TITLE:

Diagnostic utility of pericardial fluid pH in diagnosing infectious pericardial effusions among patients with moderate and large effusions undergoing pericardiocentesis at Groote Schuur Hospital: A subs-study of the IMPI trial.

By

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DECLARATION:

I, Brian Kiggundu, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABBREVIATIONS

ADA	Adenosine Deaminase.
AUC	Area under the curve
CI:	Confidence intervals
CTGF	Connective tissue growth factor
Echo	Echocardiogram
EKG	Electrocardiogram
FDG	2-[18F]-fluoro-2-deoxy-D-glucose
MTB	Mycobacterium Tuberculosis
NHLS	National Health Laboratory Service
NLR	Negative likelihood ratios
PET-CT	Positron emission tomography combined with computed tomography
PF	Pericardial fluid
PLR	Positive likelihood ratios
RIF	Rifampicin
TBP	Tuberculous pericarditis.
TB	Tuberculosis
TGF	Transforming growth factor beta
UIFNγ	Un-stimulated interferon gamma

ABSTRACT

Diagnosis of infectious pericardial disease has been challenging in the developing world despite improvement of treatment modalities. The diagnostic utility of pH in diagnosing infectious pericardial fluid is unknown, yet this concept is well studied in pleural fluid. This cross-sectional diagnostic study evaluated the diagnostic utility of pH in infectious compared to non-infectious pericardial effusions in a high-burden setting.

Methods: Patients of ≥18 years with moderate to large effusion between the 1st February 2016 and 31st May2018 were enrolled at Groote Schuur Hospital in Cape Town, South Africa. After safe pericardiocentesis, pH was measured with a blood gas analyzer. Mycobacterium tuberculosis culture and/or gene Xpert for TB and/or bacteria culture and/or microscopy served as the reference standard for definite infectious pericardial effusions. We calculated sensitivity, specificity, positive and negative predictive values, negative and positive likelihood ratios for an *a priori* pH cut off of 7.35. Receiver operating characteristic curve analysis was used for selection of ideal pH cut off.

RESULTS

Using a set sensitivity of 70% we estimated that we needed to recruit a sample size of 149 subjects for a 95% confidence interval and power of 80%. We screened 200 patients, and excluded 60 because they did not meet the appropriate exclusion criteria.

The prevalence of infectious pericarditis was 27.1% (n/N=34/140) as confirmed by the reference standard. We found the median pH (IQR) was 7.30(7.20-7.30) for definite infection, 7.30(7.30-7.35) for probable infection and 7.50(7.40-7.55) for non-infectious effusions p value <0.01 (test for trend). At a cut off of <7.35, the sensitivity was 89.5(95% CI: 75%.5-97.1%) and the specificity was 72.5% (95% CI: 62.8%-80.9%). The ideal ROC-determined cut off for pH that would give maximum sensitivity and specificity was ≤7.30 and the maximum sensitivity and specificity at optimum cut off are 86.8% (95% CI:71.9 - 95.6) and 86.8% (95% CI:71.9 - 95.6), respectively. The area under the curve at this cut-off point is 0.86 (95% CI 0.79 to 0.9), p<0. 001.

CONCLUSION:

In conclusion, pericardial PH offers diagnostic utility for infectious causes of pericardial effusions using both a PH of 7.35 and an ideal cut-off of 7.30. We recommend that given the simplicity of the test it should be adopted in evaluation of patients with pericardial effusions.

CHAPTER ONE: INTRODUCTION

1.1 PERICARDIUM AND ITS ROLE IN HEALTH

The pericardium is one of the serosal cavities of mammals.(1) It is a fibrous—serosal conical sac enclosing the roots of the aorta and the pulmonary artery. (2) Pericardium isolates the heart from the adjacent tissues, allowing its free movement within the boundaries of the pericardial cavity and is filled with a small amount of fluid which is called pericardial fluid. (2)

The normal pericardium contributes to important functions of the heart. It is necessary for: (1) lubricating the moving surfaces of the heart, (2) stabilizing the heart anatomic position, (3) isolating the heart from the adjacent anatomical structures, prohibiting the adhesion formation, the inflammatory or neoplastic extension, (4) limiting heart dilatation during diastole, reducing the endomyocardial tension, (5) preventing cardiac hypertrophy in pressure overload conditions, (6) reducing the right ventricular impulse work in left ventricular overload conditions, (7) the ventriculoarterial blood retrogression prevention during high end- diastolic ventricular pressures, (8) the preservation of the negative intrathoracic pressure, which is crucial for blood filling of atria, (9) the nervous stimulation response and regulation of the cardiac frequency and arterial blood pressure, (10) the formation of a hydrostatic compensation system ensuring that end- diastolic pressure remains the same at all hydrostatic levels and the Frank–Starling mechanism is functional. (3)

1.1.1 Global burden of pericardial disease

Pericardial disease remains an important cause of mortality and morbidity in the world. (4) Pericarditis makes up about 5% of admissions in accident and emergency units in the developed world. (5)

The etiology of pericardial disease in developed countries has been found to be mainly idiopathic in over 89% of patients, with the remainder being due to infections and malignancy (6). A study done in Western Europe revealed 5% of the patients to have infectious pericarditis, which is different from Imazio et al's study in Western Europe where 20% of cases were due to infectious pericarditis. (7) Over the years the incidence and prevalence of

pericarditis has been difficult to measure even in developed settings, but subclinical pericarditis has been found in 1% of autopsies. (8)

There is a growing burden of pericarditis in Africa and most especially in the sub-Saharan Africa. This is mainly due to the huge burden of HIV/AIDS which predisposes patients to TB in various organs/sites including the pericardium i.e. TB pericarditis, and other opportunistic infections. (9) In developing countries infectious pericardial effusions are more prevalent among patients with pericardial disease than idiopathic causes, as in the case of the developed world. A study done by Reuter et al (10) in a large academic hospital in South Africa revealed that 71.6% of the effusions were of an infectious cause. In another study done by Pandie et al, 86.1% of the patients at another large academic hospital in South Africa had infectious pericarditis.(11) A series of these published studies, as described above are in Table 1 highlighting selected papers reflecting the pattern of pericardial disease in both developed and developing worlds.

With the high burden of HIV in Sub-Saharan Africa, the number of people presenting with pericardial effusion has increased. Of patients presenting with pericardial effusions 67% are HIV positive. (12)

1.2 MORBIDITY AND MORTALITY OF PERICARDIAL DISEASE

Patients admitted with acute pericarditis with advancing age and co-infections are over three times and thirteen times more likely to suffer in hospital mortality respectively.(13) In study done by Mpiko et al on Tuberculous pericarditis with and without HIV, patients with HIV had larger pericardial effusions and more cardiovascular impairment, contributing to greater morbidity and later mortality among these patients .(14) In older studies the overall mortality of patients with non TB purulent pericarditis has been found to be 77% and reduced to 55% in those who received appropriate treatment.(15) A five year retrospective study of 57 patients in Zimbabwe revealed 100 % mortality among patients with acute purulent pericarditis.(16) Another study showed the mortality of purulent pericarditis to be 100% in untreated patients in Ohio.(17) Of patients diagnosed with

constrictive pericarditis, 60% will die in the next 10 years from date of diagnosis. (18) In a study done by Mayosi et al, the overall mortality of patients with TB pericarditis was 18.05%. (19, 20)

The presumptive diagnosis of TB pericarditis has been identified as an independent risk factor for mortality, especially in HIV positive patients, in whom the risk of mortality is approximately 4.5 times higher [HR 5.35, 95% CI 1.76-16.25] than HIV uninfected controls. (21) Patients with pericarditis in the era of HIV have been found to suffer greater morbidity. (22, 23) There is increased mortality from pericarditis among patients with HIV due to tamponade and constriction. (20, 24) In a recently published IMPI trial, patients with HIV were found to have less constriction from TB pericarditis and thus fewer complications from constriction, compared to HIV negative population. (19, 20)

1.2.1 Diagnosis of pericarditis

The diagnosis of pericarditis can be made from a medical history, clinical examination, electrocardiogram and echocardiogram, although it is much more complicated establishing its etiology. (6) The clinical diagnosis of acute pericarditis is reached when a patient has two of the following: typical chest pain, pericardial rub, wide spread ST elevation and pericardial effusion. Elevated inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate are confirmatory of a diagnosis of pericarditis. (25) Features associated with high risk pericarditis (non-viral or non-idiopathic, recurrence, constriction and cardiac tamponade) include: fever >38.0 degrees, sub-acute presentation, immunosuppression, trauma, anti-coagulation, myopericarditis, severe pericardial effusion (diastolic echo free fluid more than 20mm) and tamponade. (26)

Therapeutic pericardiocentesis is recommended in patients that have symptomatic large effusions (≥ 2cm on echocardiogram in diastole). Pericardial effusions and diagnostic pericardiocentesis is recommended where there is a high suspicion of infectious and neoplastic pericardial effusions. (27) Cardiac catheterization and 2-(18F)-Fluoro-2-deoxy-D-glucose (FDG) uptake with PET-CT have also become very useful in diagnosis of pericardial disease especially in effusive pericarditis and pericardial malignancy respectively. (28, 29)

Newer point-of-care diagnostic tests have been introduced. Adenosine Deaminase (ADA) has been used in the diagnosis of TB pericarditis (TBP). A Meta-analysis showed pooled sensitivity was 0.96 and specificity was 0.96 (30). Pandie et al showed that unstimulated interferon gamma (uIFNγ) offers superior accuracy for the diagnosis of microbiologically confirmed TBP, compared to the new Xpert MTB/RIF test and the established ADA assay. (11) Magnetic resonance and CT have been found to be useful in the diagnosis of pericarditis in the presence of constriction and a mass. (31, 32) Pericardial biopsy is useful in diagnosing and determining specific etiology, especially in patients who are having recurrent pericarditis (33).

The diagnosis of infectious pericarditis (including TB) pericarditis is very challenging, and this has great impact on mobility and mortality (34). The use of simple and affordable tests may simplify and speed up the diagnosis of disease, and thus improve the outcome from disease treatment. In the study done by Pandie et al, over 87% of pericardial effusions were infectious and the outcome was worse in the non-infectious group. (11) A simple test, such as pH to differentiate infectious from non-infectious pericardial effusion, will then be very useful as it will simplify and enable timely and appropriate patient management and thus improve the outcome.

1.1 MECHANISMS OF PERICARDIAL INJURY AND CONSTRICTION:

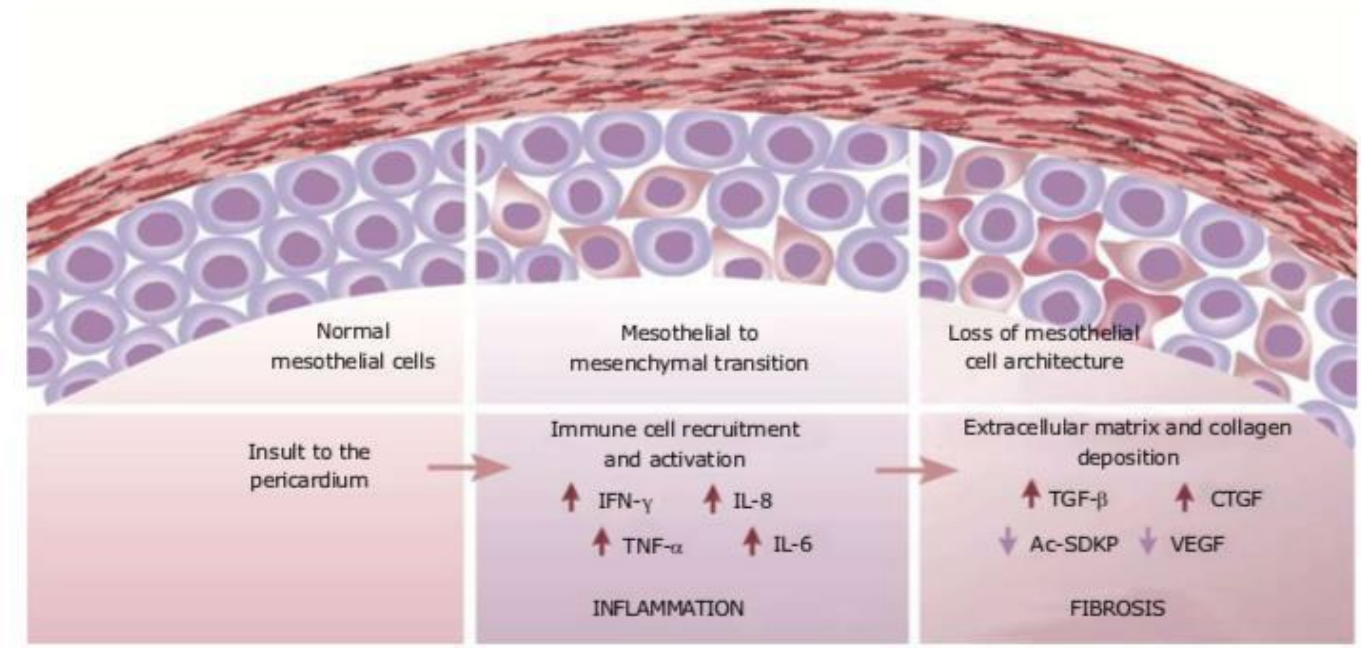

Figure 1: The diagram demonstrates the pathophysiological mechanism of pericardial injury and construction. It starts with an insult to the pericardium, this is followed by inflammatory infiltration with the release of IF-ψ IL-8, TNF-α and IL-6. This immune cell recruitment leads increase in TGF- and CTGF with resultant loss of mesothelial cell architecture and finally fibrosis which is the hall mark of constriction.

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1.3 STUDIES DONE ON PH ON PLEURAL FLUID: LESSONS LEARNT

PH has been used in pleural effusions for diagnostic purposes and therefore such studies will be useful to asses if we can adapt the use of pH in diagnosis of pericardial effusions [Table 2]. Pleural pH less than 7.30 has been found to correlate with infectious causes of pleural effusions, irrespective of the serum pH. (35). In a meta-analysis of chemistry predictors of complicated pleural effusions, pH was found to be the most effective predictor of complicated (infected) pleural effusion, [AUC:0. 92, CI: 0. 90-0.94] (36). PH as a diagnostic tool in pleural disease has been validated and recommended for use. (37) Pleural fluid studies have been done where pleural pH less than 7.20 was used to signify infectious pleural effusion. (38)

Pleural fluid pH provides more useful information for estimating the likelihood of pleurodesis failure, for which continuous likelihood ratios provide more information than binary or multilevel likelihood ratios.(39) pH less than 7.2 has been found to predict infectious pleural effusion, and is thus a guide to management of such patients.(40, 41) A study done by Light et al showed pH less than 7.2 to signify infectious pleural effusion and demonstrated that patients who were treated successfully with antibiotics showed their pH go above 7.2.(42)

Another study demonstrated that pH less than 7.3 and glucose less than 60mg/dl signify infectious pleural effusions. (43) A study by Potts et al also revealed pH less than 7.3 signified infectious pleural effusions. (44)

Ferreiro et al revealed that pH less than 7.2 signifies pleural effusions complicated by infection. (45) In a study by Fanjul et al an acidic pH less than 7.0 was found not only to be a predictor of pleural effusions complicated by infection, but was also a marker of poor outcome, thus prognostic. (46) Table 2 illustrates some of the studies described above.

1.3.1 Mechanism of low pH in pleural fluid and other serosa cavities

The pathophysiology of low pH in pleural fluid and other serosa cavities is poorly understood. However, existing theories suggest that it is due to an increased production of carbon dioxide (CO_2) by leucocytes and microbes from glucose metabolism, and by reduced diffusion of CO_2, hydrogen ions and glucose across a diseased membrane. (47)

1.3.2 Use of pH in pericardial disease

The use of pericardial pH as a guide to diagnosis has not been well validated. Animal studies have had contradictory results with the use of pH in pericardial fluid analysis, with some demonstrating a pH lower than 7.80 in neoplastic pericardial effusion, as opposed to idiopathic pericardial effusions, and others not demonstrating a difference in the two categories in dogs (48, 49). However, these studies did not assess the use of pH in differentiating between pericardial effusions of infectious and non-infectious etiology. A literature search yielded only two such studies. First, a small study of 13 surgical patients has shown that pH less than 7.08

is associated with infectious pericardial effusions. (50) Second, another small study done on 15 children with purulent pericardial effusions revealed an average pH of 7.01± 0.06. However, these children were culture negative for both pericardial effusions and blood. (51) A table summarizing these studies is given below (Table 3).

