age of the HIV-positive tuberculous group was significantly lower (p < 0.05) than that of the HIV-negative tuberculous patients [31.9 (8.4) (range 13–55) years and 41.6 (14.1) (range 13–85) years, respectively].

‘Definite’ TB (histological and/or microbiological evidence for TB) was diagnosed in 118 (73.0%) of the 162 patients classified as having tuberculous pericarditis. This included 91 patients with pericardial effusions with a positive TB culture, 13 patients with a pericardial biopsy that was diagnostic of TB, and 32 patients with a positive ZN smear and/or TB culture and/or histology in one or more extracardiac sites. A number of patients had TB demonstrated in more than one site. ‘Probable’ TB (clinical diagnosis in absence of histological and/or microbiological evidence for TB) was diagnosed in a further 44 patients. Eleven of the 162 tuberculosis patients had been on antituberculous therapy for more than 48 hours at the time of pericardial aspiration; all of them were HIV negative, including four patients with ‘definite’ TB and seven patients with ‘probable’ TB.

The corresponding median (range) ADA activity for patients with tuberculous (HIV negative) pericardial effusions, tuberculous (HIV positive) pericardial effusions, malignancy, septic pericarditis, connective tissue disease and other non-tuberculous pericardial effusions are presented in Table I. Various levels of pericardial fluid ADA activity were evaluated as a cut-off level for the diagnosis of pericardial TB and, based on ROC curves, the best results were obtained at a cut-off level of 40 U/l.

In total, 13 (out of 71) patients with non-tuberculous effusions had levels of ADA activity exceeding 40 U/l. These false positives included patients with septic pericarditis (n = 4); corresponding ADA activities of 49.1 U/l, 55.6 U/l, 138.6 U/l and 165.8 U/l (respectively), SLE (n = 1); corresponding ADA 49.0 U/l), rheumatoid arthritis (n = 1; corresponding ADA 65.3 U/l), post-traumatic pericarditis (n = 1; corresponding ADA 47.5 U/l), pericarditis of unknown origin (n = 1; corresponding ADA 50.4 U/l), non-haematological malignancies (n = 3); and haematological malignancies (n = 2). The malignancies included pericardial adenocarcinoma (ADA 47.5 U/l), squamous cell carcinoma (ADA 46.0 U/l), undifferentiated carcinoma (ADA 50.9 U/l), lymphoblastic T-cell lymphoma (ADA 166.0 U/l) and chronic myelomonocytic leukaemia (ADA 102.5 U/l).
Using the cut-off level of 40 U/l resulted in 22 false-negative pericardial effusions. Twelve of these occurred in HIV-negative and 10 in HIV-positive patients, respectively. Nine of the 12 HIV-negative patients were on anti-tuberculous therapy at the time of pericardiocentesis, whereas no HIV-positive patient was on active anti-tuberculous therapy at the time of pericardial aspiration. An additional two HIV-negative patients were also on anti-tuberculous medication; in these two the corresponding pericardial ADA activity levels were 75.0 U/l and 81.0 U/l, respectively. The corresponding median (range) ADA activity for HIV-negative TB patients on anti-tuberculous therapy, HIV-negative TB patients not on anti-tuberculous therapy, HIV-positive TB patients, and patients with non-tuberculous pericardial effusions is presented in Table II. In three patients with tuberculous pericarditis, no specific cause could be identified for the low pericardial ADA activity. The corresponding ADA activity for these three cases was 22.0 U/l, 24.0 U/l and 34.0 U/l, respectively.

After exclusion of all patients on anti-tuberculous therapy and all those categorised as pericarditis of unknown cause, an ADA activity cut-off level of 40 U/l resulted in the corresponding sensitivity, specificity, PPV, NPV and diagnostic efficiency of 90.0%, 74.0%, 90.0%, 76.0% and 86.0%, respectively.

