Gallium-68 DOTATATE – Positron Emission Tomography/ Computed Tomography (PET/CT) imaging for the detection of the role of inflammation in the pathogenesis of restenosis in patients treated with Percutaneous Balloon Mitral Valvotomy

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DEDICATIONS

This work is dedicated to my parents, Dr BS Kakooza and Mrs A Kakooza.

DECLARATION

I, **Dominic Kakooza**, declare this research report to be my own unaided work. It is being submitted for the degree of Master of Medicine in Internal Medicine in the University of the Witwatersrand, Johannesburg. It has not been previously submitted for any degree or examination at this or any other university.

Signature

Signed at Johannesburg- Parktown on the 30 day of April 2021.

PRESENTATIONS

Presenter: Professor Pravin Manga
Abstract presented at 18th annual South African Heart conference in 2017.

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LIST OF ABBREVIATIONS

ARF	Acute rheumatic fever
ASOT	Antistreptolysin O titre
BMV	Balloon mitral valvotomy
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CRP	C - reactive protein
CT	Computed tomography
DQS	Double quantum spectroscopy
ESR	Erythrocyte sedimentation ratio
FDG	Fluorodeoxyglucose
^{68}Ga	Gallium-68
ITG	Isotope technologies Garching GmbH
MBq	Megabecquerel
mCi	Millicurie
ML	Millilitre
MVA	Mitral valve area
PET	Positron emission tomography
PMBV	Percutaneous mitral balloon valvotomy
RHD	Rheumatic heart disease
ROI	Region of interest
SUV	Standard uptake value
VCAM-1	Vascular cell adhesion molecule-1
VOI	Volume of interest
µg	Micrograms

GALLIUM-68 DOTATATE – POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY (PET/CT) IMAGING FOR THE DETECTION OF THE ROLE OF INFLAMMATION IN THE PATHOGENESIS OF RESTENOSIS IN PATIENTS TREATED WITH PERCUTANEOUS BALLOON MITRAL VALVOTOMY

ABSTRACT

Background

Mitral stenosis is a well-known long-term sequel to chronic rheumatic heart disease (RHD). Percutaneous mitral balloon valvotomy (PMBV) is a non-surgical technique for dilatation of the mitral valve. Restenosis post PMBV is a common problem. The mechanism through which this process occurs is still unclear. However, it has been suggested that restenosis following PBMV may have an inflammatory aetiology based on findings of elevated inflammatory markers in patients post PBMV.

Aims and Objectives

This study aimed to evaluate the role of inflammation in mitral valve restenosis following PBMV. The study also sought to demonstrate the role of ongoing active rheumatic fever (based on serology) in patients post BMV with mitral restenosis.

Methods

This was a single centre, descriptive cross-sectional study conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between 1 April 2016 and 30 June 2016. Patients previously subjected to PBMV with evidence of restenosis, as detected by echocardiography underwent Positron Emission Tomography/ Computed Tomography (PET/CT) scan using Gallium-68 (Ga-68) DOTATATE , were recruited into the study. Twenty patients were identified and contacted, but 6 were not entered into the study; informed consent was obtained for the remaining patients. Serological testing for rheumatic fever was additionally performed in an attempt to establish a link between recurrence of rheumatic activity and inflammatory activity in the mitral valve.

Results

The study population comprised of 13 patients. Eight (61.5%) patients were females. The mean age was 46.2 ± 11.8 years. Amongst patients with confirmed restenosis in the mitral valve, 54% failed to demonstrate Ga-68 DOTATATE uptake, while only 5% of patients demonstrated serological evidence of active rheumatic fever. A negative correlation was noted between the erythrocyte sedimentation rate (ESR) and time from balloon mitral valvotomy (BMV) ($r = -0.6954$, $p= 0.0083$). This implies that as the time from BMV increases, the ESR reduces.

Conclusion

The study did not demonstrate significant Ga-68 DOTATATE uptake in mitral valves with echocardiographic evidence of restenosis post PMBV. Furthermore, in the majority of participants, serology did not confirm the role of ongoing rheumatic activity as the cause of mitral restenosis.

CHAPTER 1: PROTOCOL AND EXTENDED LITERATURE REVIEW

Background

Valvular heart disease is a common cause of cardiac disease and heart failure, with up to 20% of cases requiring cardiac surgery [1]. The incidence of the valvular heart disease (VHD) is higher in poor socioeconomic groups and developing countries than it is in the developed world [2]. The underlying aetiology in developed and developing nations differs, notably being degenerative and infective, respectively [3]. Rheumatic heart disease (RHD) is a significant cause of cardiovascular disease in younger patients in developing countries. Disease burden attributed to rheumatic fever has shown a marked decline with industrialization due to improved living conditions, which have resulted in less crowding and improvements in hygiene and sanitation. The prevalence of RHD in sub-Saharan Africa in 2015 was approximately 5.7 per 1 000 children aged 5 and 14 years [9], while in Cape Town there were 20.2 cases per 1 000 children with asymptomatic RHD. In Soweto, the incidence of symptomatic RHD was 24.7 per 100 000 amongst adults older than 13 years of age [10].

All four cardiac valves may be affected in valvular heart diseases, namely: the mitral valve, aortic valve, tricuspid valve and the pulmonary valve. Valvular heart diseases can be classified as either stenotic or regurgitant. Stenotic lesions are a result of narrowing of the valve commissure causing increased pressure requirements for the chamber above the stenotic valve to pump blood past the valve [1].

The mitral valve is one of the two atrioventricular valves (AV).It is situated on the left side of the heart, separating the left atrium from the left ventricle. The two mitral leaflets are connected by an annulus, which is a pliable fibrous ring. The function of the annulus is to contract during ventricular systole, thereby narrowing the valve area. The two leaflets (anterior and posterior) are held together by chordae tendineae and papillary muscles to form a ring [4].

Histologically, four layers make up the valve, from the outer most to the inner layer: atrialis, spongiosa, fibrosa and ventricularis. Elastic fibres and collagen make up the atrialis. The spongiosa contains extracellular matrix along its elastic fibres. The fibrosa is the layer that absorbs most of the pressure during valve closure. The final layer, the ventricularis is

continuous with the endothelial layer [5]. Normal valve leaflets and cusps have very few blood vessels, which are limited to the proximal portions of the valves.