Due to the behaviour and characteristics of pericardial fluid compared to pleural fluid, Lights' criteria have not been found to show the same applicability in differentiating exudative and transudative effusions.(49) Fluid is exudate if one of the following Light's criteria is present;

a) Effusion protein/serum protein ratio greater than 0.5

b) effusion Lactate dehydrogenase (LDH)/serum LDH > 0.6

c) LDH in effusion > 2/3 of that in serum LDH upper limit

Given all these uncertainties regarding the use of pH in pericardial disease, we carried out a study to assess the utility of pH in differentiating infectious and non-infectious causes of pericardial effusions.

1.4 PURPOSE OF THE STUDY

The overall purpose of this study was to improve the diagnosis of infectious pericarditis by introducing a simple and rapid method of identifying infectious pericardial effusions during pericardiocentesis. This study aimed to determine the diagnostic accuracy of pH estimation in the diagnosis of infectious pericarditis in patients with pericardial effusions eligible for pericardiocentesis (effusions of more than 1cm on echocardiogram in diastole) presenting to Groote Schuur Hospital. The utility of such a simple test would simplify diagnosis and positively impact prognosis.

1.4.1 Hypothesis:

We hypothesize that an acidic pH (i.e. less than 7.35) as determined by a blood gas analyser as the index test, can accurately differentiate infectious from non-infectious causes of pericardial effusion in patients with moderate to large pericardial effusions.

1.4 OBJECTIVES

1.5.1 Primary objectives

To determine the accuracy of pericardial fluid pH as a test to differentiate infectious versus non-infectious pericardial effusions

To determine the ideal pH cut off between infectious and non-infectious pericardial effusion using Receiver operating characteristics (ROC) curves.

1.5.2 Secondary objectives

To describe the diagnostic accuracy of pericardial fluid pH determination added to ADA(>35IU) in the diagnosis of tuberculous pericarditis

To determine the Sensitivity, specificity, negative predictive value, positive predictive value, negative, positive likelihood ratios and Area under the curve (AUC) using acidic pH cutoff of 7.35 as stated by NHLS

1.6 RESEARCH SETTING METHODS AND DESIGN:

This study was carried out at the Division of Cardiology, Groote Schuur Hospital (GSH) in Cape Town, one of two provincial government-funded, adult, academic tertiary health facilities that serves a population of >2 million people with a wide socioeconomic ethnic and cultural diversity from Western Cape. It was a cross sectional substudy of the IMPI 2-pilot trial, which compared fibrinolytic facilitated pericardiocentesis in patients with effusive pericarditis of all aetiologies, with routine pericardiocentesis for the reduction of constrictive pericarditis (https://clinicaltrials.gov/ct2/show/NCT02673879). Participants included in the analysis included

adults with moderate to large pericardial effusions enrolled into IMPI-2 between February 2016 to May 2018. In order to enrich the proportion of patients with non-infections pericardial effusions and normal pericardial fluid, patients under-going open cardiac surgery were also consented for the during the same period

1.6.1 ETHICAL CONSIDERATIONS:

Ethical approval was thus obtained from the Human Ethics Research Committee, University of Cape Town (HREC/REF: 475/2015 and all participants signed informed consent to participate

1.6.2 AUTHOR GUIDELINES FOR CARDIOVASCULAR JOURNAL OF AFRICA

We intend to submit to the Cardiovascular Journal of Africa whose guidelines for original articles are as follows:

Title page.

Abstract (150 words) a short inclusive statement suitable for direct electronic abstracting, identifying the purpose of the study, key methods, the main results and the main conclusion.

Key words; maximum six key words for indexing. Introduction: concise description of background, sufficient for the non-specialist to appreciate the context of the work. Clear statement of the purpose of the study.

Methods; a brief description of the study design, procedures, analytical techniques and statistical evaluation.

Results: a clear account of the study findings using quantitative language where possible and cross-referenced to the tables and figures.

Discussion: an interpretation of the study placed within the context of current knowledge, leading to specific conclusions where possible.

Acknowledgements. References, figures and tables.

1.7 REFERENCES

1. Michailova KN, Usunoff KG. Serosal membranes (pleura, pericardium, peritoneum). Normal structure, development and experimental pathology. Adv Anat Embryol Cell Biol. 2006;183:i-vii, 1-144, back cover.
2. Chinchoy. The Pericardium. 2005.
3. Holt JP. The normal pericardium. Am J Cardiol. 1970;26(5):455-65.
4. Ivens EL, Munt BI, Moss RR. Pericardial disease: what the general cardiologist needs to know. Heart. 2007;93(8):993-1000.
5. Launbjerg J, Fruergaard P, Hesse B, Jorgensen F, Elsborg L, Petri A. Long-term risk of death, cardiac events and recurrent chest pain in patients with acute chest pain of different origin. Cardiology. 1996;87(1):60-6.
6. Permanyer-Miralda G, Sagrista-Sauleda J, Soler-Soler J. Primary acute pericardial disease: a prospective series of 231 consecutive patients. Am J Cardiol. 1985;56(10):623-30.
7. Zayas R, Anguita M, Torres F, Gimenez D, Bergillos F, Ruiz M, et al. Incidence of specific etiology and role of methods for specific etiologic diagnosis of primary acute pericarditis. Am J Cardiol. 1995;75(5):378-82.
8. Troughton RW, Asher CR, Klein AL. Pericarditis. Lancet. 2004;363(9410):717-27.
9. Mayosi BM. Contemporary trends in the epidemiology and management of cardiomyopathy and pericarditis in sub-Saharan Africa. Heart. 2007;93(10):1176-83.
10. Reuter H, Burgess LJ, Doubell AF. Epidemiology of pericardial effusions at a large academic hospital in South Africa. Epidemiol Infect. 2005;133(3):393-9.
11. Pandie S, Peter JG, Kerbelker ZS, Meldau R, Theron G, Govender U, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-gamma in a high burden setting: a prospective study. BMC Med. 2014;12:101.
12. Cegielski JP, Ramiya K, Lallinger GJ, Mtulia IA, Mbaga IM. Pericardial disease and human immunodeficiency virus in Dar es Salaam, Tanzania. Lancet. 1990;335(8683):209-12.
13. Kyto V, Sipila J, Rautava P. Clinical profile and influences on outcomes in patients hospitalized for acute pericarditis. Circulation. 2014;130(18):1601-6.
14. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. Heart Fail Rev. 2013;18(3):367-73.
15. Rubin RH, Moellering RC, Jr. Clinical, microbiologic and therapeutic aspects of purulent pericarditis. Am J Med. 1975;59(1):68-78.
16. Mynors JM, Reichman B. Pericarditis--a five year study in the African. Cent Afr J Med. 1973;19(2):19-22.
17. Kauffman CA, Watanakunakorn C, Phair JP. Purulent pneumococcal pericarditis. A continuing problem in the antibiotic era. Am J Med. 1973;54(6):743-50.
18. Thia A, Prunier F, Subayi JB, Baufreton C, Geslin P, Furber A. [Long term evolution of chronic constrictive pericarditis. A study of 56 patients]. Arch Mal Coeur Vaiss. 2006;99(9):775-80.
19. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in Tuberculous Pericarditis. New Engl J Med. 2014;371(12):1121-30.
20. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. N Engl J Med. 2014;371(12):1121-30.
21. Mayosi BM, Wiysonge CS, Ntsekhe M, Gumedze F, Volmink JA, Maartens G, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. S Afr Med J. 2008;98(1):36-40.
22. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. Bmc Infect Dis. 2006;6.
23. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. BMC Infect Dis. 2006;6:2.
24. Mayosi BM, Ntsekhe M, Smieja M. Immunotherapy for tuberculous pericarditis. N Engl J Med. 2014;371(26):2534.
25. Brucato A, Brambilla G, Moreo A, Alberti A, Munforti C, Ghirardello A, et al. Long-term outcomes in difficult-to-treat patients with recurrent pericarditis. Am J Cardiol. 2006;98(2):267-71.

26. Soler-Soler J, Permanyer-Miralda G, Sagrista-Sauleda J. A systematic diagnostic approach to primary acute pericardial disease. The Barcelona experience. Cardiol Clin. 1990;8(4):609-20.
27. Maisch B, Seferovic PM, Ristic AD, Erbel R, Rienmuller R, Adler Y, et al. Guidelines on the diagnosis and management of pericardial diseases - Executive summary. European heart journal. 2004;25(7):587-610.
28. Sagrista-Sauleda J, Angel J, Sanchez A, Permanyer-Miralda G, Soler-Soler J. Effusive-constrictive pericarditis. N Engl J Med. 2004;350(5):469-75.
29. Lobert P, Brown RK, Dvorak RA, Corbett JR, Kazerooni EA, Wong KK. Spectrum of physiological and pathological cardiac and pericardial uptake of FDG in oncology PET-CT. Clin Radiol. 2013;68(1):e59-71.
30. Shen YC, Wang T, Chen L, Yang T, Wan C, Hu QJ, et al. Diagnostic accuracy of adenosine deaminase for tuberculous peritonitis: a meta-analysis. Arch Med Sci. 2013;9(4):601-7.
31. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
32. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
33. Maisch B, Rupp H, Ristic A, Pankuweit S. Pericardioscopy and epi- and pericardial biopsy - a new window to the heart improving etiological diagnoses and permitting targeted intrapericardial therapy. Heart Fail Rev. 2013;18(3):317-28.
34. Cohen R, Cohen-Aubart F, Steg PG. [Acute pericarditis in the modern era: a diagnostic challenge]. Ann Cardiol Angeiol (Paris). 2008;57(1):10-5.
35. Good JT, Jr., Taryle DA, Maulitz RM, Kaplan RL, Sahn SA. The diagnostic value of pleural fluid pH. Chest. 1980;78(1):55-9.
36. Heffner JE, Brown LK, Barbieri C, Deleo JM. Pleural Fluid Chemical-Analysis in Parapneumonic Effusions - a Metaanalysis. Am J Resp Crit Care. 1995;151(6):1700-8.
37. Bhatnagar R, Maskell N. Pleural fluid biochemistry - old controversies, new directions. Ann Clin Biochem. 2014;51(Pt 4):421-3.
38. Rahman NM, Maskell NA, West A, Teoh R, Arnold A, Mackinlay C, et al. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med. 2011;365(6):518-26.
39. Heffner JE, Heffner JN, Brown LK. Multilevel and continuous pleural fluid pH likelihood ratios for evaluating malignant pleural effusions. Chest. 2003;123(6):1887-94.
40. Houston MC. Pleural fluid pH: Diagnostic, therapeutic, and prognostic value. The American Journal of Surgery.154(3):333-7.
41. Houston MC. Pleural fluid pH: diagnostic, therapeutic, and prognostic value. Am J Surg. 1987;154(3):333-7.
42. Light RW, MacGregor MI, Ball Jr WC, Luchsinger PC. Diagnostic significance of pleural fluid pH and PCO2. Chest. 1973;64(5):591-6.
43. Potts DE, Willcox MA, Good Jr JT, Taryle DA, Sahn SA. The acidosis of low glucose pleural effusions. American Review of Respiratory Disease. 1978;117(4 I):665-71.
44. Potts DE, Levin DC, Sahn SA. Pleural fluid pH in parapneumonic effusions. Chest. 1976;70(03):328-31.
45. Ferreiro L, Toubes ME, Valdes L. [Contribution of pleural fluid analysis to the diagnosis of pleural effusion.]. Med Clin (Barc). 2014.
46. Fanjul M, Garcia-Casillas MA, Lain A, Matute JA, Parente A, Corona C, et al. [Prognostic value of pH and glucose in complicated parapneumonic pleural effusion]. Cir Pediatr. 2009;22(4):173-6.
47. Good JT, Jr., Taryle DA, Sahn SA. The pathogenesis of low glucose, low pH malignant effusions. Am Rev Respir Dis. 1985;131(5):737-41.
48. Fine DM, Tobias AH, Jacob KA. Use of pericardial fluid pH to distinguish between idiopathic and neoplastic effusions. J Vet Intern Med. 2003;17(4):525-9.
49. Amelle M LM. Biochemical Analysis of Pericardial Fluid and Whole Blood in Dogs with Pericardial Effusion
. J Vet Intern Med. 2005;19:833-6.
50. Kindig JR, Goodman MR. Clinical utility of pericardial fluid pH determination. Am J Med. 1983;75(6):1077-9.
51. Ekim M, Ekim H. Diagnostic value of the biochemical tests in patients with purulent pericarditis. Pak J Med Sci. 2014;30(4):845-9.

CHAPTER TWO: PUBLICATION READY-MANUSCRIPT

Diagnostic Utility of Pericardial pH in diagnosing infectious pericardial effusions among patients with moderate to large pericardial effusions attending Groote Schuur Hospital.

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ABSTRACT

Diagnosis of infectious pericardial disease has been challenging in the developing world despite improvement of treatment modalities. The diagnostic utility of pH in diagnosing infectious pericardial fluid is unknown, yet this concept is well studied in pleural fluid. This cross-sectional diagnostic study evaluated the diagnostic utility of pH in infectious compared to non-infectious pericardial effusions in a high-burden setting.

Methods: Patients of ≥18 years with moderate to large effusion between the 1st February 2016 and 31st May2018 were enrolled at Groote Schuur Hospital in Cape Town, South Africa. After safe pericardiocentesis, pH was measured with a blood gas analyzer. Mycobacterium tuberculosis culture and/or gene Xpert for TB and/or bacteria culture and/or microscopy served as the reference standard for definite infectious pericardial effusions. We calculated sensitivity, specificity, positive and negative predictive values, negative and positive likelihood ratios for an *a priori* pH cut off of 7.35. Receiver operating characteristic curve analysis was used for selection of ideal pH cut off.

RESULTS

Using a set sensitivity of 70% we estimated that we needed to recruit a sample size of 149 subjects for a 95% confidence interval and power of 80%. We screened 200 patients, and excluded 60 because they did not meet the appropriate exclusion criteria.

The prevalence of infectious pericarditis was 27.1% (n/N=34/140) as confirmed by the reference standard. We found the median pH (IQR) was 7.30(7.20-7.30) for definite infection, 7.30(7.30-7.35) for probable infection and 7.50(7.40-7.55) for non-infectious effusions p value <0.01 (test for trend). At a cut off of <7.35, the sensitivity was 89.5(95% CI: 75%.5-97.1%) and the specificity was 72.5% (95% CI: 62.8%-80.9%). The ideal ROC-determined cut off for pH that would give maximum sensitivity and specificity was ≤7.30 and the maximum sensitivity and specificity at optimum cut off are 86.8% (95% CI:71.9 - 95.6) and 86.8% (95% CI:71.9 - 95.6), respectively. The area under the curve at this cut-off point is 0.86 (95% CI 0.79 to 0.9), p<0. 001.

CONCLUSION:

In conclusion, pericardial PH offers diagnostic utility for infectious causes of pericardial effusions using both a PH of 7.35 and an ideal cut-off of 7.30. We recommend that given this a simple test it should be adopted in evaluation of patients with pericardial effusions.

2.1 INTRODUCTION

Pericarditis is the cause of chest pain in up to approximately 5% of adult patients presenting to the emergency room for the evaluation of chest pain. (5) The etiology of pericardial disease in this population is predominantly idiopathic (6). In sub-Saharan Africa, there is a growing burden of pericarditis that is due mainly to the huge burden of HIV/AIDS which predisposes patients to Tuberculosis (TB) and other opportunistic infections in various organs/sites including the pericardium. (9) The etiology of pericardial disease in the developing world and much of sub-Saharan Africa is due to infections mainly (70%). (10, 11)

Pericarditis has been associated with high morbidity and mortality particularly when the etiology is infectious. In the context of HIV infection, patients with pericarditis have larger pericardial effusions and more cardiovascular impairment due to tamponade and heart failure, than their HIV negative counterparts , contributing to greater morbidity and later mortality among these patients in both the developed and developing world.(14, 52) (22, 23) Patients with advancing age and co-morbidities admitted with acute pericarditis are over three times more likely to suffer in-hospital mortality in the developed world.(13)

Given that the infectious etiology of pericarditis is a major determinant of the outcome, the ability be to recognize it rapidly and accurately is of great importance. However, this is very challenging, particularly in the setting of HIV, TB and in geographies where modern diagnostic laboratory facilities are scarce because of resources (34). The use of microscopy on pericardial fluid for TB is not useful. While, culture is the diagnostic goal standard but it is associated with long waiting times of up to 8 weeks. Surrogate biochemical tests for the diagnosis of TB pericarditis (TBP) such as adenosine deaminase (ADA) are also available is some clinical and research labs; a meta-analysis of the diagnostic utility of ADA showed a pooled sensitivity of 96% and a specificity of 96% (30).