In tuberculous pericardial effusions, lymphocytes dominated the inflammatory cellular infiltrate. The corresponding median (range) pericardial lymphocyte/neutrophil ratios for tuberculous (HIV negative), tuberculous (HIV positive) and non-tuberculous pericardial effusions were 2.7 (0.2–74.0) U/l, 1.9 (0.1–7.5) U/l and 0.4 (0.1–2.2) U/l, respectively. The corresponding correlation (Spearman rank) between pericardial total leukocyte, neutrophil, lymphocyte and macrophage counts were \( r = 0.30 \) (\( p = 0.07 \)); \( r = 0.25 \) (\( p = 0.13 \)); \( r = 0.31 \) (\( p = 0.07 \)); and \( r = 0.14 \) (\( p = 0.59 \)), respectively. In the tuberculous pericardial exudates, the corresponding median (range) pericardial fluid leukocyte, neutrophil and lymphocyte counts were significantly higher in effusions with ADA activity \( \geq 40 \) U/l than in pericardial effusions with ADA levels < 40 U/l (\( p = 0.03 \), \( p = 0.02 \) and \( p = 0.03 \), respectively), whereas in non-tuberculous effusions, only the total leukocyte and neutrophil counts were higher in high ADA effusions than in low ADA effusions, as summarised in Table III.

In the HIV-positive tuberculous group, the correlation between pericardial ADA activity and peripheral blood CD4 cell counts was not significant (\( r = 0.17 \); \( p = 0.20 \)). However, at very low CD4+ cell counts there was a tendency towards low levels of ADA activity. The corresponding median (range) CD4 cell counts for patients with pericardial ADA activity < 30 U/l, pericardial ADA activity < 40 U/l and pericardial ADA activity \( \geq 40 \) U/l were 59.0 (6.0–115.0) cells/µl, 183.0 (6.0–578.0) cells/µl and 219.0 (25.0–1 006.0) cells/µl, respectively. In the HIV-negative tuberculous patients and the non-tuberculous patients the corresponding correlation between pericardial ADA activity and peripheral CD4 cell counts was \( r = -0.89 \) (\( p = 0.04 \)) and \( r = -0.42 \) (\( p = 0.57 \)), respectively.

A total of 25 pericardial biopsies were performed in patients from the tuberculous group; five of these patients were HIV positive. Fifteen biopsies demonstrated granulomatous inflammation; 12 of these were accompanied by caseating necrosis. A biopsy specimen from an HIV-positive patient resembled acute purulent pericarditis; a diagnosis of pericardial TB was based on the presence of numerous acid-fast bacilli accompanied by a positive pericardial fluid TB culture, a negative Gram stain and a negative culture for non-tuberculous bacteria. The remaining nine biopsies demonstrated no characteristic histomorphological features of TB. They were categorised as serofibrinous pericarditis (\( n = 6 \)) and fibrotic pericarditis (\( n = 3 \)). For patients with pericardial

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**TABLE I. PERICARDIAL ADA ACTIVITY IN VARIOUS DIAGNOSTIC SUBGROUPS OF PERICARDITIS**

<table>
<thead>
<tr>
<th>Diagnostic groups</th>
<th>n</th>
<th>Mean 95% confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous pericarditis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV negative</td>
<td>75</td>
<td>79.6</td>
<td>69.7–89.5</td>
</tr>
<tr>
<td>HIV positive</td>
<td>76</td>
<td>76.3</td>
<td>66.6–86.0</td>
</tr>
<tr>
<td>Malignant pericardial effusion</td>
<td>20</td>
<td>39.3</td>
<td>20.0–58.6</td>
</tr>
<tr>
<td>Unremarkable pericarditis</td>
<td>9</td>
<td>21.1</td>
<td>17.4–24.8</td>
</tr>
<tr>
<td>Septic pericarditis</td>
<td>4</td>
<td>102.3</td>
<td>59.2–145.4</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>8</td>
<td>31.1</td>
<td>0.6–61.6</td>
</tr>
<tr>
<td>Other pericardial effusions</td>
<td>20</td>
<td>30.0</td>
<td>10.7–49.3</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus
p-value established by Bonferroni test, notifying difference between diagnostic group from HIV-negative tuberculous pericarditis.

**TABLE II. PERICARDIAL ADA ACTIVITY IN VARIOUS DIAGNOSTIC GROUPS OF PERICARDITIS**

<table>
<thead>
<tr>
<th>Diagnostic groups</th>
<th>n</th>
<th>Mean 95% confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous pericarditis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV negative (not on ATC)</td>
<td>64</td>
<td>88.9</td>
<td>78.5–99.3</td>
</tr>
<tr>
<td>HIV negative (on ATC)</td>
<td>13</td>
<td>24.9</td>
<td>20.0–50.1</td>
</tr>
<tr>
<td>HIV positive</td>
<td>78</td>
<td>76.3</td>
<td>66.6–86.0</td>
</tr>
<tr>
<td>Non-tuberculous pericarditis</td>
<td>61</td>
<td>36.6</td>
<td>25.9–47.3</td>
</tr>
</tbody>
</table>

HIV: human immunodeficiency virus
ATC: anti-tuberculous chemotherapy
p-value established by Bonferroni test, notifying difference between diagnostic group from HIV-negative tuberculous pericarditis not on ATC.