The mitral valve opens during diastole to allow blood flow into the left ventricle from the left atrium. During systole the valve is closed. Proper closure during systole and opening of the valve during diastole requires synchronicity of the entire valvular apparatus Failure of its function serves as the basis of mitral valve disease, the result being stenosis or regurgitation [1]. The mitral valve is the most commonly affected valve with disease processes compared to other cardiac valves and disease can be classified as either stenotic, prolapse or regurgitant [7].

Mitral stenosis is caused predominantly by rheumatic heart diseases (RHD). In the developed world, mitral stenosis occurs because of degeneration of valvular tissue, a phenomenon seen in the elderly. In a Swedish nationwide hospital-based register study with over 10 million individuals, mitral stenosis was detected in only 1 917 (0.02%) people. In contrast, findings from a multi-center hospital-based retrospective registry of African countries involving more than 3 000 patients, mitral stenosis was reported in 13.4% of patients [2].

In the early stages of the disease, patients with mild mitral stenosis may not have any apparent symptoms. With worsening stenosis, patients may present with dyspnoea on exertion, orthopnoea and paroxysmal nocturnal dyspnoea. Chest pain, haemoptysis and fatigue are symptoms that indicate increased left atrial pressure. Examination of the cardiovascular system may reveal a diastolic thrill on palpation, a loud first heart sound and a mid-diastolic murmur [1].

RHD transpires following an attack of Acute Rheumatic Fever (ARF), a disease process caused by Streptococcus pyogens, a member of the Group A Beta Haemolytic Streptococcus [11]. Beta haemolytic streptococci are divided into serogroups based on their group A carbohydrate [1]. All M strain serotypes are believed to be capable of causing ARF, in particular, M types 1, 3, 5, 6, 14, 18, 19 and 24 [1]. The M protein is in the form of an alpha-helical coiled dimer and extends on the surface of the streptococcal wall [17]. The antibodies found in human serum known to cross-react with the cardiac tissue were found to be of three groups, firstly, α-helical coiled molecules: myosin, tropomyosin and keratin, secondly, myosin and DNA and thirdly, myosin and N-acetyl-glucosamine [3].

Cardiac involvement is brought about by molecular mimicry, where antigen sensitization of Group A streptococcal carbohydrates cross-reacts with myocardial M protein [18]. Molecular mimicry also underlines the pathogenesis of cartilage, chondrocytes, synovial cells, thalamic and subthalamic involvement in acute rheumatic fever [18].

The spectrum of illness caused by repeated episodes of skin and pharyngeal infection are autoimmune driven, namely, post-streptococcal glomerulonephritis, ARF and RHD [4]. Group A beta-haemolytic pharyngitis is primarily a disease of children, usually between 5-15 years of age [14]. Only 0.3-3% of patients with acute streptococcal pharyngitis subsequently develop rheumatic fever, implicating a genetic role in disease manifestation [15]. The mitral valve is most affected by the disease process, followed by aortic valve involvement [16]. Endocardial disease is marked by an immune-mediated valvulitis that alters the anatomy of the valve [17].

Rheumatic carditis is the most serious clinical manifestation of ARF. Lymphocyte infiltration of the endothelium in the atrialis is the initiating step in rheumatic carditis [19]. The mechanism underlying lymphocyte infiltration is complex, considering that cardiac valves are innately avascular structures. Vascular cell adhesion molecule-1 (VCAM-1) is upregulated by antibodies directed against cardiac tissue, leading to the extravasation of CD4+ and CD8+ T-cells through the valves. An inflammatory cascade results, causing extensive damage to cardiac valves. Scarring with subsequent neovascularization of the affected valves occurs, which inevitably lead to their malfunctioning [19]. Clinically, patients present with pericarditis, endocarditis or valvular disease. When the mitral valve leaflets are affected, nodules develop on the valve with resultant oedema and cellular infiltrates. The inflammatory changes within the valve leaflets lead to mitral stenosis or regurgitation [19].

Initial investigations include an electrocardiogram and a chest x-ray. These are followed by transthoracic echocardiography, which is an essential tool in establishing the diagnosis of mitral stenosis. Other non-invasive imaging modalities which can be employed to evaluate Mitral Valve disease include; transesophageal echocardiography computed tomography (CT) and magnetic resonance imaging (MRI).

Echocardiographic examination reports in rheumatic mitral stenosis should include the pattern of valve involvement, presence of calcification, severity of stenosis, associated mitral

regurgitation, other visualized valvular lesions, atrial size and function [22]. The mean pressure gradient across the mitral valve and the mitral valve area are both used as markers of severity of mitral stenosis. Doppler techniques are used along with the integrated computer software to calculate the mean gradient [22]. The European Association of Echocardiography/ American Society of Echocardiography (EAE/ASE) recommends the classification of mitral stenosis severity based on the mean pressure gradient across the mitral valve into mild (< 5 mmHg), moderate (5-10 mmHg) and severe (> 10 mmHg) [5].

The two commonly used methods of assessing mitral valve area are planimetry and pressure half-time. Planimetry is performed in the short axis using a static image, where the mitral valve orifice is measured at its narrowest part during diastole. Calcification can often make this method more difficult as it may obscure the valve edges [5]. Pressure half time utilizes the rate of drop in pressure across the mitral valve as a measure of disease severity. It is the time taken from peak trans-mitral pressure to decrease by 50%. The equation 220 ÷ pressure half time is used to estimate the mitral valve area [5]. Changes seen in rheumatic mitral stenosis include thickened leaflet tips with reduced motion in diastole. Doming of the valve leaflet during diastole seen as 'hockey stick' deformity indicates commissural fusion and calcification [22]. A transesophageal echocardiogram is preferred when the transthoracic echocardiogram is suboptimal or when complex lesions are being evaluated. It is also performed to assess the feasibility and prognosticate planned PMBV.

Computed tomography (CT) has a high spatial resolution and assesses cardiac structures in a three-dimensional view. In addition to assessing mitral valve leaflets, commissural and annular calcification, CT also provides reliable dimensions of the left atrial size, detects a thrombus in the left atrial appendage and can provide radiological evidence of pulmonary oedema and pulmonary hypertension. The main disadvantage of a CT scan is the exposure to ionizing radiation to the patient [24].

Magnetic resonance imaging (MRI) uses multiple sequences to delineate both cardiac structure and function. MRI is usually employed to assess mitral valve disease when the echocardiogram window is suboptimal. There is no patient exposure to ionizing radiation. Steady state free precession (SSFP) allows excellent delineation between blood and the myocardium, increasing the reliability of the quantification of cardiac chambers and volumes. Reduced temporal resolution, high cost and long imaging hours are some of the limitations of the MRI scan [25].