Pandie et al showed that unstimulated interferon gamma (uIFNγ) offers superior accuracy for the diagnosis of microbiologically confirmed TBP, compared to the new Xpert MTB/RIF test and the established ADA assay. (11) However, many of these tests are not widely available at the rural and peri-urban clinics and community health care centers throughout sub Saharan Africa where patients present.

Given these significant limitations of currently available tests, and the need and importance of rapidly establishing that pericarditis is infectious, if evidence were made available that demonstrates that a simple test, such a pericardial fluid pH can be differentiate infectious from non-infectious pericardial effusion, it would make a significant impact for clinicians practicing in environments where access to laboratory tests is limited.

PH has been used for diagnostic purposes in pleural effusions for decades. Several studies have demonstrated a correlation between PH and infectious causes at a PH cut off 7.20 and 7.30, irrespective of the serum pH (35) (38) and pleural pH has been validated and recommended for use to evaluate pleural fluid.(37). However, the pathophysiology of low pH in pleural fluid and other serosa cavities is poorly understood. Existing theories suggest that it may be that the pH drop is due to increased production of carbon dioxide (CO_2) by leucocytes and microbes following glucose metabolism, or by reduced diffusion of CO_2, hydrogen ions and glucose across a diseased membrane. (47)

Due to the need for a simple straightforward cheap and relatively accurate diagnostic test of infectious pericarditis and the potential for pericardial pH to do so, we performed a study to assess the utility of pH in differentiating infectious and non-infectious causes of pericardial effusions. We hypothesized that pericardial fluid pH below a value of 7.35(which is the cut off used by NHLS for acidic PH) would be able accurately distinguish infectious pericarditis (tuberculous and bacterial) from normal pericardial fluid and non-infectious (malignant, idiopathic and other inflammatory)

2.1.1 Design and Methods

Consecutive patients who were 18 years and above referred to Groote Schuur Hospital between the 1st February 2016 and 31st May2018 with moderate and large effusions for percutaneous or surgical removal of pericardial fluid were recruited to participate in the study. The study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC/REF: 475/2015 and all participants signed informed consent. All participants underwent pericardiocentesis which was performed under aseptic conditions and local anesthetic by a qualified physician under echocardiographic or fluoroscopic guidance. The full protocol is given in Appendix D.

The primary study objectives were to:

1. determine if the pericardial pH could accurately differentiate infectious from normal fluid amongst patients who referred for diagnostic and/or therapeutic pericardiocentesis; and
2. to determine the ideal pH cut off between infectious and non-infectious pericardial effusion.

The secondary objective of the study was to assess the ability of pericardial fluid pH to increase the diagnostic accuracy of pericardial fluid ADA in the diagnosis of infectious effusions due to TB.

2.1.2 Research procedures and data collection methods

Once patient signed consent to participate they were then taken to the catheterization laboratory where, under aseptic conditions and local anaesthesia, a pericardiocentesis was performed percutaneously to obtain at least 50ml of pericardial fluid following set standard protocols.(53) The sample obtained was divided into five smaller samples. One sample of 10ml was put into a mycobacteria growth indicator tube liquid culture for TB culture; another sample of 5 mls was drawn into a heparinized bottle for pH measurement; another sample of 5 mls was put into a heparinized bottle for cell count and microscopy for ZN and gram stain; another sample in purple top for gene expert for TB; and the final sample of 10mls was drawn in a standard blood culture bottle to culture other

bacteria. All these samples were delivered to the NHLS laboratory immediately, to allow for timely analysis (within an hour) particularly for the pH sample which was delivered on ice to avoid delays. The NHLS laboratory was able to receive the samples at any time of the day.

We also obtained pericardial samples from the cardiothoracic surgery unit from patients undergoing cardiac surgery during the period of the study with informed consent from the patients

It was ensured that those involved in pH measurement were blinded to the results for the reference standard and those evaluating the reference standard were blinded to pH results and this was done to minimize bias as a requirement by standard guidelines of diagnostic studies.

2.1.3 Procedure for sampling and measurement for pH

Pericardial fluid samples for pH measurement were drawn into a heparinized bottle, as studies have shown no effect of pH when heparinized bottles are used.(54) A sample on ice was sent to a chemistry laboratory for the pH to be measured within one hour to avoid delays. In an experiment at the NHLS laboratory in preparation for the study (as shown in the Appendix) it was demonstrated that pH measurement using pH strips is affected by the concentration of protein in the fluid as these depend on protein dyes and for this reason, this was not used in this study.

2.1.4 Operational Definitions:

These have been summarized in table 1a in appendix B

2.1.5 Data Management

All demographic, clinical, blood, pericardial fluid, ECG and imaging data was entered into a CRF and later entered into the Redcap database on a daily basis by the study lead investigator (BK), and it was backed up on an external drive. Any incomplete data was quickly obtained and entered promptly. This data was then exported to STATA version 13.0 software for analysis.

2.1.6 Statistical considerations and data analysis plan

The objective we used was to determine the accuracy of pericardial fluid pH as a test to differentiate infectious versus non-infectious pericardial effusions. Using this objective, using a sensitivity of 70%, we needed to recruit 149 subjects for a 95% confidence interval and power of 80%.

Continuous variables were summarized using mean (SD) for normally distributed continuous variables, median (interquartile range-IQR) for non-normally distributed continuous variables while categorical variables were expressed as frequencies and percentages. Differences in median pH across groups were analyzed using Kruskal-Wallis test for differences in medians. Correlation between pH and specified variables was tested using Pearson correlation coefficient.

Diagnostic accuracy was derived using cultures inclusive of TB culture and other infective organisms and/or gene expert for TB, and /or microscopy (with various stains) as a reference standard.

For primary objective 1: We determined the median and interquartile range (IQR) pH in the infectious pericardial effusions and non-infectious pericardial effusions using Kruskal-Wallis test to assess for differences in the medians. We then used the pH cut off of 7.35, to determine the sensitivity, specificity, positive productive value, negative productive value, negative and positive likelihood ratios for each of the index tests, and pericardial pH measured by blood gas analyzer.

For primary objective 2: Using the AUC, we determined the ideal cut off of median pH for infectious pericardial effusions and non-infectious pericardial effusions.

For secondary objective 1: We determined the sensitivity, specificity, negative predictive value, positive predictive value, and negative and positive likelihood ratios of pericardial pH used with ADA, in diagnosing

Infectious pericarditis due to TB. We also used ROC to compare the AUC for pH alone, and pH used with ADA in differentiating infectious and non-infectious pericardial effusions.

2.1.7 RESULTS:

The study flow chart is given in Figure 2. 200 patients were screened, 60 patients were excluded because their effusions were not amenable to percutaneous pericardiocentesis and or had incomplete data for analysis.

140 participants met the criteria for inclusion in this substudy, the mean age was 46.4 years, 33.6% were HIV positive, 24.3% were hypertensive and 70 of these participants were from the surgical cohort. Among these participants 21.4% had a known TB diagnosis on appropriate treatment.

Of the 140 participants, 63(45%) were found to have infectious pericardial effusions and majority of these 61.9%) had definite infection as described by our reference standard. Table 1 outlines the basic clinical characteristics, important significance difference between the two groups included systolic blood pressure, mean pulse rate, venous pressure, HIV, HTN and TB status.

Table 2 outlines the basic clinical and echocardiographic characteristics between the two groups and significant differences between the two groups including peripheral oedema, size of effusion and features of tamponade.

Tables 3 and 4 outline the X-ray and laboratory findings between the two groups with significant difference found in effusion characteristics, LDH, TB microscopy, lymphocyte predominance culture, renal function ,serum electrolytes, cholesterol and glucose.

2.1.8 Diagnostic utility of pericardial PH in infectious pericardial effusions:

We found the median (IQR) pH was 7.30 (7.20-7.30) for definite infection, 7.30 (7.30-7.35) for probable infection and 7.50 (7.40-7.55) for non-infectious effusions and these were statistically different ($p < 0.05$). The prevalence of infection was 27.1% and that for definite infection was 33% (Table 5 and 6). At pH cut off <7.35, the likelihood of detecting a positive result among the truly confirmed positives was 89.5(95% CI: 75%.5-97.1%)

and the likelihood of detecting a negative result among the truly confirmed negatives was 72.5% (95% CI: 62.8%-80.9%).

Using the receiver operator curve (ROC) (Figure 3 and Table 7), the ideal cut off for pH that would give maximum sensitivity and specificity was ≤7.30 and the maximum sensitivity and specificity at optimum cut off are 86.8% (95% CI:71.9 - 95.6) and 79.4% (70.3 - 86.8). The area under the curve at this cut-off point is 0.86(95% CI 0.79 to 0.9), p<0.001.

2.1.9 Assessing whether addition of ADA to PH increases the sensitivity and specificity with positive culture as reference standard

There was no significant difference in sensitivity between a combination of ADA (>=35) and PH (<7.35) and PH (<7.35) alone, p=0.063 (Table 8). Addition of ADA appeared to lower sensitivity (77.1% versus 89.5%). However, there was a statistically significant difference in specificities with a combination of ADA (>=35) and PH (<7.35) compared to PH (<7.35) alone (p=0.002). Addition of ADA to PH increased specificity by 7.5% (81.0% from 72.5%) 95% CI, 72.1-88.0) which overlap.

2.2 DISCUSSION:

There were 3 main findings of this study. The first important finding was that pericardial pH is an accurate and simple test to differentiate between infectious (definite and probable) and non-infectious causes of pericardial effusion. Using a conventional cut-off for acidic pH of 7.35 we found that this value had a sensitivity of 89.5%, a specificity of 72.5% and an area under the curve (AUC) value of 0.81. When we used a definition of infectious pericarditis that included only samples with a confirmed bacteriological diagnosis (confirmed), the sensitivity of 89.5%, specificity of 97.4% and area under the curve value 0.93 for a pH of 7.35 improved significantly.

The second major finding of this study was that the ideal pH cut value to differentiate between infectious (definite and probable) and non-infectious causes of pericardial effusions was 7.30. Using Receiver Operating Curve (ROC), analysis we determined that a pH of 7.30 worked optimally with a sensitivity of 86.8% , a specificity of 79.4%, area under the curve(AUC) value of 0.86 and a p value of 0.001. Interestingly this cut off correlates closely with the value which is used to differentiate infectious causes of pleural effusions from non-infections pleural effusions. (35) (37)

The 3rd major finding of this study was that the addition of ADA (cut off >35IU) to pericardial fluid pH to differentiate between infectious and non-infectious causes of pericardial effusions did not increase the diagnostic accuracy. This was surprising given that ADA used alone at a cut off of 35IU, has a sensitivity of 95.7% and a specificity of 84.0% for the diagnosis of pericardial fluid TB.(55) This could be because the study was not powered enough for this objective. From this data, adding ADA with cut off of >35IU to acidic PH (<7.35) we determined an increase in specificity by 7.5% and a decrease in sensitivity by 12.4% although this was not statistically significant.

To our knowledge use of pH as a guide to diagnosing infectious effusive pericarditis has not been well utilized in pericardial disease, most likely because its value was not studied before. This study therefore is novel and the first of its kind, with largest sample size to date, to demonstrate utility of pericardial fluid PH to identify infectious pericardial effusions with a fair degree of confidence. Furthermore, where blood gas analyzers are available, preforming pericardial pH is quick and simple allowing early initiation of appropriate and timely management of patients with pericardial effusions.

The limitation of this study is

that we were not able to recruit more patients into the study to further increase the power because some of the patients opted not to participate in the study as we initially aimed to recruit 200 patients. We were also affected by the number of participants that had incomplete data as they were excluded from analysis, but this was necessary to have clean data to answer the research question.

2.3 CONCLUSION AND RECOMMENDATION:

In conclusion, this study has demonstrated that pericardial PH is a test with diagnostic utility in patients with effusive pericarditis. A pH below 7.35 is able to differentiate between infectious and non-infectious pericardial effusions with a sensitivity of 89.5% and a specificity of 72.5%. Using the ROC-Curve analysis we have been able to demonstrate that a pH of 7.30 offers the best cut off value to differentiate between infectious and non-infectious causes of pericardial effusions. We have also demonstrated that while pericardial ADA remains a good test for the diagnosis of pericardial TB, it does not add to pericardial pH for the purpose of distinguishing infectious from non-infectious pericarditis.

Given the findings of this study we recommend that pericardial pH, which is a simple test, should be adopted in the evaluation of patients with pericardial effusions in order to rapidly and easily recognize those effusions that are infectious.

2.4 REFERENCES

1. Michailova KN, Usunoff KG. Serosal membranes (pleura, pericardium, peritoneum). Normal structure, development and experimental pathology. Adv Anat Embryol Cell Biol. 2006;183:i-vii, 1-144, back cover.
2. Chinchoy. The Pericardium. 2005.
3. Holt JP. The normal pericardium. Am J Cardiol. 1970;26(5):455-65.
4. Ivens EL, Munt BI, Moss RR. Pericardial disease: what the general cardiologist needs to know. Heart. 2007;93(8):993-1000.
5. Launbjerg J, Fruergaard P, Hesse B, Jorgensen F, Elsborg L, Petri A. Long-term risk of death, cardiac events and recurrent chest pain in patients with acute chest pain of different origin. Cardiology. 1996;87(1):60-6.
6. Permanyer-Miralda G, Sagrista-Sauleda J, Soler-Soler J. Primary acute pericardial disease: a prospective series of 231 consecutive patients. Am J Cardiol. 1985;56(10):623-30.
7. Zayas R, Anguita M, Torres F, Gimenez D, Bergillos F, Ruiz M, et al. Incidence of specific etiology and role of methods for specific etiologic diagnosis of primary acute pericarditis. Am J Cardiol. 1995;75(5):378-82.
8. Troughton RW, Asher CR, Klein AL. Pericarditis. Lancet. 2004;363(9410):717-27.
9. Mayosi BM. Contemporary trends in the epidemiology and management of cardiomyopathy and pericarditis in sub-Saharan Africa. Heart. 2007;93(10):1176-83.
10. Reuter H, Burgess LJ, Doubell AF. Epidemiology of pericardial effusions at a large academic hospital in South Africa. Epidemiol Infect. 2005;133(3):393-9.