**TABLE III. RELATIONSHIP BETWEEN PERICARDIAL LEUKOCYTES AND PERICARDIAL ADA ACTIVITY**

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>n</th>
<th>ADA &lt; 40 U/l</th>
<th>n</th>
<th>ADA ≥ 40 U/l</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous effusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial total leukocytes</td>
<td>6</td>
<td>1220 (470–2210)</td>
<td>25</td>
<td>2580 (400–10260)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pericardial neutrophils</td>
<td>6</td>
<td>53 (15–88)</td>
<td>24</td>
<td>918 (158–7737)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pericardial lymphocytes</td>
<td>6</td>
<td>721 (380–1768)</td>
<td>24</td>
<td>1140 (99–2654)</td>
<td>0.03</td>
</tr>
<tr>
<td>Non-tuberculous effusions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pericardial total leukocytes</td>
<td>6</td>
<td>1655 (220–360)</td>
<td>5</td>
<td>4341 (80–24140)</td>
<td>0.02</td>
</tr>
<tr>
<td>Pericardial neutrophils</td>
<td>6</td>
<td>812 (103–1787)</td>
<td>5</td>
<td>2843 (24–19336)</td>
<td>0.04</td>
</tr>
<tr>
<td>Pericardial lymphocytes</td>
<td>6</td>
<td>504 (51–1520)</td>
<td>5</td>
<td>260 (56–1497)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

ADA: adenosine deaminase activity
Results expressed as median (range) cells/µl
p-value established by two-way ANOVA.
TB, the median (range) ADA levels for granulomatous, purulent, serofibrinous and fibrinous pericarditis were 110.0 (41.0–304.0) U/l, 101.0 U/l, 68.0 (1.3–166.0) U/l, and 101.0 (34.0–166.0) U/l, respectively. For the HIV-positive TB patients, the median (range) ADA levels for granulomatous, purulent and serofibrinous pericarditis were 93.0 (78.0–108.0) U/l, 101.0 U/l and 74.0 (51.0–97.0) U/l, respectively. These differences were statistically non-significant.

Discussion

In South Africa, the majority of patients presenting with large pericardial effusions have pericardial TB. The present study confirms that pericardial TB is responsible for approximately 70% of the cases of large pericardial effusions seen at Tygerberg Academic Hospital in the Western Cape of South Africa. Fifty per cent of all tuberculous effusions were seen in HIV-positive individuals.

We documented high levels of ADA in tuberculous pericardial effusions. In HIV-negative TB patients the most notable cause for low pericardial ADA levels was the concomitant use of anti-tuberculous chemotherapy at the time of pericardiocentesis, suggesting that anti-tuberculous therapy influences ADA activity by one or more of the following mechanisms: (i) anti-tuberculous therapy increases the metabolic breakdown of ADA (possibly by rifampicin’s effect on hepatic metabolism), and/or (ii) anti-tuberculous therapy suppresses ADA activity directly, and/or (iii) anti-tuberculous therapy affects the cell turnover of pericardial macrophages and T lymphocytes, thereby reducing ADA levels.

The median ADA activity was not significantly lower in HIV-positive than in HIV-negative TB patients and the correlation between peripheral CD4+ lymphocyte counts and pericardial ADA activity levels was non-significant. However, a relationship between severe peripheral CD4+ lymphocyte depletion and low ADA levels was noted. Seven of the 10 HIV-positive patients with low ADA tuberculous effusions (ADA activity < 40 U/l) had corresponding CD4 cell counts < 200 cells/µl. The difference between CD4 cell counts associated with pericardial ADA activity < 40 U/l and pericardial ADA activity ≥ 40 U/l was minimal (corresponding counts being 183.0 (6.0–578.0) cells/µl and 219.0 (25.0–1006.0) cells/µl, respectively (p = 0.8)). However, in the group of tuberculous patients with pericardial ADA activity < 30 U/l, the corresponding median (range) CD4 cell count of 59.0 (6.0–115.0) cells/µl was significantly decreased (p = 0.04). These results suggest that severe CD4+ lymphocyte depletion may result in low ADA activity levels, probably by impeding the CD4+ lymphocyte-dependent cellular immune response against mycobacterial antigens in the pericardial space.