Positron emission tomography (PET) integrates both functional and structural imaging (CT) to facilitate in vivo imaging of biological processes.

Other uses of PET/CT scan imaging in cardiology are; viability testing, diagnoses inflammation/ infection (Endocarditis), diagnosing Sarcoidosis and atherosclerosis [45, 46].

The most used radiopharmaceutical for PET imaging is Fluorine-18 7 fluorodeoxyglucose (F18-FDG). F18-FDG is used for both oncological and non-oncological indications, such as the identification of suitable candidates for coronary revascularization. DOTATATE is a peptide labelled with a radioisotope Gallium-68 (Ga-68) which has drawn a lot of attention for non-invasive assessment of inflammation and metastasis of neuroendocrine tumours that express somatostatin receptors [26, 27]. DOTATATE binds to cells that express somatostatin subtype 2 receptors, when labelled with a generator-derived positron-emitting isotope, Ga-68 and can be detected with a PET scan [28]. Somatostatin receptor (SSTR-2) is a G protein-coupled receptor naturally occurring in activated macrophages, kidneys, cerebellum and neuroendocrine tumors [29]. Ga-68 DOTATATE PET/CT scan imaging has been used successfully to demonstrate plaque inflammation within the coronary arteries by the detection of the SSTR-2 of activated macrophages [30]. Gallium-68 DOTATATE does not demonstrate physiologic uptake in the myocardium. The visualization of uptake in the heart is therefore always pathological. The Ga-68 DOTATATE PET scan is not routinely used to assess patients with valvular heart diseases. There are no specific interpretation criteria for diagnosing valvular heart diseases. The main limitation is the exposure of patients to ionizing radiation. Regional uptake was previously quantified with the use of FDG. We extrapolated the principles behind FDG uptake from previous studies and applied them to our study using Ga-68 DOTATATE uptake for semi-quantification of valvular uptake using the myocardium (apex) as a point of reference. Therefore, for standardization and reproducibility we used the apex that was an are well visualized in all participants to represent the cardiac uptake as a novel method like FDG uptake to that used by Israel et al [47].

Mitral stenosis is a mechanical obstruction that interferes with blood flow across the valve. The definitive management plan for treating patients with mitral stenosis involves surgical and percutaneous procedures that aim to increase or restore the valve orifice area. These include open or closed commissurotomy, mitral valve replacement (mechanical or bio prosthetic) or percutaneous mitral balloon valvotomy [31]. Medical therapy is limited to patients in sinus

rhythm. Mitral stenosis is no longer regarded to be a high-risk condition requiring infective endocarditis prophylaxis for high-risk procedures [44].

Balloon mitral valvotomy is the preferred intervention for rheumatic mitral stenosis. Balloon valvotomy is performed on pliable valves with absent or minimal calcification, valves with less than 2+ mitral regurgitation, in the absence of thrombi in the left atrium and in patients with no concomitant valvular disease or coronary artery disease [31]. The most used technique is that by which the Inoue balloon (hourglass-shaped balloon) is passed via the femoral vein to the mitral valve via a trans-septal route to access the mitral valve, as described by Inoue et al [32]. Successive dilatations are performed to achieve adequate dilation. Successful valvotomy is marked by a >1.5 cm^2 mitral valve diameter post dilatation and by no further need of other forms of intervention [33].

Mitral restenosis is thought to be an inevitable long-term outcome after PMBV, often necessitating a repeat PMBV, open commissurotomy or mitral valve replacement. Restenosis has been defined as a mitral valve area <1.5 cm^2 in a valve previously >1.5 cm^2 or ≥ 50% reduction in MVA immediately post PMBV [34]. Mitral restenosis after PMBV is time-dependent and has been reported to occur at a rate of 4-21% [35, 36].

Long term follow-up of patients treated with PMBV is essential to curb death and monitor for mitral valve restenosis. In a study by Tomai et al. evaluating long-term outcomes over a 20-year period in 482 patients who had successful PMBV, the primary endpoints were cardiovascular death, the need for mitral surgery and repeat PMBV. Cardiovascular related mortality was noted in 9.1%, while 27% of patients required mitral surgery and only 6.1% required a repeat PMBV. Male gender, concomitant atrial fibrillation, an echocardiography score (Wilkins' score) > 8 and a valve area < 1.75 cm^2 after PMBV were clinical factors identified as independent predictors of cardiovascular death, mitral surgery and repeat PMBV [37]. Similarly, Langerveld et al. also evaluated 137 patients post PMBV. Restenosis in the mitral valve was observed in 28.3% of patients after four years and independent predictors of restenosis were chronic atrial fibrillation, a small mitral valve area post PMBV and a high residual maximum transmitral gradient [2].The presence of a small mitral valve area post BMV would indicate a suboptimal procedure, this would invariably be associated with a persistent gradient. The left atrial size is a predictor of immediate successful PMBV [39]. These

predictors highlight the concept of “atrial remodeling” which is the anatomical/structural and electrophysiological changes within the left atrial that occur when enlarged. Atrial remodeling links the decompensated state of left atrial enlargement and atrial fibrillation. Had the BMV been successful reverse atrial remodeling would have taken place marked by left atrium decompression with possible reduction in left atrial size and possible resolution of atrial fibrillation [48, 49, 50]

The pathophysiological mechanisms behind restenosis post PMBV is not well established and has been a subject of much debate over the past decade. Some authors suggest that recurrent episodes of ARF may be responsible as a cause for restenosis due to the finding of elevated markers of ARF in patients found to have restenosis [40]. Other authors propose that the technique of opening a valve with a balloon may cause a tear in the posterior and anterior valve leaflets which would promote an inflammatory infiltrate and pathological healing to occur cumulating in restenosis [41]. The healing process in the region of the trauma then leads to the deposition of fibrin and thickening of the valve leaflets. Microscopic evidence of an inflammatory role in restenosis is supported by findings from a Turkish study, where polymorphonuclear leucocytes where visualized within the endothelial cells of the valve with a narrow orifice [42]. Recently, serological evidence for an inflammatory basis in mitral restenosis was established. Serum levels of tumour necrosis factor α (TNFα), highly sensitive C-reactive protein (hs-CRP), matrix metalloproteinases (MMPs) and tissue-specific inhibitors of matrix metalloproteinases (TIMPs) were elevated in patients with mitral restenosis highlighting the role of inflammation in the process of restenosis [43]. The common denominator to all theories regarding the pathophysiological processes involved in mitral valve restenosis is inflammation, be it from turbulence, recurrent ARF or pathological healing following the BMV itself. In light of the above evidence linking inflammation to mitral restenosis, we aimed to assess the clinical utility of Ga-68 DOTATATE PET/CT in imaging patients with mitral restenosis post PBMV.