11. Pandie S, Peter JG, Kerbelker ZS, Meldau R, Theron G, Govender U, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-gamma in a high burden setting: a prospective study. BMC Med. 2014;12:101.
12. Cegielski JP, Ramiya K, Lallinger GJ, Mtulia IA, Mbaga IM. Pericardial disease and human immunodeficiency virus in Dar es Salaam, Tanzania. Lancet. 1990;335(8683):209-12.
13. Kyto V, Sipila J, Rautava P. Clinical profile and influences on outcomes in patients hospitalized for acute pericarditis. Circulation. 2014;130(18):1601-6.
14. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. Heart Fail Rev. 2013;18(3):367-73.
15. Rubin RH, Moellering RC, Jr. Clinical, microbiologic and therapeutic aspects of purulent pericarditis. Am J Med. 1975;59(1):68-78.
16. Mynors JM, Reichman B. Pericarditis--a five year study in the African. Cent Afr J Med. 1973;19(2):19-22.
17. Kauffman CA, Watanakunakorn C, Phair JP. Purulent pneumococcal pericarditis. A continuing problem in the antibiotic era. Am J Med. 1973;54(6):743-50.
18. Thia A, Prunier F, Subayi JB, Baufreton C, Geslin P, Furber A. [Long term evolution of chronic constrictive pericarditis. A study of 56 patients]. Arch Mal Coeur Vaiss. 2006;99(9):775-80.
19. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in Tuberculous Pericarditis. New Engl J Med. 2014;371(12):1121-30.
20. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. N Engl J Med. 2014;371(12):1121-30.
21. Mayosi BM, Wiysonge CS, Ntsekhe M, Gumedze F, Volmink JA, Maartens G, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. S Afr Med J. 2008;98(1):36-40.
22. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. Bmc Infect Dis. 2006;6.
23. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. BMC Infect Dis. 2006;6:2.
24. Mayosi BM, Ntsekhe M, Smieja M. Immunotherapy for tuberculous pericarditis. N Engl J Med. 2014;371(26):2534.
25. Brucato A, Brambilla G, Moreo A, Alberti A, Munforti C, Ghirardello A, et al. Long-term outcomes in difficult-to-treat patients with recurrent pericarditis. Am J Cardiol. 2006;98(2):267-71.
26. Soler-Soler J, Permanyer-Miralda G, Sagrista-Sauleda J. A systematic diagnostic approach to primary acute pericardial disease. The Barcelona experience. Cardiol Clin. 1990;8(4):609-20.
27. Maisch B, Seferovic PM, Ristic AD, Erbel R, Rienmuller R, Adler Y, et al. Guidelines on the diagnosis and management of pericardial diseases - Executive summary. European heart journal. 2004;25(7):587-610.
28. Sagrista-Sauleda J, Angel J, Sanchez A, Permanyer-Miralda G, Soler-Soler J. Effusive-constrictive pericarditis. N Engl J Med. 2004;350(5):469-75.
29. Lobert P, Brown RK, Dvorak RA, Corbett JR, Kazerooni EA, Wong KK. Spectrum of physiological and pathological cardiac and pericardial uptake of FDG in oncology PET-CT. Clin Radiol. 2013;68(1):e59-71.
30. Shen YC, Wang T, Chen L, Yang T, Wan C, Hu QJ, et al. Diagnostic accuracy of adenosine deaminase for tuberculous peritonitis: a meta-analysis. Arch Med Sci. 2013;9(4):601-7.
31. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
32. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
33. Maisch B, Rupp H, Ristic A, Pankuweit S. Pericardioscopy and epi- and pericardial biopsy - a new window to the heart improving etiological diagnoses and permitting targeted intrapericardial therapy. Heart Fail Rev. 2013;18(3):317-28.
34. Cohen R, Cohen-Aubart F, Steg PG. [Acute pericarditis in the modern era: a diagnostic challenge]. Ann Cardiol Angeiol (Paris). 2008;57(1):10-5.
35. Good JT, Jr., Taryle DA, Maulitz RM, Kaplan RL, Sahn SA. The diagnostic value of pleural fluid pH. Chest. 1980;78(1):55-9.

36. Heffner JE, Brown LK, Barbieri C, Deleo JM. Pleural Fluid Chemical-Analysis in Parapneumonic Effusions - a Metaanalysis. Am J Resp Crit Care. 1995;151(6):1700-8.
37. Bhatnagar R, Maskell N. Pleural fluid biochemistry - old controversies, new directions. Ann Clin Biochem. 2014;51(Pt 4):421-3.
38. Rahman NM, Maskell NA, West A, Teoh R, Arnold A, Mackinlay C, et al. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med. 2011;365(6):518-26.
39. Heffner JE, Heffner JN, Brown LK. Multilevel and continuous pleural fluid pH likelihood ratios for evaluating malignant pleural effusions. Chest. 2003;123(6):1887-94.
40. Houston MC. Pleural fluid pH: Diagnostic, therapeutic, and prognostic value. The American Journal of Surgery.154(3):333-7.
41. Houston MC. Pleural fluid pH: diagnostic, therapeutic, and prognostic value. Am J Surg. 1987;154(3):333-7.
42. Light RW, MacGregor MI, Ball Jr WC, Luchsinger PC. Diagnostic significance of pleural fluid pH and PCO2. Chest. 1973;64(5):591-6.
43. Potts DE, Willcox MA, Good Jr JT, Taryle DA, Sahn SA. The acidosis of low glucose pleural effusions. American Review of Respiratory Disease. 1978;117(4 I):665-71.
44. Potts DE, Levin DC, Sahn SA. Pleural fluid pH in parapneumonic effusions. Chest. 1976;70(03):328-31.
45. Ferreiro L, Toubes ME, Valdes L. [Contribution of pleural fluid analysis to the diagnosis of pleural effusion.]. Med Clin (Barc). 2014.
46. Fanjul M, Garcia-Casillas MA, Lain A, Matute JA, Parente A, Corona C, et al. [Prognostic value of pH and glucose in complicated parapneumonic pleural effusion]. Cir Pediatr. 2009;22(4):173-6.
47. Good JT, Jr., Taryle DA, Sahn SA. The pathogenesis of low glucose, low pH malignant effusions. Am Rev Respir Dis. 1985;131(5):737-41.
48. Fine DM, Tobias AH, Jacob KA. Use of pericardial fluid pH to distinguish between idiopathic and neoplastic effusions. J Vet Intern Med. 2003;17(4):525-9.
49. Amelle M LM. Biochemical Analysis of Pericardial Fluid and Whole Blood in Dogs with Pericardial Effusion
. J Vet Intern Med. 2005;19:833-6.
50. Kindig JR, Goodman MR. Clinical utility of pericardial fluid pH determination. Am J Med. 1983;75(6):1077-9.
51. Ekim M, Ekim H. Diagnostic value of the biochemical tests in patients with purulent pericarditis. Pak J Med Sci. 2014;30(4):845-9.
52. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. Heart Fail Rev. 2013;18(3):367-73.
53. Jung H-O. Pericardial Effusion and Pericardiocentesis: Role of Echocardiography. Korean Circulation Journal. 2012;42(11):725-34.
54. Bou-Khalil PK, Jamaleddine GW, Debek AH, El-Khatib MF. Use of heparinized versus non-heparinized syringes for measurements of the pleural fluid pH. Respiration. 2007;74(6):659-62.
55. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. Circulation. 2005;112(23):3608-16.
56. Imazio M, Spodick DH, Brucato A, Trinchero R, Adler Y. Controversial issues in the management of pericardial diseases. Circulation. 2010;121(7):916-28.
57. www.nhls.ac.za South Africa2015 [Available from: www.nhls.ac.za.
58. Mookadam F, Moustafa SE, Sun Y, Wilson FC, Mohammed SS, Park S, et al. Infectious pericarditis: an experience spanning a decade. Acta Cardiol. 2009;64(3):297-302.
59. Sullivan KM, Dean A, Soe MM. OpenEpi: A Web-based Epidemiologic and Statistical Calculator for Public Health. Public Health Reports. 2009;124(3):471-4.
60. Rahman NM, Mishra EK, Davies HE, Davies RJ, Lee YC. Clinically important factors influencing the diagnostic measurement of pleural fluid pH and glucose. Am J Respir Crit Care Med. 2008;178(5):483-90.
61. Mishra EK, Rahman NM. Factors influencing the measurement of pleural fluid pH. Curr Opin Pulm Med. 2009;15(4):353-7.
62. Sarodia BD, Goldstein LS, Laskowski DM, Mehta AC, Arroliga AC. Does pleural fluid pH change significantly at room temperature during the first hour following thoracentesis? Chest. 2000;117(4):1043-8.

2.5 APPENDIX.

2.5.1 Appendix A: figures and tables for chapter 1

Table 1:Global pattern of pericardial disease

	Permanyer-Miralda et al (2) (n=231) 1977-83	Zayas et al (5) (n=100) 1991-1993	Imazio et al (56) (n=453) 1996-2004	Reuter et al (8) (n=233) 1995-2001	Pandie et al (28) (n=151) 2009-2012
Setting	Western Europe	Western Europe	Western Europe	Africa	Africa
Idiopathic	1999(86.0%)	78(78.0%	377(83.2%)	32(13.7%)	3(2.0%)
Specific etiology	32(14.0%)	22(22.0%)	76(16.8%)	201(86.3%)	148(98.0%)
Neoplastic	13(5.6%)	7(7.0%)	23(5.1%)	22(9.4%)	12(7.9%)
Tuberculosis	9(3.9%)	4(4.0%)	17(3.8%)	161(69.5%)	124(82.1%)
Autoimmune	4(1.7%)	3(3.0%)	33(7.3%)	12(5.2%)	1(0.66%)
Purulent	2(0.9%)	1(1.0%)	3(0.7%)	5(2.1%)	6(4.0%)

Table 2:Summary of studies on pH in pleural fluid

Author	Year	Study design	Ph. cutoff	Statistics
Light et al	1973	Crossectional, diagnostic	<7.20	P<0.05
Potts et al	1976	Prospective, diagnostic	<7.30	P<0.01
Good JT Jr et al	1980	Crossectional, diagnostic	<7.20	P<0.05
Houston et al	1987	Descriptive	<7.20	-
J E Heffner et al	1995	Prospective, diagnostic	<7.29	AUC (92)
Fanjul et al	2009	Crossectional, diagnostic	<7.16	<0.005
Ferreiro et al	2014	Descriptive	<7.20	-

Table 3:Summary of studies on pH in pericardial fluid

Author	Year	Study design	Ph. cutoff	Statistics
Laforcade et al,(n=48)	2005	Animal, descriptive study	<7.80 in neoplastic	Neoplastic vs idiopathic
Fine et al (n=37)	2003	Animal, descriptive study	No difference	Neoplastic vs idiopathic
King et al (n=13)	1983	Crossectional, diagnostic	<7.08	Infectious vs non-infectious
Ekim et al (n=15)	2014	Crossectional, diagnostic	≤7.01	Infectious vs non-infectious

2.5.2: Appendix B figures and tables for chapter 2 (manuscript)

Table1a: Table showing the operational definitions for the study in Chapter 2

Definite infection:	1)) a pericardial effusion with confirmed tuberculous or bacterial etiology confirmed by pericardial fluid culture and/or gene Xpert for TB(Mayosi et al 2005) 2 a neutrophil predominant exudative effusion with a positive pericardial fluid or blood culture for a bacteria known to cause purulent pericarditis (Mayosi et al,2005)
Probable infection	a pericardial effusion which meets alternative criteria for tuberculosis etiology (Mayosi et al., 2005) including lymphocyte predominant exudate with significantly raised ADA (>40IU) / Interferon gamma, or Tygerberg Index >6. (Mayosi et al 05); A neutrophil predominant exudative pericardial effusion without positive cultures.
Non- infectious pericardial effusion	This will be a pericardial effusion which does not meet the above criteria for definite or probable infectious pericarditis.
Acidic PH	This will be defined as pericardial fluid pH less than 7.35 according to the reference standards of the National Health Laboratory Services.(57)
Moderate-severe pericardial effusions	Effusions of more than 1cm in diastole on echocardiogram that can be safely tapped percutaneously.

Table 1b: Baseline clinical characteristics of the participants with moderate to large pericardial effusions

Characteristic	overall (N=140)	Definite Infection (n=39)	Probable Infection (n=24)	Normal (n=68)	Malignancy (n=9)	p-value
Age in years, mean (±SD)	46.4 (16)	41.8 (15.5)	40.6 (12.1)	49.8 (16.6)	56.2 (12.5)	<0.01***
TB, n (%)	30 (21.4)	20 (51.3)	8 (33.3)	2 (2.9)	0	<0.01**
HIV, n (%)	47 (33.6)	26 (66.7)	17 (70.8)	4 (5.9)	0	<0.01 **
HPT, n (%)	34 (24.3)	7 (18.0)	5 (20.8)	18 (26.5)	4 (44.4)	0.37 **
Systolic pressure, median (IQR)	120 (109-130)	113 (106-124)	111 (102-127)	125 (115-138)	120 (115-125)	<0.01*
Diastolic pressure, median (IQR)	77 (70-84)	70 (67-84)	75 (66-89)	78 (70-82)	79 (77-84)	0.18*
Pulse rate, median (IQR)	92 (73-111)	104 (93-120)	111 (102-20)	75 (69-83)	108 (98-116)	<0.01*
Venous pressure, median (IQR)	4 (3-7)	4 (3-7)	4.5 (4-7)	3 (3-4)	7 (4-8)	<0.01*
Body temp, median (IQR)	36.4 (36.1-37.1)	36.8 (36.2-38)	36.3 (35.9 - 36.7)	36.4 (36.1-36.9)	36.6 (36-37.1)	0.05*
NYHA, n (%) NYHA I NYHA II NYHA III NYHA IV	 92 (65.7) 32 (22.9) 11 (7.9) 5 (3.6)	 15 (38.5) 18 (46.2) 6 (15.4) 0	 12 (50) 9 (37.5) 1 (4.2) 2 (8.3)	 61 (89.7) 3 (4.4) 2 (2.9) 2 (2.9)	 4 (44.4) 2 (22.2) 2 (22.2) 1 (11.1)	 <0.01**
Edema, n (%)	17 (12.1)	7 (18)	5 (20.8)	2 (2.9)	3 (33.3)	<0.01 **
Rate, median (IQR)	92 (73-114)	114.5 (100-130)	110.5 (102-120)	74 (68.5-82.5)	108 (98-116)	<0.01*
Weight (kg), median (IQR)	68 (59.5-80.3)	61 (56.8-74.5)	62 (57.4-73)	69 (65.7-84.8)	74.5 (65-78.4	<0.01*

* Kwalis test for comparison of medians

** Fishers excact test

*** ANOVA

Table 2: Baseline clinical and echocardiography findings in the participants with moderate to large pericardial effusions

Characteristic	Total (N=140)	Definite Infection (n=39)	Probable Infection (n=24)	Normal (n=68)	P value
Peripheral Oedema, n (%)	16 (11.4)	7 (18.0)	4 (16.7)	2 (2.9)	0.012
Ascites, n (%)	8 (5.7)	3 (7.7)	1 (4.2)	2 (2.9)	0.553
Impaired LV function, n (%)	8 (5.7)	2 (5.1)	1 (4.2)	5 (7.4)	1.000
Size of effusion, n (%)					<0.001
Small	48 (34.3)	0	1 (4.2)	47 (69.1)	
Moderate	48 (34.3)	16 (41.0)	13 (54.2)	17 (25.0)	
Large	44 (31.4)	23 (59.0)	10 (41.7)	4 (5.9)	
Tamponade on echo, n (%)	**22 (15.7)**	**12 (30.8)**	**6 (25.0)**	**3 (4.4)**	**<0.001**
Swinging heart	12/22 (54.6)	6/12(50.0)	4/6 (66.7)	1/3 (33.3)	**0.461**
RA/RV collapse, n (%)	21/22 (95.5)	11/12 (91.7)	6/6 (100.0)	3/3 (100.0)	**1.00**
respiratory flow, n (%)	22/22 (100.0)	12/12 (100.0)	6/6 (100.0)	3/3 (100.0)	**N/A**
Dilated IVC, n (%)	4/22 (18.2)	3/12 (25.0)	0(0.0)	1/3 (33.3)	**0.416**

All comparisons are made using fishers exact test

Table 3: Baseline X-ray and laboratory characteristics of participants with moderate to large pericardial effusions

Characteristic	Total (N=140)	Definite Infection (n=39)	Probable Infection (n=24)	Normal (n=68)	Malignancy (n=9)	p-value
Rhythm, n (%) Sinus Tachycardia 1st degree heart block Normal	 56 (40.0) 1 (0.7) 82 (58.6)	 26 (66.7) 0 12 (30.8)	 19 (79.2) 0 5 (20.8)	 5 (7.4) 1 (1.5) 62 (91.2)	 6 (66.7) 0 3 (33.3)	 <0.01*
PR Segment depression, n (%)	4 (2.9)	1 (2.6)	2 (8.3)	0	1 (11.1)	0.05*
ST Elevation, n (%)	3 (2.2)	2 (5.3)	0	0	1 (11.1)	0.04*
Globular heart, n (%)	79 (56.4)	39 (100)	23 (95.8)	9 (13.2)	8 (88.9)	<0.01*
Infiltrates, n (%)	44 (31.4)	26 (66.7)	10 (41.7)	5 (7.4)	3 (33.3)	<0.01*
Effusions, n (%)	40 (28.8)	24 (63.2)	6 (25.0)	4 (5.9)	6 (66.7)	<0.01*
Normal, n (%)	59 (42.5)	4 (10.5)	1 (4.2)	53 (77.9)	1 (11.1)	<0.01*
Effusion characteristics, n (%) Blood stained Serous Serosanguinous Pus	 10 (7.1) 62 (44.3) 67 (47.9) 1 (0.7)	 2 (5.1) 1 (2.6) 35 (89.7) 1 (2.6)	 4 (16.7) 1 (4.2) 19 (79.2) 0	 1 (1.5) 60 (88.2) 7 (10.3) 0	 3 (33.3) 0 6 (66.7) 0	 <0.01*
LDH, median (IQR)	731.5 (521-1235)	834.5 (605-1996)	777 (534-1127)	257 (168-458.8)	854.5 (560.5-1329.5)	<0.01**
pH, median (IQR)	7.4 (7.3-7.5)	7.3 (7.2-7.3)	7.3 (7.3-7.35)	7.5 (7.4-7.55)	7.2 (7.2-7.3)	<0.01**
ADA, median (IQR)	46.2 (31-62.2)	54.5 (41-81.2)	45 (35-52)	16 (13.8-19)	35.6 (9-40)	<0.01**