The inverse relationship between peripheral CD4+ cell counts in HIV-negative tuberculous patients can be explained by the recruitment of peripheral CD4+ cells into the pericardial space. Pericardial CD4+ cell counts were not determined, however, to corroborate this theory.

Pericardial exudates with an ADA activity ≥ 40 U/l were associated with higher total leukocyte and higher neutrophil counts than pericardial effusions with ADA activity < 40 U/l. In the group of patients with tuberculous pericarditis, pericardial exudates with ADA activity ≥ 40 U/l were also characterised by higher lymphocyte counts than tuberculous exudates with ADA activity < 40 U/l. Pericardial macrophage counts were available for only a minority of effusions; no specific correlation between ADA levels and macrophage counts could be demonstrated in these cases.

Pericardial neutrophil counts were lower for tuberculous effusions than for non-tuberculous effusions, whereas median ADA activity was significantly higher in tuberculous than in non-tuberculous exudates. This implies that in tuberculous effusions, lymphocytes (and possibly macrophages) play the major role in the production and release of ADA, which supports data from tuberculous pleural exudates. It has been demonstrated that lymphocytes and macrophages contain similar levels of ADA activity, and that this is much higher than in other cell types or tissues. Due to insufficient pericardial macrophage counts, our study does not add additional information on the potential contribution of macrophages to the ADA activity in tuberculous exudates. Tuberculous exudates with ADA activity < 40 U/l were accompanied by significantly lower pericardial neutrophil counts than effusions with ADA activity ≥ 40 U/l, suggesting that neutrophils also contribute to ADA activity. In non-tuberculous effusions, neutrophils are probably the major source of ADA, although lymphocytes may also contribute. However, significantly higher pericardial leukocyte counts are ‘required’ to produce similar levels of ADA activity.

In the present study, the contribution of neutrophils to ADA activity in non-tuberculous effusions was most notable in the case of septic pericarditis and in effusions associated with malignancies. A differential white cell count and cytological analysis of the pericardial fluid is therefore mandatory in patients with large pericardial effusions to exclude these two conditions, which are characterised by a predominance of pericardial fluid neutrophils and malignant cell lines, respectively.

In this study, ADA levels tended to be higher in cases that showed histological evidence of granulomatous inflammation than in cases with serofibrinous pericarditis, suggesting that the cells involved in the development and maintenance of granulomatous lesions (mainly activated macrophages and T lymphocytes) were also responsible for the elevated ADA activity in the pericardial fluid, as has been previously suggested.

Conclusion

Our study adds considerable support to the use of ADA activity as a diagnostic tool for the diagnosis of pericardial TB, and gives some insight into its use in areas characterised by a high prevalence of TB and HIV co-infection. Based on ROC curves, the best results in the present study were obtained at a cut-off level of 40 U/l. In HIV-positive patients, a correlation between severe CD4 lymphocyte depletion and low ADA levels was observed, whereas in the HIV-negative patients the most notable cause for low ADA levels was the use of anti-tuberculous chemotherapy at the time of pericardiocentesis. This suggests that anti-tuberculous therapy influences the ADA activity in patients infected with TB, possibly by affecting the metabolism of ADA and/or by suppressing mycobacterial replication, thereby indirectly affecting lymphocyte and macrophage activity.
Histopathological analysis of pericardial tissue demonstrated that granulomatous pericarditis was associated with higher ADA activity than serofibrinous pericarditis. The contribution of CD4 cells in the delayed hypersensitivity response and protection against TB is well described, including the interaction with macrophages that spearheads the protective immune response against TB. Our data suggest that factors influencing the anti-tuberculous immune response also affect ADA production.

A number of non-tuberculous diseases may also present with elevated pericardial ADA activity, most importantly septic pericarditis and effusions associated with malignancies. A differential white cell count and cytological analysis of the pericardial fluid is therefore mandatory to exclude these two conditions, which are characterised by a predominance of pericardial fluid neutrophils and malignant cell lines, respectively.

We thank Dr W. van Vuuren for assistance with pericardial histology, Ms A. Jacobs for assistance with data collection and Ms N.U. Sulzer for assistance with manuscript preparation.

References