1.2 Aim and Objective

2. The aim of the study is to evaluate the presence of inflammation in the mitral valve using Ga-68 DOTATATE PET/CT imaging in patients post balloon valvotomy.
3. The primary objective is to determine the role of ongoing active rheumatic fever (based on serology) in patients post BMV with mitral stenosis.

1.3 Materials and Methods

The study will be a single-centre descriptive cross-sectional study conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between 1 April 2016 and 30 June 2016. Patients who had PBMV for severe mitral stenosis will be identified from the catheterization suite patient register located at the Division of Cardiology at CMJAH, and those to be included will be contacted telephonically.

Inclusion criteria

Patients ≥ 18 years of age with a history of a PBMV performed six months or longer prior to study inception (1 April 2016).

Patients with a mitral valve area of 0.5 cm^2 or less than that achieved immediately post PBMV

Exclusion criteria

Patients with active infective endocarditis

Patients with a history of endocarditis involving the mitral valve

Patients with a previous surgical intervention in the mitral valve other than PBMV.

The study procedure, aims and objectives of the study will be explained to the patients and informed consent will be obtained by the signing of forms approved by the Ethics Committee. All patients who have consented to participate in the study will be invited to CMJAH for a 2D trans-thoracic echocardiogram to evaluate for evidence of mitral restenosis.

1) To minimize inter-observer variation, echocardiograms will be performed by a single operator. The mitral valve orifice area by planimetry and pressure half time will be documented. y.

2) Bloods will be drawn for:
   anti-streptolysin O- titre (ASOT): ASOT <400 iu/ml
   erythrocyte sedimentation rate (ESR) analysis: ESR < 20mm/hour.

3) Study participants will be referred to the Nuclear Medicine Department at CMJAH for Ga-68 DOTATATE PET/CT imaging. An empiric activity of 185MBq of Ga-68

DOTATATE will be administered intravenously through a peripheral vein. After a delay of 60 minutes, cardiac images will be acquired with a Siemens Biograph Somaton Sensation 40 PET/CT camera for 10 minutes. Participants will be positioned supine with both arms raised above the head. The images will be interpreted by an experienced Nuclear Medicine Consultant.

Qualitative, semi-quantitative and quantitative assessments of the images will be performed to evaluate the extent of radiopharmaceutical uptake in the mitral valve. The qualitative assessment will be done by performing a visual analysis of uptake in the mitral valve, and reported as either present or absent. A semi-quantitative assessment of the uptake will be evaluated with a scoring method, comparing the degree of uptake in the mitral valve compared to the uptake in the apex of the myocardium. The apex, being the region of the myocardium with the highest uptake was chosen as the point of reference for the comparison as follows:

0= no visualized uptake in the mitral valve
1= visualized uptake in the mitral valve less than uptake in the apex
2= visualized uptake in the mitral valve equal to uptake in the apex
3= uptake in the mitral valve more than uptake in the apex
4= markedly increased uptake in the mitral valve that exceeds uptake in the apex

The standard uptake value (SUV) will be used for the quantitative assessment. The SUV measures the uptake in a region of interest normalized on the basis of a distribution volume (where patient's total body weight, lean body weight or body surface area can all serve as surrogates for body volume). The SUV is calculated by dividing the mean activity within a selected region (or volume) of interest (in mCi/ml) by the injected dose (in mCi/g). Assuming that the patient has a volumetric density of 1 g per mL, the SUV is a unit-less quantity that provides a means of comparison of radionuclide uptake in different sites.

4) The data will be entered without identifying the patient and only the researcher will know this. A data collection sheet will be used to collect study variables (appendix B)

1.4 Statistical Analysis

Study variables collected on a data collection sheet (appendix B) will be subsequently captured on Microsoft Excel (Version 16.29.1). The data will be analysed using SPSS software version 25 (originally, Statistical Package for the Social Sciences and now called Statistical Product and Service Solutions - IBM, Chicago). Categorical variables will be expressed as absolute numbers and percentages. Continuous variables with a normal distribution will be summarized as mean ± standard deviation, while variables with a skewed distribution will be reported as the median and interquartile range (25^{th} – 75^{th} percentile values). Independent sample t-test will be used to compare the mean values for the group of patients with uptake against those that do not exhibit Ga-68 DOTATATE uptake for the following measured parameters: SUV in the mitral valve, ASOT, ESR, mitral valve area (MVA) and time from BMV. A two-tailed Pearson correlation test will be done to assess for correlation between the SUV in the mitral valve and ASOT, ESR, mitral valve area and time from BMV. Differences will be considered statistically significant at a p-value < 0.05.

1.5 Ethics

Approval to conduct the study was obtained from the Human Research Ethics Committee at the University of the Witwatersrand (certificate number M150532) (appendix A).

1.6 Timing

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Preparing protocol												
Protocol assessment												
Ethics application												
Collecting data												
Data analysis												
Writing up thesis												

1.7 Funding

Funding was the researcher's responsibility. The costs included:

- Call billing costs to call the group of selected patients
- Transport money for the selected group of patients

The cost of the isotope and PET/CT scan imaging will be handled by the Nuclear Medicine Department.

1.8 Limitations

The sample size is a limitation, as is the fact that this is from a single site, and there is no control group.

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CHAPTER 2: SUBMISSIBLE ARTICLE

Gallium-68 DOTATATE Positron Emission Tomography/ Computed Tomography (PET/CT) imaging for the detection of the role of inflammation in the pathogenesis of restenosis in patients treated with percutaneous balloon mitral valvotomy

Authors

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Short title

The role of Gallium-68 DOTATATE PET/CT imaging for the detection of inflammation in mitral valve restenosis

Conflict of interest

None

Keywords

Inflammation, mitral restenosis, percutaneous mitral balloon valvotomy, Gallium-68 DOTATATE

ABSTRACT

Background

Mitral stenosis is a well-known long-term sequel to chronic rheumatic heart disease (RHD). Percutaneous mitral balloon valvotomy (PMBV) is a non-surgical technique for dilatation of the mitral valve. This method, among other non-medical approaches is used to reverse mitral stenosis. Restenosis post PMBV is a common problem. The mechanism through which this process occurs is still unclear. However, it has been suggested that restenosis following PBMV may have an inflammatory aetiology based on findings of elevated inflammatory markers in patients post PBMV.