*Fishers exact test

** Kwalis test for medians

Table 4: Table showing laboratory characteristics for patients with moderate to large pericardial effusions

Characteristic	Total (N=140)	Definite Infection (n=39)	Probable Infection (n=24)	Normal (n=68)	Malignancy (n=9)
Lymphocyte predominant, n (%)	59 (42.1)	28 (71.8)	16 (66.7)	7 (10.3)	8 (88.9)
TB microscopy result, n (%)	1/81 (1.2)	1/38 (2.6)	0	0	0
TB culture result, n (%)	17/80 (21.3)	17/38 (44.7)	0	0	0
Gene expert, n (%)	17/82 (20.7)	16/38 (42.1)	0	0	1/9 (11.1)
Potassium, median (IQR)	4.1 (3.7 – 4.4)	3.8 (3.4 – 4.2)	4.0 (3.7 – 4.4)	4.15 (3.9 – 4.5)	4.1 (3.5 – 4.9)
Lactate, median (IQR)	3.1 (1.3 – 5.65)	6.5 (4.8 – 10.8)	4.4 (3.35 – 5.1)	1.35 (1.0 – 1.85)	8.6 (5.8 – 11.2)
Glucose, median (IQR)	5.15 (3.8 – 5.9)	3.3 (1.6 – 4.4)	4.75 (4.2 – 5.25)	5.7 (5.25 – 6.5)	4.1 (3.0 – 5.8)
Cholesterol, median (IQR)	1.6 (0.9 – 2.1)	1.8 (1.6 – 2.5)	2.1 (1.8 – 2.3)	0.9 (0.6 – 1.5)	2.9 (1.9 – 3.6)
Haemoglobin, median (IQR)	10.65 (9.4 – 12.55)	10.3 (8.8 – 11.0)	9.8 (8.75 – 11.6)	11.5 (9.75 – 13.8)	11.5 (10.7 – 12.9)
Platelet count, median (IQR)	292 (222 – 405)	333 (253 – 496)	364 (217 – 468)	258 (204 – 344)	325 (208 – 330)
WBC count, median (IQR)	7.4 (5.8 – 11.0)	7.1 (5.3 – 10.0)	6.8 (5.0 – 11.3)	7.9 (6.3 – 11.3)	10.9 (9.8 – 14.7)
Creatinine, median (IQR)	71 (55.9 – 88.0)	61 (48 – 81)	69.5 (53 – 75.5)	78 (62.5 – 96.5)	73 (66 – 163)
Urea, median (IQR)	4.8 (3.9 – 7.3)	4.7 (3.6 – 7.0)	4.1 (3.5 – 5.1)	5.3 (3.9 – 7.4)	9.3 (5.2 – 11.5)
Blood culture result +ve, n (%)	4/13 (30.8)	4/8 (50.0)	0	0	0
HIV positive, n (%)	47 (33.6)	24 (61.5)	17 (70.8)	5 (7.4)	1 (11.1)
Sputum gene Xpert +ve, n (%)	5/26 (19.2)	5/10 (50.0)	0	0	0

Table 5: Sensitivity, specificity, negative predictive value, positive predictive value, negative and positive likelihood ratios using a priori acidic PH cut- off of 7.35 in pericardial fluid.

	Acidic pH cut-off of 7.35. **GS Reference (95% CI)**
Overall	**N=140**
Prevalence	34(27.1%), (20.0-35.3)
Sensitivity	89.5(75.5-97.1)
Specificity	72.5(62.8-80.9)
PPV	54.8(41.7-67.5)
NPV	94.9(87.4-98.6)
LR (positive)	3.3(2.3-4.6)
LR (negative)	0.2(0.1-0.4)
AUC	0.81(0.74-0.88)

The reference standard (GS) was any positive of blood culture, genexpert, tbmicroscopy, tbculture, sputum genexpert

Table 6: Diagnostic accuracy of pericardial PH determination on pericardial effusions eligible for pericardiocentesis as a test to differentiate between definite infectious and non-infectious pericardial effusions using a cut off of 7.35

	Pericardial PH
Overall	**N=116 (infect vs. non-infect)**
Prevalence, n (%),95% CI	38(33.0%), (24.0-42.2)
Sensitivity (95% CI)	89.5%(75.5-97.1)
Specificity (95% CI)	97.4%(91.0-99.7)
PPV95% CI	94.4%(81.3-99.3)
NPV95% CI	95.0%(87.7)
LR (positive) 95% CI	34.9(8.9-137.7)
LR (negative) 95% CI	0.1(0.0-0.3)
AUC95% CI	0.93 (0.88-0.99)

Table 7:Ideal Ph cut off using receiver operator curve (ROC)

	Acidic pH with GS Reference (95% CI)
Overall	N=140
optimal cut off point	≤7.30
Sensitivity	86.8% (71.9 - 95.6)
Specificity	79.4% (70.3 - 86.8)
AUC (p value)	0.86 (0.79 to 0.9)
Significance level P (Area=0.5)	p<0.001

*The **gold standard (GS)** was any positive of blood culture, genexpert, TB microscopy, TB culture, sputum genexpert*

Table 8: Diagnostic accuracy of Pericardial PH used in sequence with ADA in TB pericarditis

	Ph and ADA
Overall	**N=140**
Prevalence	27(25.0%),(18-33)
Sensitivity	77.1(59.9-89.6)
Specificity	81.0(72.1-88.0)
PPV	57.4(42.2-71.7)
NPV	91.4(83.8-96.2)
LR (positive)	4.1(2.6-6.3)
LR (negative)	0.3(0.2-0.5)
AUC	0.79(0.71-0.87)

Figure 2: Figure showing the study flow chart

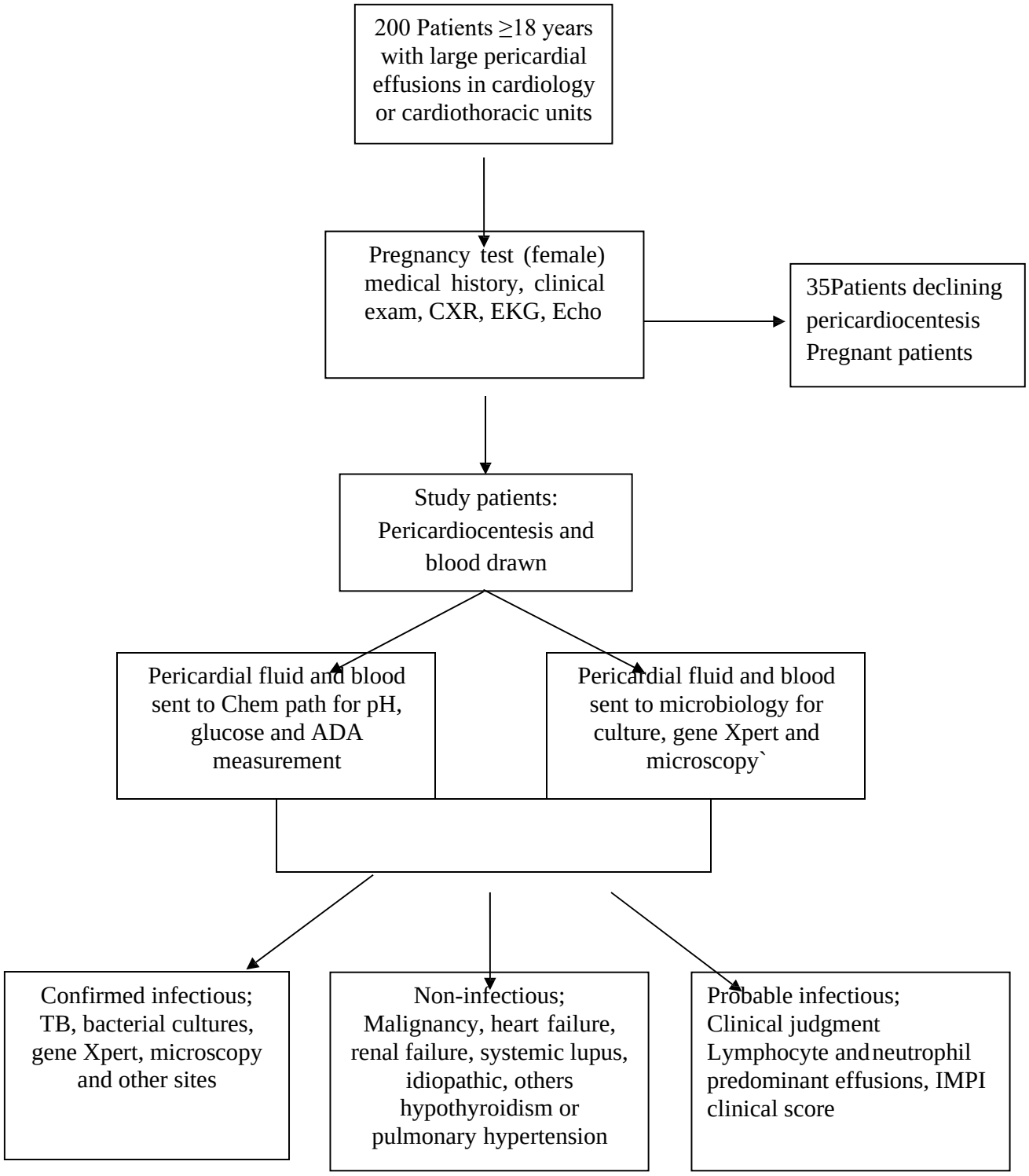
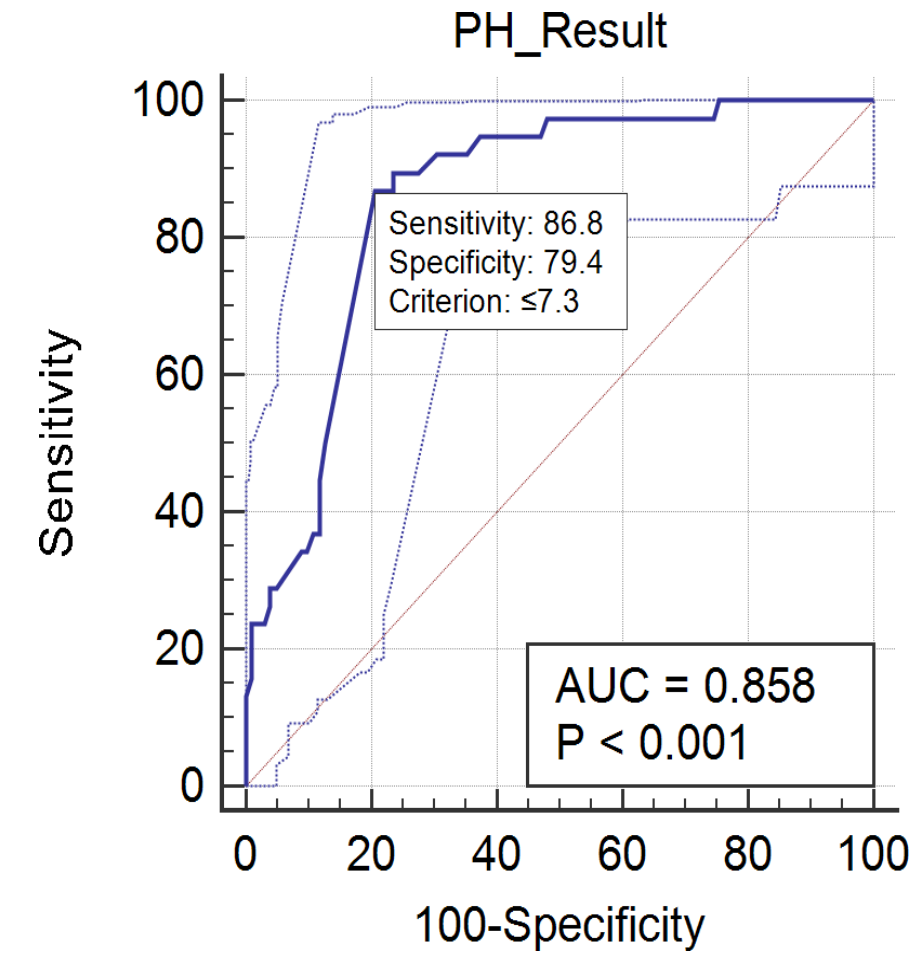


Figure 3: Showing ROC curve Ideal cut-off point for PH result with maximum sensitivity and specificity.



Sensitivity against False positive rate

95% CI of sensitivity and False positive rate

Ideal cut off-point

The optimum Ph cutoff was ≤0.730. The sentivity and specificity at this optimal cutoff was 86.8 And 79.4 respectively. The area under the ROC curve at this optimum cutoff was 0.858($p<0.01$). This means that at PH cutoff of ≤0.730, the Area under the ROC curve is significantly different from 0.5, Hence there is evidence that the laboratory test at this cutoff does has ability to distinguish between the two groups(positive /negative) ie the discriminating power or the ability of the Ph at this cutoff to categorically classify patients as true positives or true negatives is better /different from being by chance.

2.5.3: Appendix C: Proposal

TITLE:

THE INITIATIVE TO INVESTIGATE THE MANAGEMENT OF PERICARDITIS IN AFRICA (IMPI) REGISTRY; A SUB-STUDY ON DIAGNOSTIC UTILITY OF PERICARDIAL FLUID PH IN DIAGNOSING INFECTIOUS PERICARDIAL EFFUSIONS AMONG PATIENTS WITH MODERTATE AND LARGE EFFUSIONS UNDERGOING PERICARDIOCENTESIS AT GROOTE SCHUUR HOSPITAL.

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Protocol: version 0.2 24 October 2015

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ABBREVIATIONS

ADA	Adenosine Deaminase.
AUC	Area under the curve
CI:	Confidence intervals
Echo	Echocardiogram
EKG	Electrocardiogram
FDG	2-[18F]-fluoro-2-deoxy-D-glucose
MTB	Mycobacterium Tuberculosis
NHLS	National Health Laboratory Service
NLR	Negative likelihood ratios
PET-CT	Positron emission tomography combined with computed tomography
PF	Pericardial fluid
PLR	Positive likelihood ratios
RIF	Rifampicin
TBP	Tuberculous pericarditis.
TB	Tuberculosis
UIFNγ	Un-stimulated interferon gamma

OPERATIONAL DEFINITIONS

Infectious pericardial effusion: This was an effusion drained from the pericardial space of a patient, where the presence of an infective organism was identified by TB and other bacteria culture and/or gene Xpert for TB, and/or positive microscopy (with various stains) on pericardial fluid or from other sites according to another clinician's discretion.

Non-infectious pericardial effusion: This was an effusion drained from the pericardial space of a patient where the presence of an infectious organism has not been identified on TB and bacteria culture and /or gene Xpert for TB, and /or positive microscopy (with various stains) on pericardial fluid or on other sites according to another clinician's discretion.

Probable infectious pericardial effusion : This was a pericardial effusion which will have a significantly raised ADA (>40IU) /or lymphocytic or neutrophilic predominant, or pericardial effusions that were drained from pericardial space of patients with positive IMPI clinical prediction score, and of patients with positive IMPI clinical score with lymphocyte or neutrophil predominant pericardial fluid.

Acidic pH: This was defined as pericardial fluid pH less than 7.35 according to the reference standards of the National Health Laboratory Services.(57)

Moderate to severe pericardial effusions: Effusions of more than 1cm in diastole on echocardiogram that can be safely tapped percutaneously.

PURPOSE OF THE STUDY

The overall purpose of this study was to improve the diagnosis of infectious pericarditis by introducing a simpler and much quicker method of identifying infectious pericardial effusions during pericardiocentesis. This study was to determine the diagnostic accuracy of pH estimation in the diagnosis of infectious pericarditis in patients with pericardial effusions eligible for pericardiocentesis (effusions of more than 1cm on echocardiogram in diastole) presenting to Groote Schuur Hospital. The utility of such a simple test would simplify diagnosis and positively impact on prognosis.

Hypothesis

Hypothesis 1:

Using the blood gas analyzer as the index test, pH in the acidic range (i.e. less than 7.35) would signify an infectious cause of a pericardial effusion in patients with moderate to large pericardial effusions. The reference standards were MGIT (mycobacteria growth indicator tube) culture for TB, gene Xpert for TB, routine blood culture for other bacteria, and microscopy with various stains for other infectious agents.

Objectives

Primary objectives

1. To determine the diagnostic accuracy of pericardial pH determination on pericardial effusions eligible for pericardiocentesis as a test to differentiate between infectious and non-infectious pericardial effusions
2. To determine the ideal pH cut off between infectious and non-infectious pericardial effusion

Secondary objective

1. To describe the diagnostic accuracy of pericardial fluid pH determination added to ADA in the diagnosis of infectious effusions due to TB.

Outcomes

Primary outcomes

1. Median pH and difference between median pH of infectious pericardial effusions as compared to non-infectious pericardial effusions.

2. Median pericardial pH of effusions due to TB as compared to median pericardial pH of effusions due to other bacterial organisms.

3. Sensitivity, specificity, negative predictive value, positive predictive value, negative and positive likelihood ratios using acidic pH cutoff of 7.35.

4. Ideal pH cut off differentiating infectious from non-infectious pericardial effusions using Receiver operating characteristic (ROC) curves.

Secondary outcomes

1. Sensitivity, specificity, negative predictive value, positive predictive value, negative and positive likelihood ratios of pH used in sequence with ADA in diagnosing infectious effusions due to TB.

ABSTRACT

Background: Pericardial disease is known to be a contributor to morbidity and mortality worldwide. In sub-Saharan Africa as compared to the western world, infectious causes of pericardial disease are more common. Diagnosis of infectious pericardial disease has been challenging in our setting despite improvement of treatment modalities. The diagnostic utility of pH in pericardial fluid is unknown, yet this concept is well studied in pleural fluid. This study will evaluate the diagnostic utility of pH in infectious compared to non-infectious pericardial effusions.

Methods: Effective from February 2016 through May 2018, 165 consecutive patients with pericardial effusions eligible for pericardiocentesis were enrolled at a single Centre in Cape Town, South Africa but 25 were excluded from analysis due to incomplete data. Mycobacterium tuberculosis culture and/or gene Xpert for TB and/or bacteria culture and/or microscopy served as the reference standard for definite infectious pericardial effusions. Sensitivity, specificity, positive and negative predictive values, negative and positive likelihood ratios for pH cut off of 7.35 will be used and Receiver operating characteristic curve analysis will be used for selection pH ideal cut off.

Impact: This study was to evaluate the diagnostic utility of pH with the aim of introducing a simpler and quicker method of improving diagnosis of infectious pericardial effusions during pericardiocentesis.

BACKGROUND

Global burden of pericardial disease

Pericardial disease remains an important cause of mortality and morbidity in the world (4).

Pericarditis makes up about 5% of admissions in accident and emergency units in the developed world.(5) The etiology of pericardial disease in developed countries has been found to be mainly idiopathic in over 89% of patients, with the remainder being due to infections and malignancy(6). A study done in Western Europe revealed 5% of the patients to have infectious pericarditis, which is comparable to data from a study by Imazio et al in Western Europe where 20% of cases were due to infectious pericarditis.(7) Over the years the incidence and prevalence of pericarditis has been difficult to measure even in developed settings, but subclinical pericarditis has been found in 1% of autopsies. (8)

There is a growing burden of pericarditis in Africa and most especially in the sub-Saharan Africa. This is mainly due to the huge burden of HIV/AIDS which predisposes patients to TB in various organs/sites including the pericardium i.e. TB pericarditis, and other opportunistic infections.(9) In developing countries infectious pericardial effusions are more prevalent among patients with pericardial disease than idiopathic causes, as in the case of the developed world. A study done by Reuter et al (10) in a large academic hospital in South Africa revealed that 71.6% of the effusions were of an infectious cause. In another study done by Pandie et al, 86.1% of the patients at another large academic hospital in South Africa had infectious pericarditis.(11) A series of these published studies, as described above are in Table 1 highlighting selected papers reflecting the pattern of pericardial disease in both developed and developing worlds.

With the high burden of HIV in Sub-Saharan Africa, the number of people presenting with pericardial effusion has increased. Of patients presenting with pericardial effusions 67% are HIV positive.(12)

Table 1: Etiological diagnosis in the published series of acute pericarditis

	Permanyer-Miralda et al (2) **(n=231) 1977-83**	Zayas et al (5) **(n=100) 1991-1993**	Imazio et al (56) **(n=453) 1996-2004**	Reuter et al (8) **(n=233) 1995-2001**	Pandie et al (28) **(n=151) 2009-2012**
Setting	Western Europe	Western Europe	Western Europe	Africa	Africa
Idiopathic	1999(86.0%)	78(78.0%	377(83.2%)	32(13.7%)	3(2.0%)
Specific etiology	32(14.0%)	22(22.0%)	76(16.8%)	201(86.3%)	148(98.0%)
Neoplastic	13(5.6%)	7(7.0%)	23(5.1%)	22(9.4%)	12(7.9%)
Tuberculosis	9(3.9%)	4(4.0%)	17(3.8%)	161(69.5%)	124(82.1%)
Autoimmune	4(1.7%)	3(3.0%)	33(7.3%)	12(5.2%)	1(0.66%)
Purulent	2(0.9%)	1(1.0%)	3(0.7%)	5(2.1%)	6(4.0%)

Morbidity and mortality of pericardial disease

Patients admitted with acute pericarditis with advancing age and co-infections are over three times and thirteen times more likely to suffer in hospital mortality respectively.(13) In the event of HIV infection, patients with pericarditis have larger pericardial effusions and more cardiovascular impairment, contributing to greater morbidity and later mortality among these patients.(52) A study of 130 patients with infectious pericarditis demonstrated a mortality of 67% among patients treated medically, and 24% among those treated surgically. This difference in proportion was statistically significant.(58) Overall mortality of patients with non TB purulent pericarditis has been found to be 77% and reduced to 55% in those who received appropriate treatment.(15) A five year retrospective study of 57 patients in Zimbabwe revealed 100 % mortality among patients with acute purulent pericarditis.(16) Another study showed the mortality of purulent pericarditis to be 100% in untreated patients in Ohio.(17) Of patients diagnosed with constrictive pericarditis, 60% will die in the next 10 years from

date of diagnosis.(18) In a study done by Mayosi et al, the overall mortality of patients with TB pericarditis was 18.05%.(19)

The presumptive diagnosis of TB pericarditis has been identified as an independent risk factor for mortality, especially in HIV positive patients, in whom the risk of mortality is approximately 4.5 times higher [HR 5.35, 95% CI 1.76-16.25].(21) Patients with pericarditis in the era of HIV have been found to suffer greater morbidity. (22) There is increased mortality due to pericarditis among these patients due to cardiac tamponade and constriction.(20) However in the recently published IMPI trial, patients with HIV were found to have less constriction from TB pericarditis and thus fewer complications from constriction, compared to HIV negative population.(19)

Diagnosis of pericarditis

The diagnosis of pericarditis can be made from a medical history, clinical examination, electrocardiogram and echocardiogram, although its much more complicated establishing its etiology.(6) The clinical diagnosis is reached when a patient has two of the following: typical chest pain, pericardial rub, wide spread ST elevation and pericardial effusion. Elevated inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate are confirmatory of a diagnosis of pericarditis. (25) Features associated with high risk pericarditis (non-viral or non-idiopathic, recurrence, constriction and cardiac tamponade) include: fever >38.0 degrees, sub-acute presentation, immunosuppression, trauma, anti-coagulation, myopericarditis, severe pericardial effusion (diastolic echo free fluid more than 20mm) and tamponade.(26)

Therapeutic pericardiocentesis is recommended in patients that have symptomatic large (effusions ≥ 2cm on echocardiogram in diastole). Pericardial effusions and diagnostic pericardiocentesis is recommended where there is a high suspicion of infectious and neoplastic pericardial effusions.(27) Cardiac catheterization and FDG uptake with PET-CT have also become very useful in diagnosis of pericardial disease especially in effusive pericarditis and pericardial malignancy respectively.(28, 29)

Newer point-of-care diagnostic tests have been introduced. Adenosine Deaminase (ADA) has been used in the diagnosis of TB pericarditis (TBP). A Meta-analysis showed pooled sensitivity was 0.96 and specificity was 0.96 (30). Pandie et al showed that unstimulated interferon gamma (uIFN γ) offers superior accuracy for the diagnosis of microbiologically confirmed TBP, compared to the new Xpert MTB/RIF test and the established ADA assay. (11) Magnetic resonance and CT have been found to be useful in the diagnosis of pericarditis in the presence of

constriction and a mass.(31) Pericardial biopsy is useful in diagnosing and determining specific etiology, especially in patients who are having recurrent pericarditis (33).

The diagnosis of pericarditis is very challenging, especially infectious pericarditis and TB pericarditis, and this has great impact on mobility and mortality (34). The use of simple and affordable tests may simplify and speed up the diagnosis of disease, and thus improve the outcome from disease treatment. In the study done by Pandie et al, over 87% of pericardial effusions were infectious and the outcome was worse in the non-infectious group.(11) A simple test, such as pH to differentiate infectious from non-infectious pericardial effusion, will then be very useful as it will simplify and enable timely and appropriate patient management and thus improve the outcome.

Studies done on pH on pleural fluid: lessons learnt

PH has been used in pleural effusions for diagnostic purposes and therefore this study will be useful to asses if we can adapt the use of pH in diagnosis of pericardial effusions. Pleural pH less than 7.30 has been found to correlate with infectious causes of pleural effusions, irrespective of the serum pH. (35). In a meta-analysis of chemistry predictors of complicated pleural effusions, pH was found to be the most effective predictor of complicated (infected) pleural effusion, [AUC:0. 92, CI: 0. 90-0.94] (36). PH as a diagnostic tool in pleural disease has been validated and recommended for use.(37) Pleural fluid studies have been done where pleural pH less than 7.2 was used to signify infectious pleural effusion.(38)

Pleural fluid pH provides more useful information for estimating the likelihood of pleurodesis failure, for which continuous likelihood ratios provide more information than binary or multilevel likelihood ratios.(39) pH less than 7.2 has been found to predict infectious pleural effusion, and is thus a guide to management of such patients.(40) A study done by Light et al showed pH less than 7.2 to signify infectious pleural effusion and demonstrated that patients who were treated successfully with antibiotics showed their pH go above 7.2.(42)

Another study demonstrated that pH less than 7.3 and glucose less than 60mg/dl signify infectious pleural effusions.(43) A study by Potts et al also revealed pH less than 7.3 signified infectious pleural effusions.(44) Ferreiro et al revealed that pH less than 7.2 signifies pleural effusions complicated by infection.(45) In a study by Fanjul et al an acidic pH less than 7.0 was found not only to be a predictor of pleural effusions complicated by infection, but was also a marker of poor outcome, thus prognostic.(46) Table 2 below illustrates some of the studies described above.

Table 2: pH studies in pleural fluid with their cutoffs from different studies:

Author	Year	Study design	Ph. cutoff	Statistics
Light et al	1973	Crossectional, diagnostic	<7.20	P<0.05
Potts et al	1976	Prospective, diagnostic	<7.30	P<0.01
Good JT Jr et al	1980	Crossectional, diagnostic	<7.20	P<0.05
Houston et al	1987	Descriptive	<7.20	-
J E Heffner et al	1995	Prospective, diagnostic	<7.29	AUC (92)
Fanjul et al	2009	Crossectional, diagnostic	<7.16	<0.005
Ferreiro et al	2014	Descriptive	<7.20	-

Mechanism of low pH in pleural fluid and other serosa cavities

The pathophysiology of low pH in pleural fluid and other serosa cavities is poorly understood. However, existing theories suggest that it is due to an increased production of carbondioxide (CO_2) by leucocytes and microbes from glucose metabolism, and by reduced diffusion of CO_2, hydrogen ions and glucose across a diseased membrane.(47)

Use of pH in pericardial disease

The use of pericardial pH as a guide to diagnosis has not been well indicated. Animal studies have had contradictory results with the use of pH in pericardial fluid analysis, with some demonstrating a pH lower than 7.8 in neoplastic pericardial effusion, as opposed to idiopathic pericardial effusions, and others not demonstrating a difference in the two categories in dogs (48, 49). However, these studies did not assess the use of pH in differentiating between pericardial effusions of infectious and non-infectious etiology. A small study of 13 surgical patients has shown that pH less than 7.08 is associated with infectious pericardial effusions.(50) Another small study done on 15 children with purulent pericardial effusions revealed an average pH of 7.01± 0.06. However, these children were culture negative for both pericardial effusions and blood.(51) These studies are illustrated in the Table 3 below.

Table 3: Studies done on pericardial fluid pH

Laforcade et al, (n=48)	2005	Animal, descriptive study	<7.80 in neoplastic	Neoplastic vs idiopathic
Fine et al (n=37)	2003	Animal, descriptive study	No difference	Neoplastic vs idiopathic
King et al (n=13)	1983	Crossectional, diagnostic	<7.08	Infectious vs non-infectious
Ekim et al (n=15)	2014	Crossectional, diagnostic	≤7.01	Infectious vs non-infectious

Due to the behavior and characteristics of pericardial fluid compared to pleural fluid, Lights' criteria have not been found to show the same applicability in differentiating exudative and trunsudative effusions.(49)

Due to these uncertainties in the use of pH in pericardial disease, we will carry out a study to assess the utility of pH in differentiating infectious and non-infectious causes of pericardial effusions.

METHODS

Study design:

This will be a cross sectional diagnostic accuracy study in patients with pericardial effusions selected for pericardiocentesis at Groote Schuur Hospital.

Characteristics of the study population

Study population

Patients 18 years and above attending Groote Schuur hospital.

Target population

This will be patients above 18 years with moderate to severe pericardial effusions (>1cm depth in diastole on echocardiogram).

Inclusion criteria

Patients above 18 years who will give informed consent to participate in the study attending Groote Schuur Hospital during the study period.

Patients who will have moderate or large pericardial effusions, which are effusions of more than 1cm in diastole on echocardiogram.

Exclusion criteria

Patients who will withhold their consent for the procedure of pericardiocentesis.

Sample size

Using a proportion of 86.1 % found by Pandie et al, and for a confidence interval of 95% and alpha of 5 %, the sample size should be 184. (11) The different calculated sample sizes for different confidence intervals are illustrated in Table 4 below.

We estimated the sample size using proportions for crossectional studies.(59)

Our assumptions were a population size of over 1 000 000 and a confidence limit as a percentage of 5%.

Équation

Sample size n = [DEFF*Np (1-p)]/ [(d2/Z21-α/2*(N-1) + p*(1-p)]

DEFF=design effect, N= population size, p=hypothesized frequency of outcome factor in population, d=confidence limits as percentage of 100, α = size of critical region

Our target will be to recruit 200 patients for the study, as we aim to have a more than 5% increase in estimated sample size to cater for errors in sampling and measurements of outcome.

Table 4: Sample size (n) for various confidence levels

Confidence intervals	Estimated sample size
80%	79
90%	130
95%	184
97%	226
99%	318
99.9	519
99.99	725

$$n_{Se} = \frac{Z^2_{\frac{\alpha}{2}} \widehat{Se}(1 - \widehat{Se})}{d^2 \times Prev}$$

Where n_{se}= sample size, Se=sensitivity, Prev= Prevalence, d= precision estimate

We estimated the sample size for our set sensitivity of 70% using the above equation, we will need to recruit 149 subjects for a 95% confidence interval and power of 80%.

A sample size of 200 was planned but we were only able to collect 165 and 25 were excluded from analysis due to incomplete data leaving a total of 140 samples.

Recruitment and enrolment

Consenting patients referred to Groote Schuur Hospital cardiology division, with moderate to severe pericardial effusions safe for pericardiocentesis (more than 1cm in diastole on echocardiogram), were consecutively recruited and enrolled into the study by the lead investigator (BK), starting on 2nd February 2016. We also obtained pericardial fluid from patients undergoing cardiac surgery from the cardiothoracic surgeon as part of the normal pericardial fluid. These control samples were only drawn from cardiac surgery patients who had given informed consent. We obtained 43 samples as controls, and the majority of these effusions were from patients with non-diseased pericardium. These were added to our sample size to increase the number of normal effusions. This was thought to give the study greater capacity to distinguish between infectious and non-infectious/normal pericardial effusions by increasing the ratio of non-infectious to infectious pericardial effusions.

The patients were examined for blood pressure, weight, height, pulse rate and jugular venous pressure (JVP). They had their blood drawn to measure renal function and full blood count, and they had a chest X-ray, a 12-lead electrocardiogram and an echocardiogram.