Aims and Objectives

This study aimed to evaluate the role of inflammation in mitral valve restenosis following PBMV. The study also sought to demonstrate the role of ongoing active rheumatic fever (based on serology) in patients post BMV with mitral stenosis.

Methods

This was a single centre, descriptive cross-sectional study conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between 1 April 2016 and 30 June 2016. Patients previously subjected to PBMV with evidence of restenosis as detected by echocardiography underwent Positron Emission Tomography/ Computed Tomography (PET/CT) scan using Gallium-68 (Ga-68) DOTATATE were recruited into the study. Twenty patients were identified and contacted, but 6 were not entered into the study; informed consent was obtained for the remaining patients. Serological testing for rheumatic fever was additionally performed in an attempt to establish a link between recurrence of rheumatic activity and inflammatory activity in the mitral valve.

Results

The study population comprised of 13 patients. Eight (61.5%) patients were females. The mean age was 46.2 ± 11.8 years. Amongst patients with confirmed restenosis in the mitral valve, 54% failed to demonstrate Ga-68 DOTATATE uptake, while only 5% of patients demonstrated serological evidence of active rheumatic fever. A negative correlation was noted between the erythrocyte sedimentation rate (ESR) and time from balloon mitral

Conclusion

The study did not demonstrate significant Ga-68 DOTATATE uptake in mitral valves with echocardiographic evidence of restenosis post PMBV. Furthermore, in the majority of participants, serology did not confirm the role of ongoing rheumatic activity as the cause of mitral restenosis.

INTRODUCTION

Rheumatic heart disease (RHD) is a major cause of cardiovascular disease in children and young adults residing in developing countries. RHD is most prevalent in sub-Saharan Africa. An estimated 33 million people globally currently live with RHD [1]. The incidence of symptomatic RHD in individuals older than 14 years of age in Soweto was 24.7 per 100 000 population per annum [2]. In Cape Town, the prevalence of asymptomatic RHD in school children, as detected by echocardiography was 20 cases per 1 000 children [3]. Investigators with estimated that the prevalence may be as high as 30 per 1000 school going children when assessed with the use of echocardiography [15].

Although all cardiac valves may be involved in the rheumatic process, the mitral valve is most affected [4]. Mitral valve stenosis is one of the most common long-term complications of RHD, second only to mitral regurgitation. Mitral stenosis may manifest clinically up to twenty years following the initial attack of rheumatic fever [5]. Interventional procedures for symptomatic patients with mitral stenosis include closed or open mitral commissurotomy, mitral valve replacement (mechanical or bioprosthetic) and, percutaneous mitral balloon valvotomy (PMBV) [6, 7]. Balloon mitral valvotomy is the preferred intervention for rheumatic mitral stenosis offering immediate symptomatic relief [2].

Mitral restenosis is thought to be an inevitable long-term outcome post PMBV, often necessitating repeat PMBV, open mitral commissurotomy or mitral valve replacement. Restenosis is defined as a mitral valve area less than $1.5cm^2$ or a $\geq 50\%$ loss of the initial gain in valve area [9]. The mechanism behind restenosis is not well established. A traumatic injury to the mitral valve leaflet during PMBV with a resultant inflammatory process during the healing process has been found to result in mitral restenosis. The healing process in the region of the trauma then leads to the deposition of fibrin and thickening of the valve leaflets [10]

Gallium-68 (Ga-68) DOTATATE is a radiopharmaceutical that binds and localizes to cells that express somatostatin subtype-2 receptors such as neuroendocrine tumours [11]. Activated macrophages are cells typically seen in chronic inflammatory processes that express somatostatin subtype 2 receptors [1]. Thus, we hypothesized that Ga-68 DOTATATE imaging could be used to assess the role of inflammation in mitral valve restenosis post PMBV.

The aim of the study is to evaluate the role of inflammation in patients with mitral valve restenosis following PMBV using the radioisotope Ga-68 DOTATATE in PET/CT imaging. The study also sought to demonstrate the role of ongoing active rheumatic fever (based on serology) in patients post BMV with mitral stenosis.

MATERIALS AND METHODS

This was a single-centre descriptive cross-sectional study conducted at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between 1 April 2016 to 30 June 2016. Approval to conduct the study was obtained from the Human Research Ethics Committee at the University of the Witwatersrand (appendix B). A database of patients that underwent PBMV for severe mitral stenosis at CMJAH was obtained from the cardiology department and reviewed by the primary investigator. Patients who were ≥ 18 years of age with a history of a previous PBMV performed more than six months prior to study inception as well as echocardiographic evidence of restenosis were included in the study. Patients with a mitral valve area of 0.5 cm^2 or less than that achieved immediately post PBMV were also included. We excluded patients with active infective endocarditis or a history of endocarditis involving the mitral valve as well as those with a previous surgical intervention in the mitral valve other than PBMV.

Twenty patients were contacted telephonically and the study objectives were explained. Six patients were not recruited into the study (figure 1). Patients were requested to come to CMJAH for evaluation. After informed consent was obtained, an echocardiographic assessment of the mitral valve to screen for restenosis was conducted in each patient. The mitral valve orifice area by planimetry and pressure half time was assessed. Echocardiography was also used to exclude endocarditis. The transthoracic echocardiogram was performed by a single cardiology fellow to minimize intra-observer error.

Biochemistry

Blood samples for an antistreptolysin O titre (ASOT) and erythrocyte sedimentation ratio (ESR) were drawn from all patients with evidence of mitral restenosis. This served to

highlight a subset of patients in which repetitive episodes of rheumatic fever could have played a role in restenosis.

PET/CT image acquisition

Patients with evidence of restenosis as detected by echocardiography underwent positron emission tomography/computed tomography (PET/CT) scan using Gallium-68 (Ga-68) DOTATATE. Imaging was performed at the Nuclear Medicine department at CMJAH. The radiopharmaceutical (Gallium-68 DOTATATE) was prepared by radiographers within the department, following a set protocol for DOTATATE preparation (appendix C**)**. An empiric activity of 185MBq of Ga-68 DOTATATE was administered intravenously through a peripheral vein. After a delay of 60 minutes, cardiac images were acquired with a Siemens Biograph Somaton Sensation 40 PET/CT camera for 10 minutes. Patients were positioned supine with both arms raised above the head. This was done to prevent the arms from blocking the view of the heart. The PET scan was acquired in 2D mode and corrected for attenuation using low dose CT. The CT was also used for anatomical correlation of PET findings as well as for diagnostic evaluation. CT slice thickness was set at 5 mm.