Research procedures and data collection methods

Fully consenting patients were recruited into the study with purpose of obtaining the following information:

Demographic variables: age, sex, weight, height and home address.

Clinical variables: Pulse rate, Blood pressure, jugular venous pressure (JVP), height in meters and history of constitutional symptoms.

Investigations: electrocardiogram, history of constitutional symptoms, pericardial and serum glucose, cholesterol, sodium, potassium and pH levels, HIV status, chest x-ray and echocardiography findings. We also included relevant microscopy and culture results.

All these data will be recorded by the lead investigator and entered into RedCap and subsequently exported to STATA for analysis.

Patients were then be taken to the catheterization laboratory where, under aseptic conditions and local anesthesia, a pericardiocentesis was performed percutaneously to obtain at least 50ml of pericardial fluid following set standard protocols.(53)

The sample obtained was divided into five smaller samples. One sample of 10ml was put into a mycobacteria growth indicator tube liquid culture for TB culture; another sample of 5 mls was drawn into a heparinized bottle for pH measurement; another sample of 5 mls was put into a heparinized bottle for cell count and microscopy for ZN and gram stain; another sample in purple top for gene expert for TB; and the final sample of 10mls was drawn in a standard blood culture bottle to culture other bacteria. All these samples were delivered to the NHLS laboratory immediately, to allow for timely analysis (within an hour) particularly for the pH sample which was delivered on ice to avoid delays. The NHLS laboratory was able to receive the samples at any time of the day. The study schedule is illustrated in Table 5 below.

We also obtained pericardial samples from the cardiothoracic surgery unit from patients undergoing cardiac surgery during the period of the study with informed consent from patients, as described in the section on recruitment and enrolment.

It was ensured that those involved in pH measurement were blinded from the results for the reference standard.

Study overview

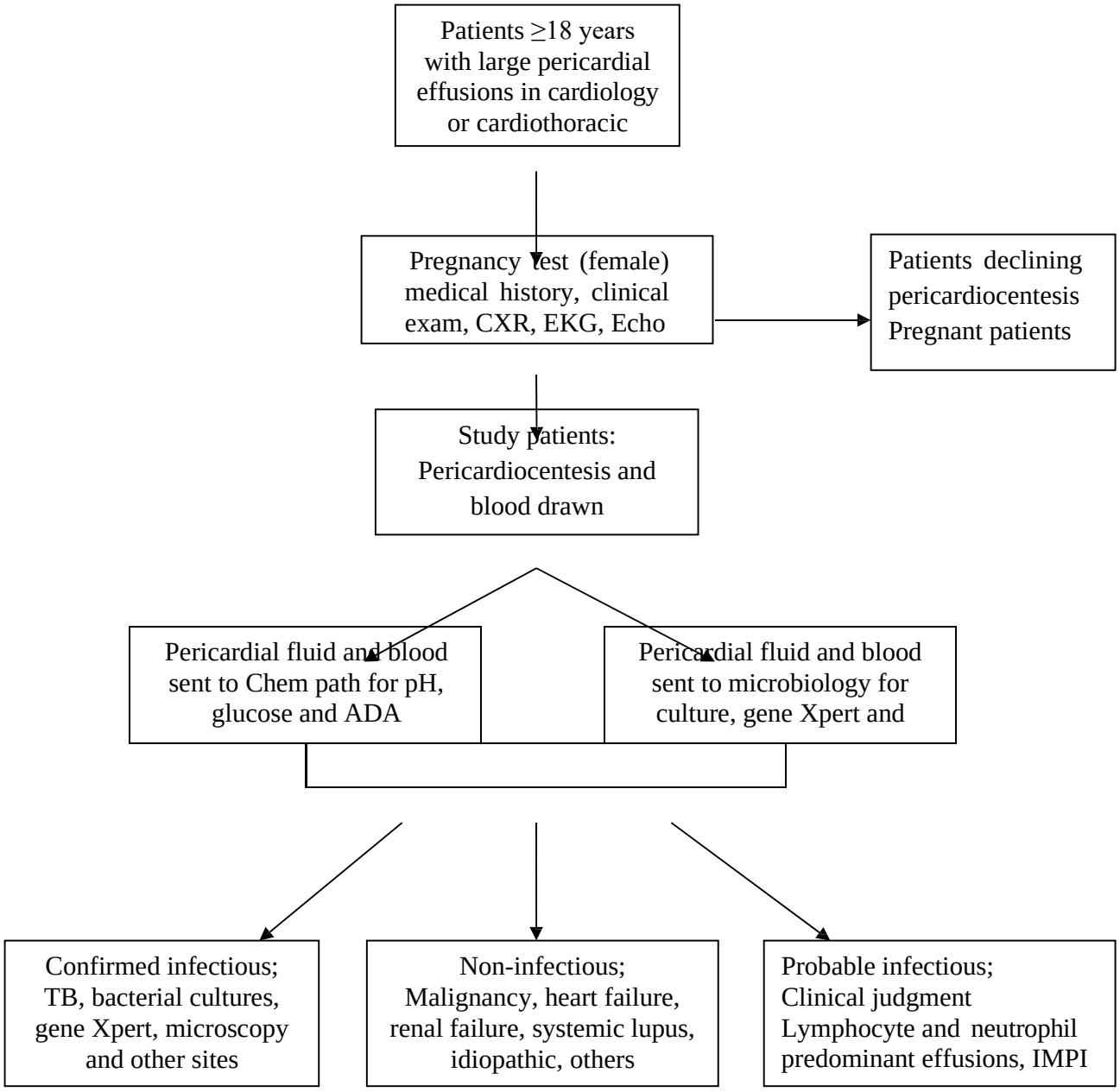

Table 5: Study schedule

`	Day 0 (≤1 hour)	Immediately	2 to 10 days	2 to 4 weeks
Consent (study)	✓			
Consent (Pericardiocentesis)	✓			
Medical History & Exam	✓			
Pericardiocentesis	✓			
Drawing blood	✓			
Chest X-ray		✓		
EKG		✓		
Echocardiogram	✓			
pH (blood & Pericardial)	✓			
Glucose, Cholesterol, Lactate, Protein, Na^+, $K,^+$ Cl^- (Blood & Pericardial)	✓			
Blood culture results			✓	
ADA measurement		✓		
TB culture results				✓
Total PF(pericardial fluid)	**50mls**			
Total blood	**10mls**			

Procedure for sampling and measurement for pH;

Samples for pH measurement were drawn into a heparinized bottle, as studies have shown no effect of pH when heparinized bottles are used.(54) We sent the sample to a chemistry laboratory for the pH to be measured within one hour on ice to avoid delays. pH was measured with a blood gas analyzer. pH on fluid has been found to

remain stable within the first hour at room temperature from a study which disputes the myth that fluid has to be collected and kept on ice at zero degrees but we delivered it on ice because it creates a sense of urgency on sample processing .(60) We also ensured that the other samples are kept at room temperature and pH measurement will be done within one hour of sample collection.(61) We chose to use this method of collection because the studies that suggested pH changes at room temperature were small studies, but larger and more recent studies showed no significant change in pH at room temperature over an hour, with four measurements done at 15,30,45 and 60 minutes respectively.(62) In an experiment that we conducted in NHLS laboratory in preparation for our study (as shown in the Appendix) we demonstrated that pH measurement using pH strips is affected by the concentration of protein in the fluid as these depend on protein dyes and for this reason we did not use them in this study.

Data management

Data was entered into a CRF and later entered into the Redcap database on a daily basis by the study lead investigator (BK), and it was backed up on an external drive. Any incomplete data was quickly obtained and entered promptly. This data was then exported to STATA version 11.0 program for analysis.

Data analysis

Continuous variables were expressed as mean (SD) while categorical variables were expressed as number and group percentages. Differences in pH between groups were analyzed using unpaired Student t test and one-way Anova. Correlation between pH and specified variables was quantified using Pearson correlation coefficient.

Diagnostic accuracy was derived using cultures inclusive of TB culture and other infective organisms and/or gene expert for TB, and /or microscopy (with various stains) as reference standard.

For primary objective 1: We determined the mean pH in the infectious pericardial effusions, and mean pH in non-infectious pericardial effusions, using the STATA version 11.0. We also determined the median pH of infectious and non –infectious pericardial effusions. We then use the pH cut off of 7.35, as stated by NHLS, to determine the sensitivity, specificity, positive productive value, negative productive value, negative and positive likelihood ratios for each of the index tests, and pericardial pH measured by blood gas analyzer.

For primary objective 2:_Using the AUC, we will determine the ideal cut off of median pH for infectious pericardial effusions and non-infectious pericardial effusions and determine AUC.

For secondary objective 1: We determined the sensitivity, specificity, negative predictive value, positive predictive value, and negative and positive likelihood ratios of pericardial pH used with ADA, in diagnosing Infectious pericarditis due to TB. We also used ROC to compare the AUC for pH alone, and pH used with ADA in differentiating infectious and non-infectious pericardial effusions.

We also proceeded to determine the median pH of pericardial effusions due to TB, and effusions due to other bacteria.
Median pH for infectious and non-infectious pericardial effusions was also determined.

ETHICAL CONSIDERATIONS

Description of risks and benefits

Eligible patients had percutaneous pericardiocentesis and the risks, which include pain, arrhythmias and myocardial puncture, were explained to all participants. The only adverse event was pain which was managed promptly with local anaesthetic.

The benefits of the study to the patients were: urgent lifesaving pericardiocentesis, and timely diagnosis, treatment and appropriate referrals to alleviate their medical condition.

Informed consent process

The principal researcher explained to each patient the study question we were trying to answer, the importance of the study, the benefits and the risks, the exact procedure of percutaneous pericardiocentesis and what would happen after their contact with the study team. This was done in English, and a study nurse or/and a translator would translate and explain to those patients who did not speak English. Patients who were temporarily cognitively impaired were consented through a legally accepted representative or legal guardian. The consenting patients were given a form to sign in the presence of a witness.

Privacy and confidentiality

Participant information was be handled in great confidence. Access to information was limited to the study investigators, and in case of presentations or publications no patient identifiers would be displayed.

Reimbursement for participation

Patients were recruited while in Groote Schuur hospital for the study and for that reason there was no need for reimbursement

Emergency care and insurance for research related injuries

The patients were seen and managed by a team of doctors at Groote Schuur Hospital and thus we minimized the risks of injuries and any patients' injuries were managed in the hospital immediately.

The UCT no fault insurance clause applied to this study as incorporated in the informed consent form.

What happens at the end of the study?

The results from the study were to be published in a reputable journal.

We ensured that patients got appropriate management for the causes of their pericardial disease and timely referrals where need is required.

REFERENCES

1. Michailova KN, Usunoff KG. Serosal membranes (pleura, pericardium, peritoneum). Normal structure, development and experimental pathology. Adv Anat Embryol Cell Biol. 2006;183:i-vii, 1-144, back cover.
2. Chinchoy. The Pericardium. 2005.
3. Holt JP. The normal pericardium. Am J Cardiol. 1970;26(5):455-65.
4. Ivens EL, Munt BI, Moss RR. Pericardial disease: what the general cardiologist needs to know. Heart. 2007;93(8):993-1000.
5. Launbjerg J, Fruergaard P, Hesse B, Jorgensen F, Elsborg L, Petri A. Long-term risk of death, cardiac events and recurrent chest pain in patients with acute chest pain of different origin. Cardiology. 1996;87(1):60-6.
6. Permanyer-Miralda G, Sagrista-Sauleda J, Soler-Soler J. Primary acute pericardial disease: a prospective series of 231 consecutive patients. Am J Cardiol. 1985;56(10):623-30.
7. Zayas R, Anguita M, Torres F, Gimenez D, Bergillos F, Ruiz M, et al. Incidence of specific etiology and role of methods for specific etiologic diagnosis of primary acute pericarditis. Am J Cardiol. 1995;75(5):378-82.
8. Troughton RW, Asher CR, Klein AL. Pericarditis. Lancet. 2004;363(9410):717-27.
9. Mayosi BM. Contemporary trends in the epidemiology and management of cardiomyopathy and pericarditis in sub-Saharan Africa. Heart. 2007;93(10):1176-83.
10. Reuter H, Burgess LJ, Doubell AF. Epidemiology of pericardial effusions at a large academic hospital in South Africa. Epidemiol Infect. 2005;133(3):393-9.
11. Pandie S, Peter JG, Kerbelker ZS, Meldau R, Theron G, Govender U, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous pericarditis compared to adenosine deaminase and unstimulated interferon-gamma in a high burden setting: a prospective study. BMC Med. 2014;12:101.
12. Cegielski JP, Ramiya K, Lallinger GJ, Mtulia IA, Mbaga IM. Pericardial disease and human immunodeficiency virus in Dar es Salaam, Tanzania. Lancet. 1990;335(8683):209-12.
13. Kyto V, Sipila J, Rautava P. Clinical profile and influences on outcomes in patients hospitalized for acute pericarditis. Circulation. 2014;130(18):1601-6.
14. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. Heart Fail Rev. 2013;18(3):367-73.
15. Rubin RH, Moellering RC, Jr. Clinical, microbiologic and therapeutic aspects of purulent pericarditis. Am J Med. 1975;59(1):68-78.
16. Mynors JM, Reichman B. Pericarditis--a five year study in the African. Cent Afr J Med. 1973;19(2):19-22.
17. Kauffman CA, Watanakunakorn C, Phair JP. Purulent pneumococcal pericarditis. A continuing problem in the antibiotic era. Am J Med. 1973;54(6):743-50.
18. Thia A, Prunier F, Subayi JB, Baufreton C, Geslin P, Furber A. [Long term evolution of chronic constrictive pericarditis. A study of 56 patients]. Arch Mal Coeur Vaiss. 2006;99(9):775-80.
19. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in Tuberculous Pericarditis. New Engl J Med. 2014;371(12):1121-30.
20. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, et al. Prednisolone and Mycobacterium indicus pranii in tuberculous pericarditis. N Engl J Med. 2014;371(12):1121-30.
21. Mayosi BM, Wiysonge CS, Ntsekhe M, Gumedze F, Volmink JA, Maartens G, et al. Mortality in patients treated for tuberculous pericarditis in sub-Saharan Africa. S Afr Med J. 2008;98(1):36-40.
22. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. Bmc Infect Dis. 2006;6.
23. Mayosi BM, Wiysonge CS, Ntsekhe M, Volmink JA, Gumedze F, Maartens G, et al. Clinical characteristics and initial management of patients with tuberculous pericarditis in the HIV era: the Investigation of the Management of Pericarditis in Africa (IMPI Africa) registry. BMC Infect Dis. 2006;6:2.
24. Mayosi BM, Ntsekhe M, Smieja M. Immunotherapy for tuberculous pericarditis. N Engl J Med. 2014;371(26):2534.
25. Brucato A, Brambilla G, Moreo A, Alberti A, Munforti C, Ghirardello A, et al. Long-term outcomes in difficult-to-treat patients with recurrent pericarditis. Am J Cardiol. 2006;98(2):267-71.