PET/CT image interpretation

The scans were reviewed and interpreted by an experienced nuclear medicine specialist. Qualitative, semi-quantitative and quantitative assessments of the images were performed to evaluate the extent of radiopharmaceutical uptake in the mitral valve. The qualitative assessment was done by performing a visual analysis of uptake in the mitral valve and was reported as either present or absent. A semi-quantitative assessment of the uptake was evaluated with a scoring method compared the degree of uptake in the mitral valve compared to the uptake in the apex of the myocardium. The apex, being the region of the myocardium with the highest uptake was arbitrary chosen as the point of reference for the comparison as follows:

0= no visualized uptake in the mitral valve

1= visualized uptake in the mitral valve less than uptake in the apex

2= visualized uptake in the mitral valve equal to uptake in the apex

3= uptake in the mitral valve more than uptake in the apex

4= markedly increased uptake in the mitral valve that exceeds uptake in the apex

The standard uptake value (SUV) was used for the quantitative assessment. The SUV measures the uptake in a region of interest normalized on the basis of a distribution volume (where patient's total body weight, lean body weight or body surface area can all serve as surrogates for body volume). The SUV is calculated by dividing the mean activity within a selected region (or volume) of interest (in mCi/ml) by the injected dose (in mCi/g). Assuming the patient has a volumetric density of 1 g per mL, the SUV is a unit-less quantity that provides a means of comparison of radionuclide uptake in different anatomical sites.

STATISTICAL ANALYSIS

Study variables were collected on a data collection sheet (appendix B) and subsequently captured on Microsoft Excel (Version 16.29.1). The data was analysed using SPSS software version 25 (originally, Statistical Package for the Social Sciences and now called Statistical Product and Service Solutions - IBM, Chicago). Categorical variables were expressed as absolute numbers and percentages. Continuous variables with a normal distribution were summarized as mean ± standard deviation, while variables with a skewed distribution were summarized as the median and interquartile range (25^{th} – 75^{th} percentile values). Confidence intervals were calculated at 95% interval levels and differences were considered statistically significant at a *p*-value < 0.05. Independent sample t-test was used to compare the mean values for the group of patients that had isotope uptake against those that did not have isotope uptake for the following measured parameters: SUV in the mitral valve, ASOT, ESR, mitral valve area (MVA) and time from BMV. A two-tailed Pearson correlation test was done to assess for correlation between the SUV in the mitral valve and ASOT, ESR, mitral valve area and time from BMV.

RESULTS

The study population comprised 13 patients (Figure 1). There were eight (61.5%) women and the mean age was 46.2 ± 11.8 years. The majority of the patients were African (84.6%). Ga-68 DOTATATE uptake in the mitral valve was visualized in 6 patients (46.1%). The rest of the baseline characteristics are shown in Table 1. The mean time post PBMV was 9.5 ± 3.9 years. The mean SUV in the mitral valve of patients with evidence of Ga-68 DOTATATE uptake was 1.37 ± 0.41 (p=0.0000).

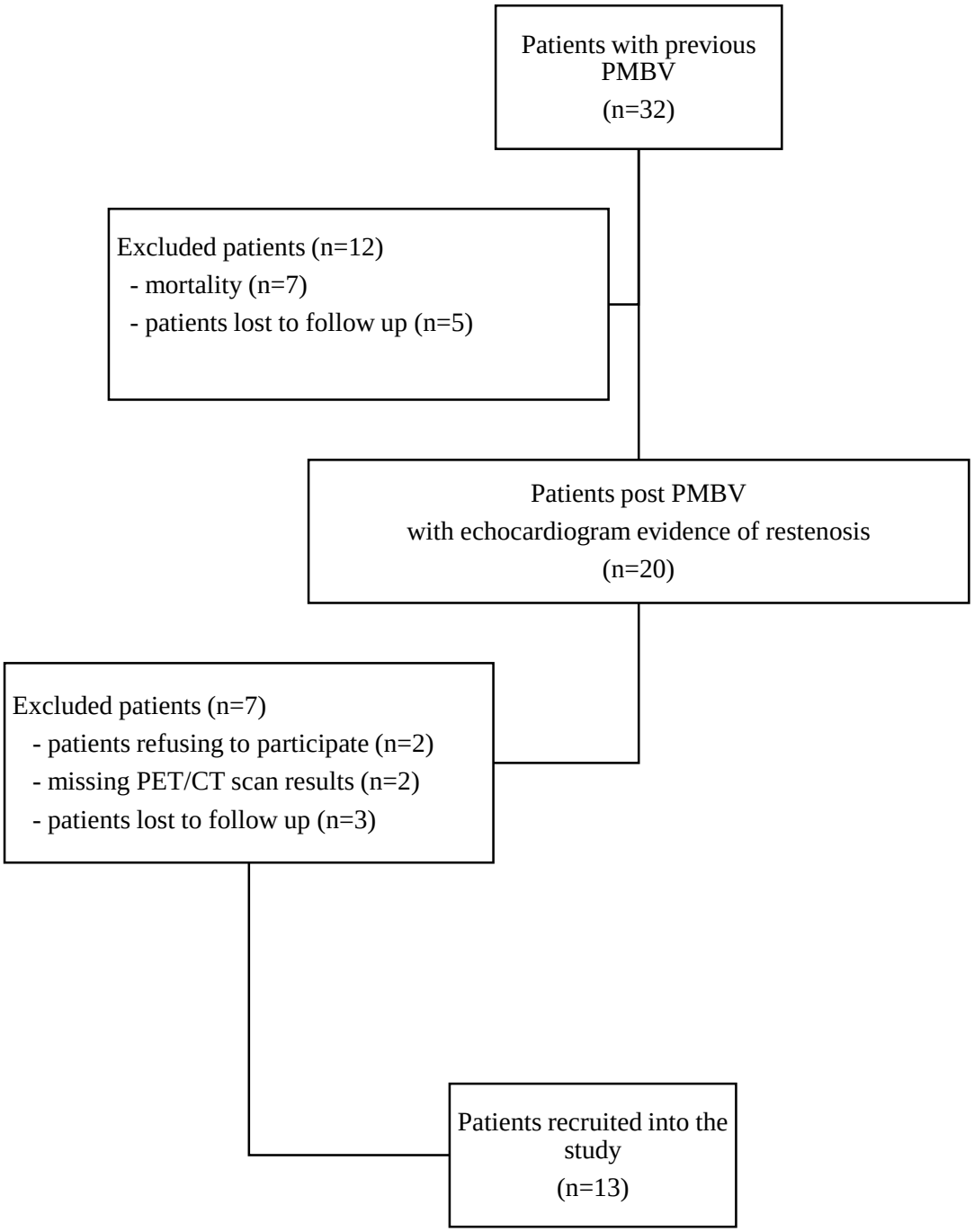

Figure 1 Flow chart of the study

Table 1 Demographic and clinical characteristics of patients with mitral restenosis stratified according to Gallium-68 DOTATATE uptake in the mitral valve

Variable	Overall population (n=13)	Uptake in mitral valve (n=6) (46.1%)	No uptake in mitral valve (n=7) (53.8%)	p-value
Age (years)	*46.2 ± 11.8	*49.5 ± 12.8	*43.4 ± 11.1	0.379
Female, n (%)	*8 (61.5)	*4 (66.7)	*4 (57.1)	0.725
Ethnicity, n (%)				0.097
African	*11 (84.6)	*4 (66.7)	*7 (100.0)	
Mixed ancestry	*2 (15.4)	*2 (33.3)	*0 (0.0)	
Laboratory indices				
ESR	*21.2 ± 15.5	*25 ± 14.4	*17.9 ± 16.7	0.4305
ASOT	#149.5 (29 - 109)	#187.5 ± 254.2	#116.9 ± 194.9	0.5919
Mitral valve area (cm^2)	*0.68 ± 0.17	*0.70 ± 0.17	*0.65 ± 0.19	0.6773
Time from BMV (years)	*9.5 ± 3.9	*8.3 ± 4.3	*10.4 ± 3.5	0.3518

Data shown as mean ± standard deviation (SD) for continuous variables with a normal distribution and median and interquartile range (p25 -p75) for continuous variables with a skewed distribution. For categorical variables, data is represented as absolute numbers and percentages. ASOT: anti-streptolysin O titre; BMV: balloon mitral valvotomy; ESR: erythrocyte sedimentation rate; MVA: mitral valve area; SUV: standard uptake value

Footnote* Mean ± SD

Median with IQR

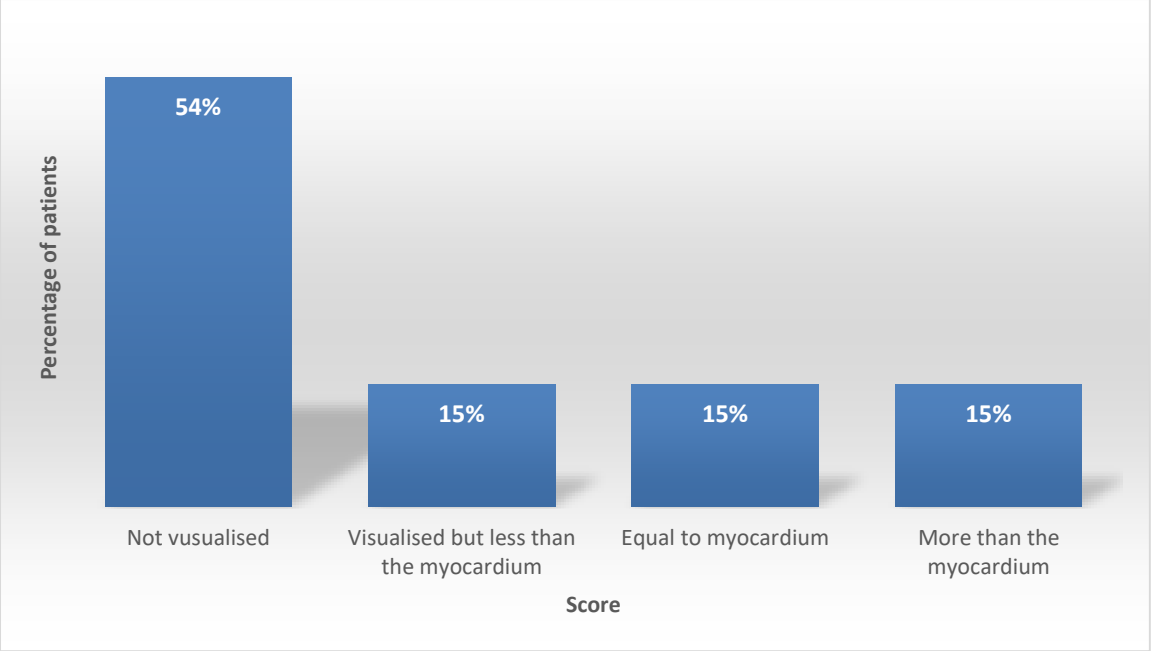

Figure 2 Percentage of patients demonstrating Gallium-68 DOTATATE uptake in the mitral valve on visual analysis of images

Correlation between the SUV in the mitral valve and ASOT, ESR, mitral valve area and time from BMV was assessed. A significant correlation was noted between ESR and time from BMV ($r = -0.695$, $p = 0.0083$). The rest of the results are presented in Table 2.

Table 2 Pearson's correlation coefficients between laboratory indices, SUV in the mitral valve, mitral valve area and time from balloon mitral valvotomy

	Mitral valve SUV	**ASOT**	**ESR**	**Mitral valve area**	**Time from BMV**
Mitral valve SUV	1.0000				
ASOT	0.0022	1.0000			
p-value	0.9943				
ESR	-0.0489	0.5245	1.0000		
p-value	0.8740	0.0657			
Mitral valve area	0.1600	-0.2798	-0.0326	1.0000	
p-value	0.6015	0.3546	0.9157		
Time from BMV	-0.0923	-0.3041	-0.6954	0.0296	1.0000
p-value	0.7642	0.3123	0.0083	0.9236	

DISCUSSION

The study attempted to demonstrate through the use of imaging, the role of inflammation in the mitral valve as the cause of restenosis post PMBV. The mean age in our cohort was 46 years, in keeping with findings by Palacios et al. who found a mean age of 49 years in 35 patients who had PBMV for mitral restenosis [7]. Furthermore, similar to our study, there was a female preponderance in the study by Palacios and colleagues. The higher prevalence of RHD in females is unclear and hypothesized to be related to social factors such as involvement in child-raising hence increased susceptibility to Streptococcus pyogenes infection as well as the pregnancy state, imposing a higher cardiac burden [4].