26. Soler-Soler J, Permanyer-Miralda G, Sagrista-Sauleda J. A systematic diagnostic approach to primary acute pericardial disease. The Barcelona experience. Cardiol Clin. 1990;8(4):609-20.
27. Maisch B, Seferovic PM, Ristic AD, Erbel R, Rienmuller R, Adler Y, et al. Guidelines on the diagnosis and management of pericardial diseases - Executive summary. European heart journal. 2004;25(7):587-610.
28. Sagrista-Sauleda J, Angel J, Sanchez A, Permanyer-Miralda G, Soler-Soler J. Effusive-constrictive pericarditis. N Engl J Med. 2004;350(5):469-75.
29. Lobert P, Brown RK, Dvorak RA, Corbett JR, Kazerooni EA, Wong KK. Spectrum of physiological and pathological cardiac and pericardial uptake of FDG in oncology PET-CT. Clin Radiol. 2013;68(1):e59-71.
30. Shen YC, Wang T, Chen L, Yang T, Wan C, Hu QJ, et al. Diagnostic accuracy of adenosine deaminase for tuberculous peritonitis: a meta-analysis. Arch Med Sci. 2013;9(4):601-7.
31. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
32. Alter P, Figiel JH, Rupp TP, Bachmann GF, Maisch B, Rominger MB. MR, CT, and PET imaging in pericardial disease. Heart Fail Rev. 2013;18(3):289-306.
33. Maisch B, Rupp H, Ristic A, Pankuweit S. Pericardioscopy and epi- and pericardial biopsy - a new window to the heart improving etiological diagnoses and permitting targeted intrapericardial therapy. Heart Fail Rev. 2013;18(3):317-28.
34. Cohen R, Cohen-Aubart F, Steg PG. [Acute pericarditis in the modern era: a diagnostic challenge]. Ann Cardiol Angeiol (Paris). 2008;57(1):10-5.
35. Good JT, Jr., Taryle DA, Maulitz RM, Kaplan RL, Sahn SA. The diagnostic value of pleural fluid pH. Chest. 1980;78(1):55-9.
36. Heffner JE, Brown LK, Barbieri C, Deleo JM. Pleural Fluid Chemical-Analysis in Parapneumonic Effusions - a Metaanalysis. Am J Resp Crit Care. 1995;151(6):1700-8.
37. Bhatnagar R, Maskell N. Pleural fluid biochemistry - old controversies, new directions. Ann Clin Biochem. 2014;51(Pt 4):421-3.
38. Rahman NM, Maskell NA, West A, Teoh R, Arnold A, Mackinlay C, et al. Intrapleural use of tissue plasminogen activator and DNase in pleural infection. N Engl J Med. 2011;365(6):518-26.
39. Heffner JE, Heffner JN, Brown LK. Multilevel and continuous pleural fluid pH likelihood ratios for evaluating malignant pleural effusions. Chest. 2003;123(6):1887-94.
40. Houston MC. Pleural fluid pH: Diagnostic, therapeutic, and prognostic value. The American Journal of Surgery.154(3):333-7.
41. Houston MC. Pleural fluid pH: diagnostic, therapeutic, and prognostic value. Am J Surg. 1987;154(3):333-7.
42. Light RW, MacGregor MI, Ball Jr WC, Luchsinger PC. Diagnostic significance of pleural fluid pH and PCO2. Chest. 1973;64(5):591-6.
43. Potts DE, Willcox MA, Good Jr JT, Taryle DA, Sahn SA. The acidosis of low glucose pleural effusions. American Review of Respiratory Disease. 1978;117(4 I):665-71.
44. Potts DE, Levin DC, Sahn SA. Pleural fluid pH in parapneumonic effusions. Chest. 1976;70(03):328-31.
45. Ferreiro L, Toubes ME, Valdes L. [Contribution of pleural fluid analysis to the diagnosis of pleural effusion.]. Med Clin (Barc). 2014.
46. Fanjul M, Garcia-Casillas MA, Lain A, Matute JA, Parente A, Corona C, et al. [Prognostic value of pH and glucose in complicated parapneumonic pleural effusion]. Cir Pediatr. 2009;22(4):173-6.
47. Good JT, Jr., Taryle DA, Sahn SA. The pathogenesis of low glucose, low pH malignant effusions. Am Rev Respir Dis. 1985;131(5):737-41.
48. Fine DM, Tobias AH, Jacob KA. Use of pericardial fluid pH to distinguish between idiopathic and neoplastic effusions. J Vet Intern Med. 2003;17(4):525-9.
49. Amelle M LM. Biochemical Analysis of Pericardial Fluid and Whole Blood in Dogs with Pericardial Effusion
. J Vet Intern Med. 2005;19:833-6.
50. Kindig JR, Goodman MR. Clinical utility of pericardial fluid pH determination. Am J Med. 1983;75(6):1077-9.
51. Ekim M, Ekim H. Diagnostic value of the biochemical tests in patients with purulent pericarditis. Pak J Med Sci. 2014;30(4):845-9.

52. Ntsekhe M, Mayosi BM. Tuberculous pericarditis with and without HIV. Heart Fail Rev. 2013;18(3):367-73.
53. Jung H-O. Pericardial Effusion and Pericardiocentesis: Role of Echocardiography. Korean Circulation Journal. 2012;42(11):725-34.
54. Bou-Khalil PK, Jamaleddine GW, Debek AH, El-Khatib MF. Use of heparinized versus non-heparinized syringes for measurements of the pleural fluid pH. Respiration. 2007;74(6):659-62.
55. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. Circulation. 2005;112(23):3608-16.
56. Imazio M, Spodick DH, Brucato A, Trinchero R, Adler Y. Controversial issues in the management of pericardial diseases. Circulation. 2010;121(7):916-28.
57. www.nhls.ac.za South Africa2015 [Available from: www.nhls.ac.za.
58. Mookadam F, Moustafa SE, Sun Y, Wilson FC, Mohammed SS, Park S, et al. Infectious pericarditis: an experience spanning a decade. Acta Cardiol. 2009;64(3):297-302.
59. Sullivan KM, Dean A, Soe MM. OpenEpi: A Web-based Epidemiologic and Statistical Calculator for Public Health. Public Health Reports. 2009;124(3):471-4.
60. Rahman NM, Mishra EK, Davies HE, Davies RJ, Lee YC. Clinically important factors influencing the diagnostic measurement of pleural fluid pH and glucose. Am J Respir Crit Care Med. 2008;178(5):483-90.
61. Mishra EK, Rahman NM. Factors influencing the measurement of pleural fluid pH. Curr Opin Pulm Med. 2009;15(4):353-7.
62. Sarodia BD, Goldstein LS, Laskowski DM, Mehta AC, Arroliga AC. Does pleural fluid pH change significantly at room temperature during the first hour following thoracentesis? Chest. 2000;117(4):1043-8.

2.10: Appendix D: Guidelines for Mphil and PH experiment and Lights criteria and IMPI score and budget

Lights Criteria

	Exudate	Transudate
Fluid/Serum Protein	>0.5	<0.5
Fluid/Serum LDH	>0.6	<0.6
Serum LDH	>200IU	<200IU

★LDH; Lactate dehydrogenase

IMPI Clinical Prediction Score

Age	>50years
HIV status	Positive
Constitutional symptoms	Present

pH laboratory experiment done by Dr. Brian Kiggundu and Prof. David Marais:

We undertook a table Ph experiment with Prof. Marais today (14th/July/2015)

The aim was to do a quick evaluation of the effect of protein on pH indicator strips and paper strips.

As discussed earlier in the Chem path meeting, pH estimation by pH indicator strips and indicator paper is affected by protein and thus errors in pH estimation.

Given the fact that we shall be measuring pH on exudates, which have high protein, we may have these errors.

We evaluated how well a urine dipstick and pH paper indicator does with a change in protein concentration.

We measured pH using dipstick and pH Paper indicator at different protein concentration by diluting serum with a buffer from a protein concentration of 70g/l to 1.1g/l. We discovered that the pH paper indicator showed much higher pH than expected and with dipsticks we got varying pH readings at high protein concentration, but more consistent readings at lower protein concentration.

We also found it difficult to read exact pH values, which are in between colors for example 7.5. pH also changed with time; it became higher.

Conclusion was that pH indicator strips are affected by protein content in fluid and it is difficult to differentiate between pH readings that are in between two colors. However, if we can get strips that have user-friendlier cut offs to allow more discrimination between pH values, then we can use the pH strips, since we shall be comparing them to the blood gas analyzer. If they are useless at the end of the study, we will draw that conclusion.

We will also ensure that pH is measured immediately.

BUDGET

Item	**Cost per Patient**	**Quantity**	**Total**
Pericardial determination pH by blood gas analyzer	R60.00	200	R12, 000.00
Pericardial fluid biochemistry (protein, glucose, lactate, sodium, potassium, chloride, cholesterol)	R90.00	200	R18, 000.00
pH by pH meter	R100.00	200	R20, 000.00
Printing	R4.00	250	R9, 000.00
Total			**R59, 000.00**
Miscellaneous @ 10% of total cost			R5, 900.00
Grand Total			**R64, 900.00**

MPhil: Diagnostic utility of pericardial pH in diagnosing infectious pericardial effusions at Groote-Schuur Hospital

II: Monograph format.

I: Publication format

The dissertation must include a manuscript in publication-ready format and should be structured as follows:

Chapter 1: Introduction

1.1 Context

In this section the student should put the research in medical, social, geographical and economic perspective by providing the relevant background, research setting and rationale of the research. This should **not** be a full literature review, but the candidate may need to enlarge on the introduction in the publication-ready manuscript to facilitate a clear motivation of how their research contributes to advancing scientific knowledge with particular referance to the research setting. If there are aspects of the methodology that merit more detail than was afforded in the submitted paper, a subsection titled "methodological aspects" should be included. References quoted in this chapter should appear at the end of the chapter, not at the end of the thesis.

1.2 Ethical considerations

In this section the student should discuss how the ethical considerations were addressed and state the relevant HREC approval number(s).

Note: The word count for part 1.1 and 1.2 combined should not exceed 2000 words.

1.3 Author guidelines of the Journal for which the paper has been formatted

Unless specially motivated, the journal chosen should allow for at least 2000 words (not more than 5000 words) excluding abstract, tables, figures and references. The "Instructions to Authors" of the journal must be appended. The journal chosen for publication must be appropriate to the subject matter of the dissertation – the reason for choice should be motivated here. The journal must also be listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the South African Department of Education: (http://www.lib.uct.ac.za/medical/index.php?html=/libs/accredjnls.htm&libid=24)

Chapter 2: Publication-ready manuscript

The study is presented in the form of a manuscript of an article for a named peer reviewed journal, meeting all the requirements set out in the "Instructions for Authors" of that journal, including the word count and referencing style. Unless specially motivated, the journal chosen should allow for at least 2000 words (not more than 5000 words) excluding abstract, tables, figures and references. The "Instructions to Authors" of the journal must be appended. The co-authors should be listed in the appropriate order, and each of their contributions to the manuscript stated. The journal chosen for publication must be appropriate to the subject matter of the dissertation and listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education (http://www.lib.uct.ac.za/medical/index.php?html=/libs/accredjnls.htm&libid=24); *other journals with similar review processes, particularly South African journals may be acceptable if permission is obtained from the PMC Chair after appropriate motivation is provided.*

Note 1: In this format, the candidate need not have submitted the article for publication, nor is the acceptance of the article for publication a requirement for passing the degree. However, the norm is to publish the study with the supervisor(s) as co-author(s), and candidates are strongly encouraged to submit their manuscript for publication after examination of the minor dissertation.

NOTE 2: IF THE RESEARCH IS A FULL SYSTEMATIC REVIEW, THERE IS NO NEED FOR A SEPARATE CHAPTER 1 – THE REVIEW SHULD BE SUBMITTED AS ONE CHAPTER.

II: Published (or accepted for publication) paper format

A manuscript that has already been published or accepted for publication in a journal that is listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education (*other journals with similar review processes, particularly South African journals may be acceptable if permission is obtained from the PMC Chair after appropriate motivation is provided),* may be submitted **if the candidate was the first author, the candidate's contribution was completed under supervision during his/her registration for the degree, and the paper is in line with the educational aims and scope of research described in the first part of this document.**

The dissertation must be submitted in similar format to the publication-ready format – the only differences being: a separate literature review is not required; the accepted publication is submitted as a single chapter following the same format as described above under "Chapter 2"; and the reviewer comments from the journal should be attached as an appendix. *When this format is used, the contributions of all the authors must be very clearly stated under a sub-heading in the "Acknowledgments and contributions" section in the first part of the thesis.*

III: Standard monograph format

Some disciplines and constituent Colleges of the Colleges of Medicine of South Africa require a standard monograph presented in a comprehensive and scholarly style to be submitted as part of the examination. The length is typically 16 000 to 20 000 words in length, but may vary. If the length is not stipulated, the monograph should be 6000 – 16000 words, excluding references and tables.

A recommended structure for the body of the dissertation is as follows;

Chapter 1: Introduction and Literature review

(see guidelines above)

Chapter 2: Methods

Material and methods of the study must be fully described and factually presented.

Chapter 3: Results
Chapter 4: Discussion and conclusions

Appendices (relevant to all formats)

All other ***relevant*** supporting documents should be appended, including:

- Questionnaire/data capture instrument(s)
- Consent forms and related participant information sheets
- Technical appendices and relevant additional tables not included in the main manuscript.

  These should be accompanied by a brief narrative.

- Ethics approval letters (except for a full systematic review) and other relevant permissions
- **Instructions to authors if format I or II is submitted**
- **Reviewer comments if format II is submitted**

Language and writing

Clear, grammatically correct English is essential.

Supervisors may assist candidates in developing scientific communication skills but they are not required to do detailed editing or correction of spelling, grammar, or style. Training in scientific writing is available at the Health sciences Writing Centre. Registrars need to make an appointment via the website: http://www.writingcentre.uct.ac.za/about/healthsciences

Candidates should refer to Form D4, Guidelines on the Layout and Style of the Dissertation or Thesis. As long as the dissertation is readable and internally consistent, any of a number of styles are acceptable. For a publication-ready manuscript, references should be formatted according to the instructions to authors for the journal selected, and candidates should use the same style throughout their dissertation. For a monograph format manuscript, the Harvard style for referencing is recommended, but not compulsory. For reference management, Refworks or Endnote can be downloaded from the ICTS or UCT library website.

Candidates should look at previous examples of Master's dissertations in the library. Master's dissertations are available in the Health Sciences Library. A search will need to be done to obtain a list of titles and authors. This search can be done using search words (e.g. dissertation, health, health sciences, etc.). The librarian can be asked for assistance. Some of these dissertations are available via: http://www.medical.lib.uct.ac.za/hsl/theses-dissertations

Annual approval

After 1 year, apply to HREC for continuing approval Form FHS016 (for intervention study) or FHS017 (for record review) or submit a study closure form, FHS010, if the study is complete. If registration in MMED III is required for more than one year then complete form D2(b) and submit to Post Grad Office when re-registering.

Submission of dissertations

On completion, the dissertation and a Turn-it-in originality report must be submitted to the Faculty Postgraduate Office. The candidate should inform the Faculty Officer one month in advance of the intention to submit, using Form D8 (Intention to submit) online with PeopleSoft system and should subsequently submit their dissertation using the same system **– guidelines for this process and the use of Turn-it-in are on the Mmed/Mphil Vula Website and detailed guidelines are also available in the UCT student help document: " Digital submission of a thesis/dissertation for examination and library access"**. This document is available online at http://www.uct.ac.za/usr/current_students/postgrad/digital_upload_dissertations_theses.pdf

Supervisors will be requested by the Faculty Postgraduate Officer to submit a letter supporting submission, and clearly specifying whether the format of submission, so that the appropriate instructions are sent to the examiners. This letter should be supplied by the primary supervisor. If this supervisor is external, the internal supervisor must be kept informed at every stage of the process.

Please note: In the event that any of your external examiners request a hard copy of your dissertation/ thesis, you will be required to supply this. The Faculty office will inform you should this be necessary.

Specific submission requirements may be set by individual disciplines or constituent Colleges of the CMSA, and registrars are obliged to ensure that their research projects and dissertations meet these specific requirements. UCT Dissertation submission deadlines:

1. March 15th for June graduation
2. August 15th for December graduation

Note on fees: To avoid attracting fees, dissertations need to be submitted before the beginning of the first quarter (first day of academic year), and before the start of the second semester (mid July) to qualify for a 50% fee rebate.

Examiners

The full dissertation will be submitted for examination through the Postgraduate Office to two examiners (nominated by the supervisors and HOD) – at least one examiner must be external to UCT. An internal examiner must not be involved in the research.

MPhil Minor dissertation guidelines As approved in PC; September 2017

It is the supervisors' responsibility to submit names of three potential examiners (or two examiners who have already agreed to examine pending approval of the Post Graduate Office) to the Faculty Officer when the candidate is ready to submit. Appointment of examiners from outside South Africa is encouraged. These nominations need to be approved by the Deputy Dean: Postgraduate Affairs on behalf of the Faculty Board and submitted to the Faculty Board for ratification via a Dean's Circular. Details required for each examiner are: academic qualifications, postal and/or physical address, telephone and fax numbers and e-mail address, and one paragraph description of their standing in the relevant field (drawn from their CV if need be). The examiners will be sent a copy of these guidelines as well as a guideline for marking. *The candidate may not be informed of the identity of the examiners*. After the outcome of the minor dissertation has been finalised, the examiners' identities are made known if the examiners have indicated that they do not object to this.

Publication agreement

The university has a moral responsibilty to publish all research undertaken when publication is stated as an anticipated output. A candidate who fails to submit a manuscript to a journal for publication within 1 year of submission of their thesis, must accept that their supervisor(s) are entitled to publish their data on their behalf, with the student as co-author - this should be stated in the memorandum of understanding.