Recurrent episodes of rheumatic fever have been implicated to be responsible for mitral valve restenosis post PMBV [14]. There was no correlation between ESR and ASOT with the

mitral valve area. Only 5% of patients demonstrated serological evidence of active rheumatic fever, leading us to conclude that ongoing rheumatic activity was unlikely to be responsible for mitral valves restenosis in our cohort. Mitral restenosis after PMBV is often thought to be a time-dependent occurrence. In our study, the mean duration post PMBV was 9.5 years. This finding cannot be translated to the amount of time it took to develop restenosis as our patients were not followed up, and restenosis was assessed at a single time point.

Ga-68 DOTATATE was the radiopharmaceutical agent of choice because of its ability to bind and internalize in macrophages expressing somatostatin subtype-2 receptors. The results obtained in this study could not attribute inflammation as the causative factor leading to valve restenosis. We hypothesize that perhaps the number of activated macrophages was too few in the mitral valve with evidence of restenosis or the valve leaflets did not have resident activated macrophages.

During the interpretation of Ga-68 DOTATATE PET/CT images, we used an arbitrary SUV cut off point that is not supported by previous research studies. Although 54% of patients with echocardiographic evidence of mitral restenosis were found to have Ga-68 DOTATATE uptake in the mitral valves, the clinical utility of this imaging modality in delineating inflammation in valve tissues remains to be proven.

The main limitation of the study is the small sample size. Further multi-centre studies with a large number of patients are required to evaluate the true clinical utility of Ga-68 DOTATATE PET/CT when imaging patients with valvular heart diseases suspected to have underlying inflammation. This could potentially expand the use of cardiac imaging in evaluating inflammation in patients suspected to have mitral restenosis following PMBV. Secondly, the study should have been designed such that there is a control group, consisting of patients with previous PBMV, but no evidence of restenosis. This would have allowed better comparison of SUV and patterns of Ga-68 DOTATATE uptake in the mitral valve. Lastly, not all ethnic groups were represented in the study sample. The study results may not be generalizable across all ethnic groups. This may be investigated in future studies.

LIMITATIONS

This was a single-center study with no control group and small sample size. In addition, blood samples for ASOT and ESR were only drawn from patients with evidence of mitral restenosis.
The study should have been designed such that there is a control group, consisting of patients with previous PBMV, but no evidence of restenosis.

Cardiac uptake and quantification done to date utilize FDG, in our study DOTOTATE was used and our results were based on us extrapolating that the principals would be similar. Future studies would be needed to validate our method.

This is novel study and as such there is a lack of standardized guidelines/protocols for the use of PET/CT in VHD with regards to DOTATATE.

CONCLUSION

Within the limitations of this study, the study did not demonstrate significant Ga-68 DOTATATE uptake in mitral valves with echocardiographic evidence of restenosis post PMBV. Furthermore, in the majority of participants, serology did not confirm the role of ongoing rheumatic activity as the cause of mitral restenosis.

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Appendix A: Ethics approval form

R14/49 Dr. Dominic Kakooza

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150532

NAME: (Principal Investigator)	Dr. Dominic Kakooza
DEPARTMENT:	Internal Medicine Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Ga DOTATATE-PET/CT Scan Imaging for the Detection of the Inflammation in the Pathogenesis of Restenosis in Patients with Rheumatic Mitral Valve Stenosis Treated with Percutaneous Balloon Mitral Valvotomy.
DATE CONSIDERED:	29 May 2015
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Manga
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	10/07/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Data collection sheet

Study number: **Date:**

Hospital number: **Race:**

Gender: **Date of PMBV:**

Contact number:

Mitral valve area (cm^2)	
Restenosis: Y/N	
ASOT	
ESR	
PET/CT Scan	
Isotope uptake Y/N	
SUV in the mitral valve	**SUV in the apex**

Appendix C: Gallium-68 DOTATATE preparation

The process of preparation of the radiotracer Gallium-68 DOTATATE took place on the morning of the procedure. An automated system linked to $^{69}Ge/^{68}Ga$ generator for the preparation was used. The complex stepwise process cumulates to an end product of Gallium-68 being labelled with the peptide (DOTATATE).

1850MBq of Gallium-68 and 25µg of DOTATATE were introduced into the ITG (Isotope technologies Garching GmbH) fluid labelling module. Following the admixture of the two substrates, a separation phase then isolates labelled and non-labelled DOTATATE, the unlabelled being removed. During the process, approximately 555MBq of Gallium-68 decays therefore only 1295MBq of Gallium-68 was ultimately available to be paired with DOTATATE.

Lastly a quality control measure was undertaken to remove impurities of uncomplexed Gallium-68 utilizing a silica gel plate. Once the preparation was ready it was used within thirty minutes to minimize decay.

Appendix D: SUV explained

SEMI-QUANTITATION OF TRACER UPTAKE IN PET IMAGES BASED ON COLOUR SCALE

SUV is the most commonly used index for the quantification of uptake in PET or PET/CT scans. It is a semi-quantitative measurement of the uptake in a region of interest normalized on the basis of a distribution volume (where patient's total body weight, lean body weight or body surface area can all serve as surrogates for body volume).

The SUV is calculated by dividing the mean activity within a selected region (or volume) of interest (in mCi/ml) by the injected dose (in mCi/g).

$$\text{SUV} = \frac{\text{Activity in ROI or VOI (mean or max)/volume of ROI or VOI}}{\text{Total injected activity/Volume surrogate}}$$

Assuming the patient has a volumetric density of 1 g per mL, the SUV is a unit-less quantity that provides a means of comparison of FDG uptake between patients.

Images can be viewed over a grey scale or colour scale range.
The GQS 10 is a fixed 10 step colour scale. This "rainbow" colour scale has relatively abrupt changes in colour, which enable easy differentiation of uptake intensity in the low, mid or high range.

By looking at PET images in SUV units, patient's images were compared on the same colour scale.

Appendix E: Consent Form

Charlotte Maxeke Johannesburg Academic Hospital

Department of Internal Medicine

Division of Cardiology

I, ... hereby consent to participation on the study:

Gallium-68 DOTATATE PET/CT scan imaging for the detection of the role of Inflammation in the pathogenesis of restenosis in patients with rheumatic mitral valve stenosis treated with percutaneous balloon mitral valvotomy.

I hereby consent to my blood being taken, to an echocardiogram being performed and to a PET scan being done with the administration of an intravenous isotope.

The nature and possible risks have been explained to me by the study investigator/other (specify name) …… in my preferred language and in a way that I understand.

Signature of participant..... Date: ..../..../20..

Time: ...:...

Witness 1: .....

Witness 2: .....